Voila, a New *Drosophila* Courtship Variant that Affects the Nervous System: Behavioral, Neural, and Genetic Characterization

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In *Drosophila melanogaster*, a specific *PGAL4* transposon induces the *Voila*¹ genetic variant and produces multiple phenotypes. Homozygous *Voila*^{1/1} flies rarely reach adulthood, whereas heterozygous *Voila*^{1/+} adult males show strong bisexual behavior. Males with a single copy of *Voila*¹ driving the feminizing transgene *UAS-transformer* show very reduced sexual activity but no overall effect on their behavior. *Voila*¹ is specifically expressed in the nervous system. In the CNS, it is expressed mainly in the mushroom bodies and, to a lesser

extent, in the antennal lobes. In the peripheral nervous system, *GAL4* expression is almost entirely restricted to the gustatory sensilla. Using chromosomal deficiencies, the behavioral alteration was genetically mapped to the same location as the *PGAL4* element (86E₁₋₂). The multiple behavioral effects of the *Voila* genetic variant are discussed in light of its expression in the nervous system and its genetic basis.

Key words: *Drosophila*; courtship behavior; *PGAL4*; gustation; mushroom bodies; transformer

Understanding the function of genes and the neural circuitry that underlies complex behaviors has been the focus of increasing scientific interest over the past decade. In *Drosophila melanogaster*, studies of courtship offer an explanation of the ways genes influence the execution of complex behaviors (Greenspan, 1995).

Sexually mature *D. melanogaster* males perform elaborate courtship behaviors in response to conspecific females (Sturtevant, 1915). Mature males generally court female and immature flies, but in some circumstances they also vigorously court conspecific mature males (Ferveur et al., 1995, 1997; Zhang and Odenwald, 1995; Yin Hing and Carlson, 1996). During a courtship bout, the male orients toward his partner and taps her (or his) abdomen with his forelegs. Next, he will vibrate one wing, thereby producing a species-specific song. Then, he may lick the genitals of his partner, curl his abdomen, and attempt copulation. These stereotypic precopulatory behaviors are sex-specific and genetically determined (Hall, 1994).

In *D. melanogaster*, very few genes are known that affect only courtship. In the genetic hierarchy of sex differentiation, *Sex-lethal* (*Sxl*) and *transformer* (*tra*) control sexual behavior as part of their larger influence on somatic sex determination (Sturtevant, 1945; Tompkins and McRobert, 1989, 1995). Downstream of *tra*, *doublesex* and *fruitless* (*fru*) control two independent pathways of sexual differentiation (Taylor et al., 1994; Villela and Hall, 1996).

fru acts specifically on the CNS (Ryner et al., 1996), and various *fru* mutant alleles diversely affect male courtship behavior (Ito et al., 1996; Villella et al., 1997). One of the most conspicuous behavioral phenotypes exhibited by *fru* males is the courtship chain of male flies in which individuals are simultaneously courters and courted. Recently, the *dissatisfaction* (*dsf*) gene has been shown to specifically affect—downstream of *tra* but independently of *dsx*—sex-specific courtship behaviors and neural differentiation in flies of both sexes (Finley et al., 1997).

A pioneering technique that produced mosaic flies with XX and XO tissues was initially used to map the neural structures that control male courtship (Hall, 1977, 1979; von Schilcher and Hall, 1979) and female receptivity to male courtship (Tompkins and Hall, 1983). It is now possible to feminize small subsets of the male brain by ectopically expressing the female form of the sex-determination gene *tra* (*UAS-tra*), under control of *PGAL4* enhancer-trap lines. The partial feminization of the antennal lobes (ALs) or the mushroom bodies (MBs) or both causes mosaic males to engage in bisexual courtship (Ferveur et al., 1995; O'Dell et al., 1995). These results suggest that MBs and ALs differ functionally between the sexes. The MBs, which are neurally connected with the ALs, contain a sexually dimorphic number of neurons (Technau, 1984) and express male-specific genes such as *roXI*, independently of *tra* activity (Amrein and Axel, 1997; Meller et al., 1997). The prothoracic legs, the maxillary palps, and the antenna also show a different number of chemosensilla in the two sexes (Nayak and Singh, 1983; Singh and Nayak, 1985; Venard et al., 1989; Stocker, 1994). In particular, primary afferents from the leg sensilla project with a sexually dimorphic pattern into the thoracic ganglia (Possidente and Murphy, 1989).

