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Genetics

Structural brain mutant of *Drosophila melanogaster* with reduced cell number in the medulla cortex and with normal optomotor yaw response

(cell degeneration/developmental plasticity/orientation behavior/Golgi staining/genetic mosaics)

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ABSTRACT **KS58, one out of six known alleles of the small optic lobes (*sol*) gene in *Drosophila melanogaster*, reduces the cell number in the medulla cortex by degeneration of ganglion cells in the pupae to about 50%. Also, about half the volume of the medulla and lobula complex neuropils is missing. Many Golgi-stained cells in the mutant optic lobes resemble their homologues in wild type. However, special classes of transmedullary columnar neurons projecting to the lobula or to both lobula and lobula plate are not seen in the mutant. Some neurons linking the lobula complex to the central brain send branches to the medulla (the branches do not exist in wild type); some other types seem to be missing. The fate mapping of the KS58 focus reveals a location ventral to the head bristles and in *sine oculis* (*so*) flies the mutation further reduces the rudiments of the optic lobes normally seen. Therefore the *sol* phenotype is not induced by mutant eyes and the primary gene action seems to be on nervous tissue. The structural alterations of the small optic lobes are reflected in visual orientation behavior. The optomotor yaw response, however, is almost quantitatively preserved. The respective neural network should still be present in the mutant optic lobes.**

Due to the rarity of structural brain mutants their usefulness for the understanding of anatomy, development and function of the insect brain is unexplored. In the past 12 years abundant behavioral mutants have been selected (1–3), but only few of them were later shown to be structural brain mutants (4). Recently, however, Heisenberg and Böhl (5) isolated mutants of *Drosophila melanogaster* by mere inspection of brain morphology. We have chosen one with reduced optic lobes for further analysis.

The visual behavior of flies and the structure of their compound eyes and optic lobes are well studied (6–8). On the photoreceptor level the genetic approach has already been successfully applied (9–11). However, up to now only one mutant with normal eyes and structurally altered optic lobes has been described (4). It affects the giant horizontal (H) and vertical (V) cells of the lobula plate. This mutant was shown to be behaviorally defective in the optomotor yaw response, corroborating the idea that the H cells are part of the optomotor pathway. They are thought to integrate the outputs of the elementary movement detectors (EMDs), which seem to be situated in the medulla (12). Due to the enormous complexity of the medulla neuropil, no neural circuitry has as yet been assigned to the EMDs. Mutants with reduced cell number in the medulla could simplify the search for them. The small optic lobes (*sol*) mutant characterized in this paper seems to be well suited for such investigations because it still shows normal optomotor yaw responses.

MATERIALS AND METHODS

Stocks. sol^{KS58}, sol^{KS84}, and sol^{KS160} were all isolated in our laboratory (5); *sine oculis* (without eyes, *so*), the double mutant sol^{KS58}; *so* and the wild-type strains Berlin and Kapelle (10) were used for histological and behavioral studies. For mapping of the *sol* gene we used the marker strain *y cv v f* provided by M. M. Green. In order to produce gynandromorphs, males of the triple mutant w^a *f* sol^{KS58} were crossed with *R(1)2 In(1)*w^{vc}*/In(1)dl49 y w lz* females provided by J. A. Campos-Ortega.

Histological Techniques. For mapping of the *sol* gene and for the genetic mosaic analysis the histological mass procedure for simultaneous handling of about 20 brains developed by Heisenberg and Böhl (5) was used. For neuropil staining we applied the Holmes–Blest method (13) after fixation with Carnoy's solution. Sections were 7 μm thick. Cells were counted on photographs of semi-thin sections (1.5 μm) of adult flies and of pupae about 33 hr after pupation. Heads or pupae were fixed in 1% osmium tetroxide and 1% glutaraldehyde at pH 7.3. Sections were stained for 1 min with a 1:1 mixture of 1% azure blue and of 1% methylene blue in 1% sodium tetraborate at 50°C.

Golgi staining of single cells was carried out as suggested by Colonnier (14). After embedding in Durcupan, 35 μm sections were cut.

Optomotor Yaw Response. The optomotor yaw responses were recorded under open loop conditions, using the styrofoam ball device of Buchner (15). For stimulus conditions see Heisenberg and Götz (2) and figure legends.