Mate recognition relies on sexually dimorphic signals and especially on cuticular hydrocarbons that act as sex pheromones (Antony and Jallon, 1982; Jallon, 1984). *Drosophila* males are able to simultaneously perceive female and male predominant pheromones: female pheromones stimulate male sexual excitation, whereas male-specific molecules tend to inhibit male excitation

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(Ferveur and Sureau, 1996). Pheromones are mainly detected by contact during tapping and licking, pinpointing the crucial role of gustatory organs borne by the prothoracic legs and the proboscis (Ferveur, 1997). A recent experiment shows that the sexual orientation of mosaic XY flies is independent of their pheromonal feminization (Ferveur et al., 1997).

Here we describe the behavioral, neuronal, and genetic characteristics of the new genetic variant *Voila*, which may play a crucial role in pheromonal perception and male courtship behavior. *Voila* is the first reported case of a complete block of courtship correlated with manipulation of a restricted part of the nervous system.

MATERIALS AND METHODS

Fly stocks and genetics. Fly stocks were maintained at 25°C in a 12 hr dark/light cycle. Crosses were performed using standard techniques. A description of the chromosomes and mutations used in this study can be found in Lindsley and Zimm (1992).

The *PGAL4* DB345 insertion line (carrying *Voila*¹) was isolated in an enhancer-trap screen that focused on the fly chemosensory system (DB345 was kindly provided by Damina Balmer, Fribourg). Because the *Voila*¹ insert is recessive lethal, the *Voila*¹ chromosome was maintained balanced over the chromosome *TM3*, *Sb Ser*. To produce (shi) *Voila*^{1/+}, a strain built with the genetic background of the *shibire*^{ts} strain (shi), *Voila*^{1/TM3} males were crossed with shi females, and subsequent heterozygous *Voila*^{1/+} male progeny were backcrossed to shi females for five successive generations. Neither males nor females from the (shi) *Voila*^{1/+} strain carry the X chromosome associated with the thermosensitive *shi*^{ts} mutation. We also backcrossed *Voila*¹ into other genetic backgrounds: flies from these strains yielded the same behavioral tendency, although it was weaker than in (shi) *Voila*^{1/+}.

Genetic mapping of the behavioral defect caused by the *Voila*¹ insert was performed with deficiencies covering the chromosomal region between 86C and 87C (for information about their breakpoints and origin, see Reuter et al., 1987).

Females from the shi strain and Canton-S (CS) males were chosen as control objects because of their clear behavioral phenotypes (Ferveur and Sureau, 1996; Ferveur et al., 1997).

Behavioral tests. Behavioral tests were performed using a protocol modified from Ferveur and Sureau (1996). Briefly, all behavioral assays were performed on 4-d-old males (kept alone after eclosion) that were individually aspirated into an observation chamber (2.8 cm diameter, 0.5 cm height). After 10 min, a 4-d-old virgin shi female or a CS male, used as the object fly, was introduced. The courtship index value (*CI*) is the percentage of time that the subject male spends courting during a 10 min observation period. We noted the occurrence of different stereotypical male behaviors: tapping, wing vibration, licking the genitalia, and attempted copulation. We also noted the frequency with which subject males attempted copulation with an object fly. Object flies were generally decapitated (except when otherwise indicated).

Decapitation prevents reciprocal courtship and standardizes the duration of courtship, because no copulation occurs with decapitated females (Ferveur et al., 1995). Courtship indices were arc sin-transformed before being compared statistically. The data were not always normally distributed, probably because there was a high proportion of males that did not show any homosexual courtship (*CI* = 0). The effect of the genotype of both flies (courter and courted) and their interaction was measured with a two-factor ANOVA. Using the same procedure, the effect of decapitation was separately tested on male or female object flies. We decided not to mix the three factors in the same analysis because the "decapitation-intact" factor produces very different behavioral consequences in the two sexes: copulation occurs only in intact females.

Locomotor activity was measured in similar environmental conditions. We averaged the total number of lines, drawn under the chamber, crossed by each fly (locomotor activity units = LAU) [modified from Tompkins et al. (1982)]. For each experiment, four single flies were sequentially observed for five periods of 20 sec, every 2 min. At least 25 flies per genotype were studied. Locomotor activity values were normally distributed and thus were compared with a Student's *t* test.

The sexual receptivity of females was measured by latency to copulation when paired with control males. Logarithmically transformed ($\log_e$) latencies were statistically compared between experimental and control females (Mann–Whitney *U* test).

Histochemistry and immunocytochemistry. The *Voila*^{1/TM3} strain was crossed to a *UAS-lacZ* strain (Brand and Perrimon, 1993), to a *UAS-tau* strain (Ito et al., 1997), or to a *UAS-GFP* strain (Brand, 1995). The transactivated β -galactosidase was visualized either histochemically or immunocytochemically. In the first case, heads, thoraces, and abdomens of the *Voila*¹ *UAS-lacZ* heterozygotes were dissected in Millonig's buffer, fixed in 1% glutaraldehyde (in Millonig's buffer), and stained for β -galactosidase activity with a solution containing 5–10 mg X-Gal/ml DMSO (Brand and Perrimon, 1993). The CNS, heads, thoraces, wings, and legs were mounted in Faure's solution. For immunocytochemistry, a monoclonal anti- β -galactosidase antibody (Promega, Madison WI) was applied 1:2000 overnight at 4°C to 10 μ m cryosections and then stained with the Vectastain ABC Kit (Vector Laboratories, Burlingame CA). The tau protein in *Voila*¹ *UAS-tau* heterozygotes was tagged by a monoclonal anti-tau antibody (Sigma, St. Louis MO) (1:2000) and stained in the same way as for β -galactosidase. GFP patterns were visualized in a Bio-Rad MRC 1024 confocal microscope.

In situ chromosomal mapping. Polytene chromosome squashes from *w*; *Voila*^{1/+} heterozygotes were prepared using the procedure of Zuker et al. (1985). The position of the *PGAL4* insertion was determined using the biotinylated DNA probe of the pBM292 plasmid, which contains the *GAL4* sequence. *In situ* hybridization to polytene chromosomes was performed according to Smith et al. (1990). The DNA of the pBM292 plasmid was biotinylated using a nick-translation system (BRL, Bethesda, MD) and biotin-11-dUTP and detected by the binding of streptavidin-coupled alkaline phosphatase (Enzo Biochemicals).

RESULTS

The bisexual orientation of *Voila*¹ segregates with the chromosome 3 carrying the *PGAL4* transposon

The strain DB345 is an enhancer-trap strain that we induced by *PGAL4* mutagenesis (Brand and Perrimon, 1993). Males with a single copy of the *PGAL4* DB345 transposon show abnormal courtship behavior: they actively court both virgin females and mature males. The locus containing the *PGAL4* insertion is associated with altered male sexual orientation and was named *Voila* (*à la voile et à la vapeur*, French slang for a bisexual human).

Mutant males of the original strain *Voila*^{1/+} and of the balanced strain *Voila*^{1/TM3}, exhibited a vigorous bisexual courtship toward both intact and decapitated flies (Fig. 1). *Voila*^{1/TM3} males were still able to discriminate between intact females and males: their courtship intensity was higher with females ($CI_f = 83$) than with males ($CI_m = 35$). Furthermore, *Voila*^{1/TM3} males attempted to copulate more often with intact female (83%) than with intact male partners (18%). When paired with decapitated targets, *Voila*^{1/TM3} males also showed very vigorous bisexual courtship responses ($CI_f/CI_m = 66/38$); 73% and 29%, respectively, of these males attempted to copulate with decapitated female or male flies.