RESULTS

Isolation and Genetic Mapping of the *sol* Mutants. Among the X-chromosomal mutants isolated by Heisenberg and Böhl (5) three (KS58, KS84, and KS160) with obviously reduced optic lobes fall in one complementation group. The mutant KS58 was mapped by using the morphological trait. Its position is to the right of forked (*f*) (60 ± 1). Three mutants sent to us by J. R. Merriam (PC79, KS91, and EE111) belong to the same complementation group and were shown to have small optic lobes as well. They were isolated due to poor fast phototaxis, and two of them were mapped to the right of *f* by using this behavioral defect (J. R. Merriam, personal communication). We tentatively consider the six mutants to be alleles of one gene, *sol*. The penetrance of the six alleles is 100%, but they differ in the degree

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Abbreviations: EMD, elementary movement detector; H cell, horizontal cell; Sut, small field unilateral tristratified amacrine cell; Tm, transmedullary neuron; V cell, vertical cell.

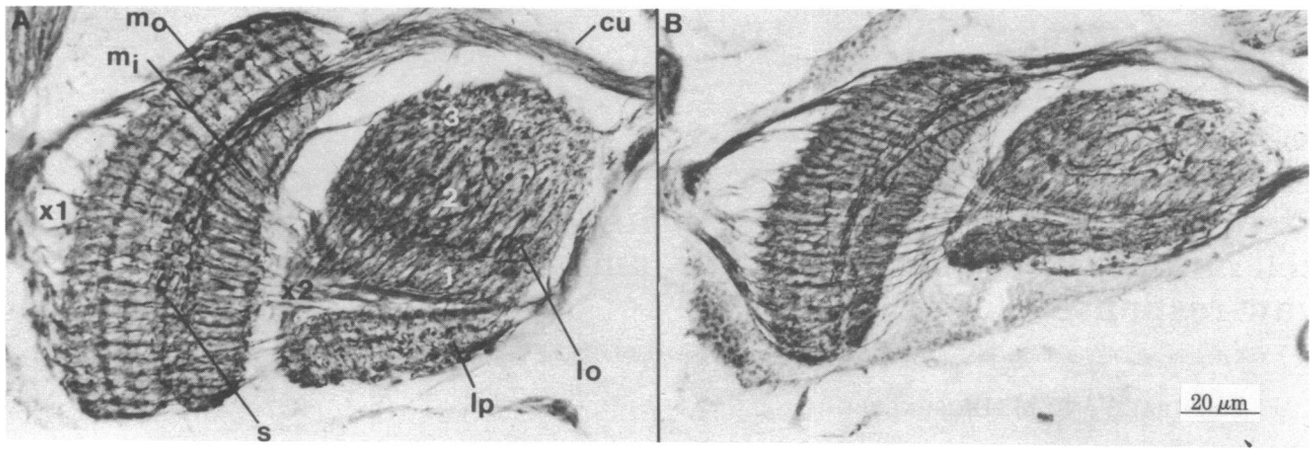

FIG. 1. Silver-stained horizontal sections through the neuropils of wild-type (*A*) and sol^{KS58} (*B*) optic lobes. With regard to the mutant medulla note that the reduction in size goes along with a reduction in the thickness of the individual columns, whereas the number of columns is normal. The characteristic tangential striation of the outer wild-type medulla is missing in the mutant. Lobula and lobula plate of sol^{KS58} are greatly reduced along the posterior–anterior axis. Whereas the first layer (1) of the lobula seems little affected (see also Fig. 2), the second (2) and third (3) layers are drastically reduced in the mutant. It is mainly in the latter two layers that in wild-type transmedullary neurons (Tm and Y cells) contact lobula columnar neurons projecting to the central brain. cu, Cucatti bundle; lo, lobula; lp, lobula plate; m_i, medulla proximal to the serpentine layer; m_o, medulla distal to the serpentine layer; s, serpentine layer; x1, first optic chiasma; x2, second optic chiasma.

of the reduction of the optic lobes. This study is mainly concerned with the allele sol^{KS58}, which reduces the volumes of medulla and lobula complex neuropils by about 50% (Fig. 1).

Counts of Cell Bodies in the Optic Ganglia of sol^{KS58} and Wild Type. Most cells forming the medulla neuropil stem from the outer optic anlage (16), which generates the lamina cells and the cells of the medulla cortex proper. In accordance with cell counts by Hofbauer (17), we counted about 40,000 cell bodies in the wild-type medulla cortex. The respective number for the sol^{KS58} mutant is about 20,000. The cell number in the lamina is not affected.