The chromosome 3 carrying the *Voila*¹ insert, outcrossed in the background of the control *shibire* strain [(shi) *Voila*^{1/+}; see Materials and Methods], segregated with a strong male bisexual orientation: (shi) *Voila*^{1/+} males exhibited courtship responses that were very similar to those of *Voila*^{1/TM3} males. With intact targets, their courtship indices (CI_f/CI_m) were 93/49 (81/43, with decapitated flies); 84% and 21% of male flies attempted to copulate with intact female and male partners, respectively (95% and 18%, with decapitated targets). In comparison, control CS males performed highly active heterosexual courtship with females, but very little homosexual courtship ($CI_f/CI_m = 72/3$ with intact flies and 47/3 with decapitated flies).

Statistical analysis reveals that the genotype of the courter and the sex of the courted, but not their interaction, significantly affect the intensity of courtship (Table 1). This suggests that subject males from the three strains show the same degree of sexual discrimination (ability to discriminate the sex of their partner). Also, decapitation exerts a significant behavioral effect when tested with object females, but not with males.

Figure 1. Courtship behavior of *Voila*¹, *Voila*¹ *UAS-tra*, and control males. The courtship directed toward intact and decapitated male or female targets is expressed as the courtship index (*A*) or as the frequency of complete courtship (*B*). For each genotype, the courtship of 35 and 20 males was measured with male and female targets, respectively. For description of genotypes, see Material and Methods. *A*, Courtship index values were measured as the percentage of time spent courting by the subject male, during a 10 min observation period. The height of each bar indicates the mean ($\pm$ SE) for the corresponding genotype. *B*, The frequency of complete courtship was calculated from the percentage of subject males that attempted copulation with an object fly.

*Voila*¹/*TM3* females did not significantly differ for sexual receptivity ($U = 708.5$; $n = 37/44$; $p = 0.32$) or for locomotor activity ($t = 0.624$; $df = 82$; $p = 0.53$) when compared with control CS females.

The genetic feminization driven by *Voila*¹ yields males with very reduced sexual excitation

Male flies carrying a single copy of the *Voila*¹-*PGAL4* insertion together with the feminizing transgene *UAS-transformer* (*UAS-tra*) (Ferveur et al., 1995) exhibited very little courtship toward females and males: $CI_f/CI_m = 3/2$ with intact flies and 16/15 with decapitated flies (Fig. 1). Furthermore, *Voila*¹ *UAS-tra* males very rarely attempted to copulate with targets of either sex.

The behavioral defect in *Voila*¹ *UAS-tra* is not caused by a general alteration of behavior, because the locomotor activities of

both *Voila*¹/*TM3* and *Voila*¹ *UAS-tra* males are similar (113 ± 6 and 109 ± 8 LAU; see Materials and Methods). Moreover, the locomotor activity of both of these male genotypes is significantly higher than for CS males (68 ± 4 LAU; $t = 6.45$; $df = 93$; $p < 0.0001$).

*Voila*¹ *UAS-tra* females were not significantly different from control females (+/*UAS-tra*; +/*TM3*) either for sexual receptivity ($U = 1062$; $n = 40/58$; $p = 0.49$) or for locomotor activity ($t = 0.649$; $df = 56$; $p = 0.52$).

Mapping the bisexual behavior caused by *Voila*¹

The *PGAL4* insertion in the *Voila*¹ strain was mapped *in situ*, using a *GAL4* DNA probe, to the chromosomal region 86E₁₋₂ (Fig. 2*A*).

Using a set of chromosomal deficiencies *Df*(3*R*) covering the

Table 1. Effect on male courtship index of the genotype of the courter fly (subject), of the state of the courted (object), and of their interaction

Subject fly (genotype)	Object fly (treatment/sex)	Courtship index (arc sin transformation)			
		Effect	df	<i>F</i>	<i>p</i>
<i>Voila</i> /TM3 (shi) <i>Voila</i> /+ Canton-S	Intact female or male	Subject	205	63.71	0.00001
		Object		417.80	0.00001
		Interaction		1.65	0.19
	Decapitated female or male	Subject	257	74.14	0.00001
		Object		223.58	0.00001
		Interaction		0.39	0.68
	Intact or decapitated female	Subject	194	55.04	0.00001
		Object		23.83	0.00002
		Interaction		0.08	0.93
	Intact or decapitated male	Subject	268	78.17	0.00001
		Object		1.23	0.27
		Interaction		3.59	0.03

Sex and decapitation of the object were separately tested with a two-factor ANOVA. For genotypes of subject flies, see Materials and Methods.

region 86C to 87C (Fig. 2*B*), we tested whether the male bisexual orientation observed in the *Voila*¹ strain was caused by the *PGAL4* insertion. The courtship indices of five males with various *Df(3R)/TM3* genotypes toward control females and males were measured and compared with control *w; + /TM3* males (Fig. 2*C*). The data shown in Table 2 reveal that male flies carrying a single copy of the chromosomal region 86D₄–E₁₉ exhibited abnormal sexual behavior. Among all strains carrying deficiencies, only *Kx-1/TM3* and *T-32/TM3* males differed significantly from control males in their homo- and heterosexual courtship (Table 2). Furthermore, males from these two strains did not discriminate male and female objects, unlike males carrying other deficiencies that clearly showed a strong heterosexual orientation (Fig. 2*C*). The significant interaction between both male genotypes *Kx-1* and *T-32*, with regard to the sex of their courted object, suggests that various chromosomal aberrations around the point of *Voila* insertion can affect male courtship differently. Indeed, we have found that *Kx-1* and *T-32* males differ for both their hetero- and homosexual courtship (ANOVA; Newman–Keuls *post hoc* test: *p* = 0.008 and 0.029, respectively).

We also found that *Voila* homozygotes are lethal, and this defect was also mapped to the chromosome interval 86D₄–E₁₉ (data not shown).

***Voila* expression pattern in the adult brain and thoracic ganglia**

Using X-gal as a substrate in *Voila*¹ *UAS-lacZ* flies, similar patterns of expression were observed in the brain of male and female flies. The most intense staining occurs in Kenyon cells, the intrinsic elements of the MBs (Fig. 3) (Balmer, 1994). All of the Kenyon cell components were clearly visible, including the densely clustered cell bodies in the calyx region and their elaborate fiber pathways in the pedunculus and the MB lobes. Other strongly labeled interneurons were present in the lamina, and a number of other cell bodies and fibers expressed *lacZ* in the brain and in the subesophageal ganglion (SOG).

Anti- β -galactosidase immunocytochemistry showed similar patterns and revealed additional elements. Staining was present in a number of cell bodies lateral and ventral to the ALs that form dense arborizations in the AL neuropil. In addition, a few labeled fibers of unknown identity were found in the antennal commis-

sure. Outside the ALs, expression was observed in a subset of cells in the pars intercerebralis and their projections in the median bundle, as well as in processes in the giant commissure. Afferent staining in the labial nerve (Fig. 3) (see below) was correlated with label in gustatory centers in the SOG (Stocker and Schorderet, 1981; Nayak and Singh, 1985). The *tau* reporter gene pattern was similar to the anti- β -galactosidase pattern and showed in addition that some of the cells ventral to the ALs projected to the inner antennocerebral tract. This suggested expression in relay interneurons that link the ALs with higher brain centers.

In the ventral ganglia, strong labeling of a large number of elements obscured the sensory projections from legs and wings (see below).

Expression pattern in the PNS of the adult head

In *Voila*¹ flies crossed with *UAS-lacZ*, *UAS-tau*, or *UAS-GFP*, reporter gene expression was observed in specific elements of the head sensory system (Fig. 3). In the labial palps, there was massive label in each taste bristle (Ray et al., 1993) but not in taste pegs (Falk et al., 1976). The simultaneous staining of bristle shafts and afferent axons suggests that reporter gene expression resided in the sensory neurons. However, an additional expression in sheath cells remains possible.

In the pharynx, massive staining was present in the labral sense organ and in the ventral cibarial sense organ (Stocker and Schorderet, 1981; Nayak and Singh, 1983; Balmer, 1994; Stocker, 1994), both of which are putative gustatory sensilla. Less intense label occurred in fishtrap bristles, and there was no expression in the dorsal cibarial sense organ. Staining in certain head nerves was caused by either labeled afferents (e.g., from taste bristles in the labial nerve) or a subset of peripheral nerve glia (e.g., at the base of the maxillary palps or in motor nerve arborizations).