Inspection of mutant pupae 33 hr after pupation, when proliferation of ganglion cells is completed, reveals extensive cell degeneration in the medulla cortex (G. Technau, personal communication). At this time the cell number in the mutant medulla cortex still amounts to about 70% of the number counted in wild-type pupae.

The neurons of the lobula complex and some neurons contributing to the medulla neuropil (see Fig. 3) are formed by the inner optic anlage. Their number is about equal in mutant and wild type 33 hr after pupation. However, degeneration of ganglion cells in the cortex of the mutant lobula complex seems to be slightly more frequent than in wild type, at least at the developmental stage investigated.

The sol^{KS58} Optic Lobe Phenotype Is Not Induced by Mutant Eyes. In several mutants recently analyzed by Meyerowitz and Kankel (18), defects in the optic lobes are induced by genetically mutant eyes. This is not the case with sol^{KS58}; i.e., the mutation acts on the optic lobes of *so* flies missing any ommatidia by abolishing the medulla rudiment, leaving only a drastically reduced lobula complex. Furthermore, we screened 127 genetic mosaics produced by the ring-X-method (Fig. 2). In 12 cases wild-type eyes were associated with mutant optic lobes phenotype or vice versa. Twenty-eight mosaic eyes were found above phenotypically uniform optic lobes. Detailed fate mapping using bristles as external landmarks showed that the focus for the sol^{KS58} optic lobes phenotype maps about 11 units [sturts] ventral to the ocellar and the posterior orbital bristles. These results are compatible with a primary gene action on the central brain or on the optic lobes.

Shape of Single Neurons in the Optic Lobes of sol^{KS58}. In total over 600 wild-type and mutant Golgi stain-impregnated brains have been inspected. Only the main results can be summarized here. Some classes of transmedullary cells (Tm and Y cells), which project deeply into the lobula in wild-type flies, were never stained in the mutant. However, because it is difficult to prove the absence of cells with Golgi studies, we base our arguments on the cells we have seen. Among those we have to differentiate between normal, wild-type-like cells and cells with a somehow altered appearance.

A neuron has been called wild-type-like when its dendritic aborizations and axonal projections are in the same relative positions and of the same relative extent as their wild-type counterparts. Their reduced size in absolute terms is accounted for by the reduction in ganglion size. Neurons are called abnormal when they do not meet the above criteria. In extreme cases the identification of such cells may be difficult.

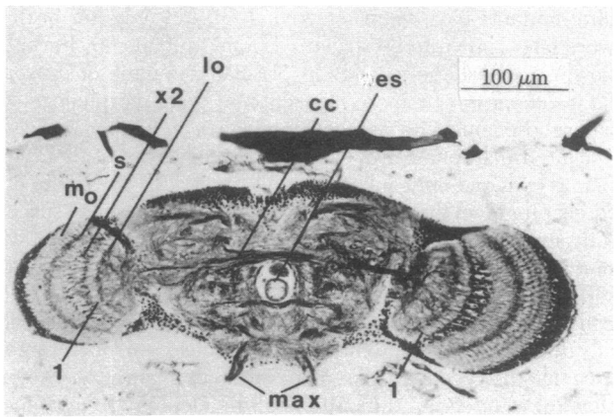

FIG. 2. Silver-stained frontal section through the brain of a genetic mosaic displaying the sol^{KS58} optic lobes phenotype on the left and the wild-type phenotype on the right. In this gynander the head cuticle landmarks and the eyes were male on the left side and female on the right. The projections from the left lobula to the central brain seem to be thinner than those from the right lobula. The volume of the central brain hemisphere associated with the reduced optic lobes in such left–right gynanders is reduced by about 12% compared to the other hemisphere. This might not be explained by only sexual dimorphism of the brain. cc, Central commissure; es, esophagus; max, maxillary labellar nerve; other abbreviations as in Fig. 1.