In the third antennal segment, reporters were expressed in all of the trichoid and basiconic sensilla, including their shafts, which were labeled. The *tau* pattern revealed additional expression in sensilla of the sacculus (Shanbhag et al., 1995) and perhaps in coeloconic sensilla. With none of the reporters was expression visible in afferents from the third segment, suggesting that *GAL4* expression is localized in trichogen cells rather than in sensory neurons. In the maxillary palps, expression resided in the basi-

Figure 2. Mapping of *Voila*¹. *A*, *In situ* chromosomal mapping of the *PGAL4* insertion in salivary gland of *w; Voila*^{1/+} larva. *B*, Deficiencies used to map the bisexual courtship observed in *Voila*^{1/TM3} males. Deficiencies are aligned with the salivary gland chromosome map (Lindsley and Zimm, 1992). Bars represent the extent of the deficiencies, and the hatched region represents uncertainty as to breakpoint position. The double-headed line indicates the chromosomal region 86D₄–E₁₉. *C*, Deficiency mapping of the behavioral phenotype of *Voila*^{1/TM3}. Courtship responses of *w; Df(3R)/TM3* males toward decapitated male or female targets were measured. The height of each bar indicates the mean ($\pm$ SE) courtship index measured in at least 25 individuals per genotype (15 for *w; +/TM3*). All deficiencies were tested over the *TM3, Sb Ser* balancer chromosome from the *Voila*^{1/TM3} strain. Sexual discrimination (difference between the intensity of homo- and heterosexual courtship) was statistically tested (two-factor ANOVA, Newman–Keuls test) for each genotype. ****p* < 0.001. ns, Not significant.

Table 2. Effect on male courtship behavior of different deficiencies of chromosome 3

Subject fly (genotype)	Object fly (sex)	Courtship index (arc sin transformation)			
		Effect	df	F	p
All Df(3)R deficiencies and w; +/TM3	Female or male	Subject	349	10.97	0.00001
		Object		107.78	0.00001
		Interaction		7.98	0.00001
w; Kx-1, w; T-32, and w; +/TM3	Female or male	Subject	150	18.40	0.00001
		Object		23.72	0.00001
		Interaction		11.12	0.00003
w; 86D, w; P-21, w; T-45, and w; +/TM3	Female or male	Subject	233	2.52	0.0588
		Object		112.31	0.00001
		Interaction		0.10	0.96

Deficiencies are represented on Figure 2. All object flies were decapitated. Two-factor ANOVA was performed as in Table 1.

conic sensilla (Balmer, 1994) and some of the distal mechanosensory bristles. Again, the lack of stained afferents argues against the presence of GAL4 in sensory neurons. Weak labeling occurred in the Johnston's organ in the second antennal segment.

These data suggest that in olfactory sensilla of the antenna and the maxillary palps, GAL4 is expressed in trichogen or other sheath cells, whereas in the gustatory sensilla of the labial palps and the pharynx, GAL4 expression is neuronal. We found no evidence of sexually dimorphic patterns in these organs.

Expression pattern in wings and legs

Strong *lacZ* expression was associated with ~40 taste bristles on the wing margin (costal vein and radial 1 vein), which are arranged in two rows (Hartenstein and Posakony, 1989; Stocker, 1994). Staining was clearly absent from the mechanosensory bristles, which outnumber the taste bristles by a factor of 4–5 (Fig. 3). Additional labeling occurred in certain wing campaniform sensilla. Label associated with afferents from taste bristles and campaniform sensilla suggests that the expression was neuronal in both cases.

The tibia and tarsus bear two types of bristles, mechanosensory and gustatory (Nayak and Singh, 1983; Nottebohm et al., 1992). On the forelegs, the latter are slightly more abundant in males than in females (Nayak and Singh, 1983). In *Voila*¹ *UAS-lacZ*, expression was present exclusively in elements associated with taste bristles (Fig. 4). This correlation was particularly obvious in the tibia, which bears a few well spaced taste bristles among many mechanosensory bristles. Strong staining in the leg nerve suggests that the expression is neuronal rather than associated with sheath cells, similar to gustatory sensilla in the head or wings. The sexual dimorphism of foreleg taste bristles was reflected by a stronger general label of tarsi and of the leg nerve in males.