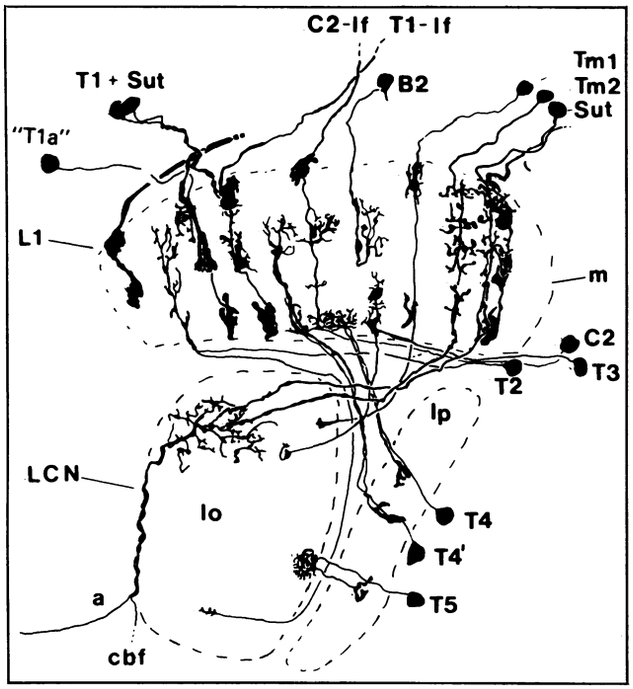

FIG. 3. Composite of camera lucida drawings of some Golgi-stained columnar neurons occurring in the optic lobes of *sol*^{KS58}. The cells shown can be homologized with neurons of *Drosophila* wild type or other diptera (see, e.g., ref. 8). Diagnostic means are the position of the cell bodies and the extent and general appearance of dendritic and axonal arborizations. L1 designates the medulla ending of a lamina monopolar cell no. 1. In the medulla cortex the cell bodies of the medulla intrinsic neurons B2, "T1a," and Sut are found. "T1a" sends a short dendritic branch into the first optic chiasma. T1 cells link lamina and medulla. The cell body fibers of T1 and Sut of the same column are always closely associated. Tm1 and Tm2 are examples of transmedullary neurons projecting to the lobula. The cell bodies of C cells (C2 is shown) and of T2 and T3 are formed by the inner optic anlage, as are the cells of the lobula plate cortex—e.g., T4 and T5 cells. T4′ is a variant of T4 in the mutant. One lobula columnar neuron (LCN) with its axon (a) projecting to the central brain and its cell body fiber (cbf) is drawn. lf, Linking fiber; m, medulla neuropil; lo, lobula neuropil; lp, lobula plate neuropil.

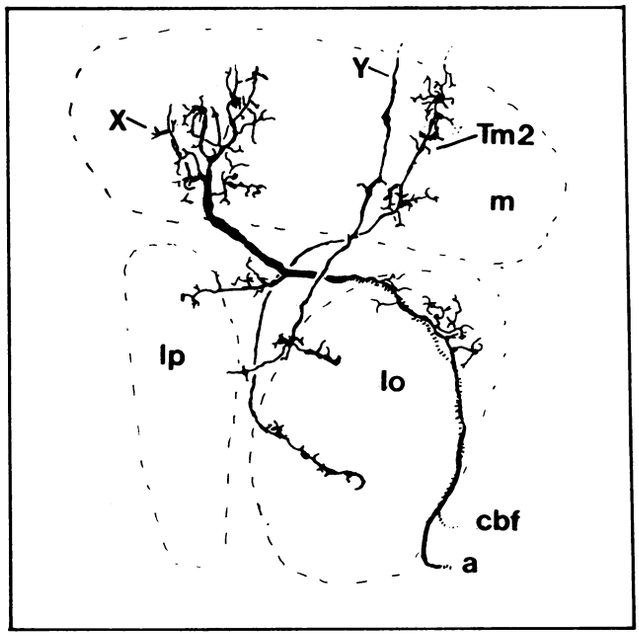

FIG. 4. Camera lucida drawing of three Golgi-stained neurons in the optic lobes of one *sol*^{KS58} fly: A transmedullary cell (Tm2), a midget Y cell (Y), and a neuron (X) with dendritic arborizations in medulla (m), lobula plate (lp), and lobula (lo) projecting to the central brain. Such a cell was never seen in wild-type flies, but several times in the mutant. a, axon; cbf, cell body fiber.

It is of special importance that among the wild-type-like cells several neurons are found that have their cell bodies in the medulla cortex. As mass impregnations have shown, at least four of those (T1, Tm1, a small-field unilateral tristratified amacrine cell (Sut), and "T1a," see Fig. 3) occur in neighboring visual columns. This suggests that these cells are as frequent in the mutant as they are in wild type. In fact, electron microscopy of the mutant lamina has shown T1 terminals in all cartridges inspected. These results imply that the reduced cell number of the mutant medulla cortex is due to the absence of defined cell classes of columnar neurons.