DISCUSSION

Of several hundred *PGAL4* enhancer-trap strains screened, we focused on the strain carrying the *PGAL4* insertion in the *Voila* locus, which causes two different male courtship behaviors: (1) an increase in bisexual excitation with one copy of the transposon and (2) a loss of heterosexual behavior when *PGAL4* drives the feminizing *UAS-transformer* transgene. Bisexual courtship maps to the same location as the *PGAL4* insertion (86E₁₋₂).

Is there a relation between bisexual courtship and excitability in *Voila*¹/TM3 males?

Heterozygous *Voila*¹ males generally displayed more vigorous homo- and heterosexual courtship than control strain males.

Their courtship duration with control object females was twice that with control object males. However, their ability to discriminate the sex of their partner was similar to that of control CS males (Table 1). The locomotor activity of *Voila*¹/TM3 males was higher than that of control males, suggesting that their both increased sexual excitation and locomotor activity are caused by their higher excitability. It is possible that a higher locomotor activity simply increases the probability of encounters between sexual partners (Cobb et al., 1987). The fact that *Voila*¹ *UAS-tra* males also showed a very high locomotor activity suggests that all males carrying a single copy of *Voila*¹ exhibit a hyperactive locomotor activity. However, feminized *Voila*¹ *UAS-tra* males had a very reduced courtship, demonstrating that male sexual excitation can be affected without locomotor behavior being altered. These data suggest that both behaviors are related but are controlled by different neural sites.

Mushroom bodies and courtship

In the CNS, the MBs and to a lesser extent the ALs of *Voila*¹ showed GAL4 expression. MBs are known to control courtship and complex behaviors in insects. For example, the ablation of MBs in the male cricket increases its locomotor activity (Huber, 1955). Furthermore, the electric stimulation of a neural structure close to the MBs elicits courtship behavior in male crickets and grasshoppers (Wadepuhl and Huber, 1979). Feminization of MBs in *Drosophila* can alter pheromone discrimination and male sexual orientation (Ferveur et al., 1995; O'Dell et al., 1995). Our study suggests that the *Voila* insert increases excitation during male courtship. A difference in the amount of VOILA product (one dose instead of two)—or the accumulation of a mutated product—in the MBs could increase male locomotor activity. We propose that two doses of VOILA are normally required in the MBs to control male-specific behavior. Preliminary results performed both with excisions of the *Voila* transposon and with deletions covering that locus suggest that the mutating effects of *Voila* are not related to the dosage of the GAL4 protein (M. Balakireva and J.-F. Ferveur, unpublished data).

Genetic feminization of the peripheral gustatory system in *Voila*¹ and reduced heterosexual orientation

Adult *Voila*¹ flies express GAL4 very specifically in the afferent neurons of most gustatory organs: the labial palps, several pharyngeal gustatory organs, and perhaps all the wing and leg taste sensilla. *Voila*¹ expression underlines the clear sexual dimorphism in the number of gustatory sensilla on the prothoracic legs (Fig. 4). The gustatory organs on the front legs and the proboscis are thought to play a crucial role during tapping and licking behaviors (Venard et al., 1989; Ferveur and Sureau, 1996; Ferveur, 1997). In contrast, expression of *Voila*¹ in olfactory organs does not appear to reside in sensory neurons.

*Voila*¹ *UAS-tra* males show a high locomotor activity but very reduced hetero- and homosexual courtship. Our preliminary data with *Voila*¹ *UAS-tra* males show high courtship responses with both male and female targets carrying low levels of or no pheromones. These results suggest that female pheromones, which normally have a stimulatory effect, inhibit the courtship of feminized *Voila*¹ *UAS-tra* males. Genetic feminization of the afferent gustatory neurons in *Voila*¹ *UAS-tra* males may have changed their perception properties or may have altered their primary sex-specific projection in the thoracic ganglia (Possidente and Murphey, 1989) or in the brain. It is impossible to measure whether sexual discrimination is altered in the CNS of these males (Ferveur et al., 1995).

Figure 3. Expression of *Voila*¹ in the adult nervous system of 4-d-old flies. *A*, Head cuticle with a complete proboscis. *B*, Frontal section (10 μ m) of the brain. *C*, Anterior wing margin. *D*, Frontal view of a whole brain. Expression of the β -galactosidase in *Voila*¹-*UAS-lacZ* males (*A*, *C*, *D*). Anti-tau labeling on *Voila*¹ *UAS-tau* male (*B*). The main peripheral nervous structures (shown on *A*) are the third antennal segment (*A*), the labial palps (*LB*), the labral sense organ (*LSO*), the ventral cibarial sense organ (*V*), and the maxillary palps (*MX*). The arrow on *C* indicates neuronal expression associated with a wing gustatory sensillum. In the brain, structures strongly labeled with TAU (shown on *B*) are the mushroom bodies (*MB*; arrow on *D*) and the antennal lobes (*AL*). Scale bar, 25 μ m.

because their courtship responses are too weak. However, the influence of the different neural structures in which *Voila* is expressed can be explored by producing mosaics using the FLP/FRT technique (Golic, 1991).

The behavioral phenotype of *Voila* is complex

Male flies carrying various aberrations (deficiency, insertion) in the chromosomal region 86C–87C, when compared with control males, showed differences for both their homo- and heterosexual orientation. Further genetic experiments, including *PGAL4* remobilization, will help to dissect the influence of the *Voila* locus on different aspects of male courtship. The comparison of *Kx-1* and *T-32* deficiencies provides a potential starting point for such a dissection: heterozygous males of either genotype lacking the chromosomal segment 86E₂₋₁₉ showed very reduced heterosexual excitation and, probably as a consequence, reduced sexual discrimination (Fig. 2). However, the fact that *Kx-1* males showed significantly higher courtship indices than *T-32* flies suggests that the proximal region (86D₄–E₂) of the locus (or its product), which is deleted only in *Kx-1* flies, normally decreases male courtship. We thus predict that heterozygous *Voila*¹/*TM3* males, which show

high excitation and normal sexual discrimination, are altered only for the proximal part of the *Voila* locus.

Comparison of *Voila*¹ with other courtship mutations

In *Drosophila melanogaster*, some mutant genes have already been shown to alter male courtship behavior. For example, *dissatisfaction* (*dsf*) mutant males exhibit a reduced sexual discrimination (Finley et al., 1997). The most intensely studied male courtship mutant is *fruitless* (*fru*). Different *fru* mutant alleles have revealed both genetic and phenotypic complexity at this locus (Villella et al., 1997), as for *Voila*. The original *fru* allele (*fru*¹) shows a decrease in male sexual discrimination (Hall, 1978), whereas a *PlacZ* targeted allele (*fru*^{satori}) shows almost no sexual excitation or discrimination (Ito et al., 1996). The *fru* locus codes for several transcripts, some of which are sex-specific and found in restricted neural structures, including the ALs (Ito et al., 1996; Ryner et al., 1996).

*Voila*¹ carries a *PGAL4* transposon and thus is open to investigation by all the genetic features offered by the *PGAL4* enhancer-trap system. First, *PGAL4* insertion in the *Voila*¹ allele has two mutational effects: (1) it is a recessive lethal, and (2) it dominantly enhances hetero- and homosexual courtship behaviors and loco-

Figure 4. *lacZ* Expression of *Voila*¹ in female (*A*) and male (*B*) prothoracic legs. Each picture is a composition showing the tibia (*top*) and upper tarsal segments (*bottom*) of *Voila*¹ *UAS-lacZ* 4-d-old flies. Arrowheads indicate some of the taste bristles that are always associated with neuronal labeling. Scale bar, 25 μ m.

motor activity. Moreover, ectopic expression of the feminizing *UAS-tra* transgene with *Voila*¹ drastically decreases male heterosexual excitation without any other observed behavioral effect. *Voila*¹ should be a useful tool for driving the expression of cloned genes to dissect the biological basis of courtship behavior and pheromone perception in *Drosophila*.

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