Fig. 3 also shows some other cell types frequently encountered in the mutant optic lobes (see figure legend for details). Abnormal neurons in the mutant optic lobes are often characterized by unusual extension of their dendritic or telodendritic aborizations. T4′, a variant of the bushy T cell (T4), is an example. Its dendritic tree in the medulla is not restricted to the most proximal layer; it grows instead, as does T2, beyond the serpentine layer.

Another class of modified neurons in the mutant needs special attention. As opposed to their wild-type counterparts, some neurons linking the lobula complex to the central brain send branches into the medulla neuropil (Fig. 4). Among those cells is a giant V cell of the lobula plate. This has been seen twice in Golgi-stained brains and is obvious in frontal sections stained with the Holmes–Blest method (Fig. 5).

Visual Behavior. In spite of its reduced cell number the medulla of *sol* still mediates visual behavior. Here we present the data for the optomotor yaw response, which is essentially normal (Figs. 6 and 7), even under threshold conditions for low light intensity and for high temporal frequency (ω/λ). Only at low temporal frequency does movement detection seem to be somewhat impaired. Other pieces of visual behavior such as landing response, orientation behavior, and figure–ground discrimination (19) are characteristically modified. For instance a

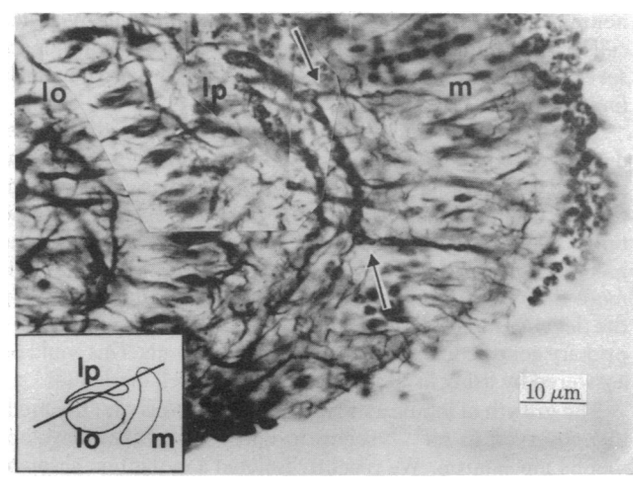

FIG. 5. Silver-stained frontal section through the left optic lobes of a *sol*^{KS58} fly. It shows a giant V cell of the lobula plate branching (arrows) into the posterior part of the medulla. The two V cells stained in Golgi preparations had similar branches. (*Inset*) Plane of section in a horizontal view. Abbreviations as in Fig. 2.

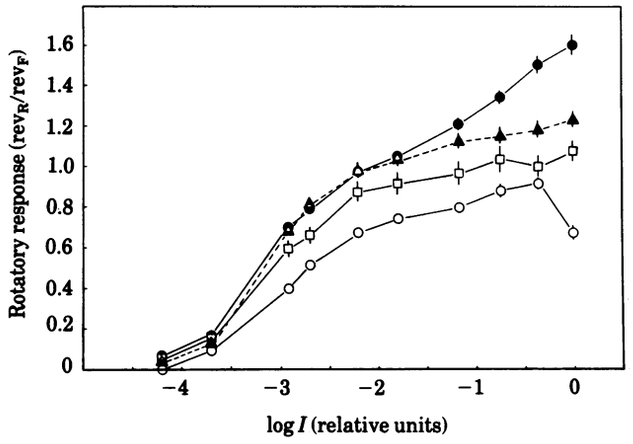

FIG. 6. Optomotor yaw responses as functions of light intensity I for wild-type flies (□), sol^{KS58} (●), sol^{KS84} (○), and sol^{KS160} (▲). Fixed flies walked on a styrofoam ball in the center of a rotating striped drum with the spatial wavelength $\lambda = 45°$ and the temporal frequency $\omega/\lambda = 1.6$ Hz. The rotatory response R is expressed as the quotient of the rotations of the styrofoam ball around its vertical axis (rev_R) and its horizontal axis perpendicular to the longitudinal axis of the fly (rev_F). The error bars indicate the SEM of at least 74 repetitive measurements. In some cases they are too small to be shown. The response function of wild type is an average from three flies, mutant data are from individual flies, the small optic lobe phenotypes of which were determined by subsequent histology.

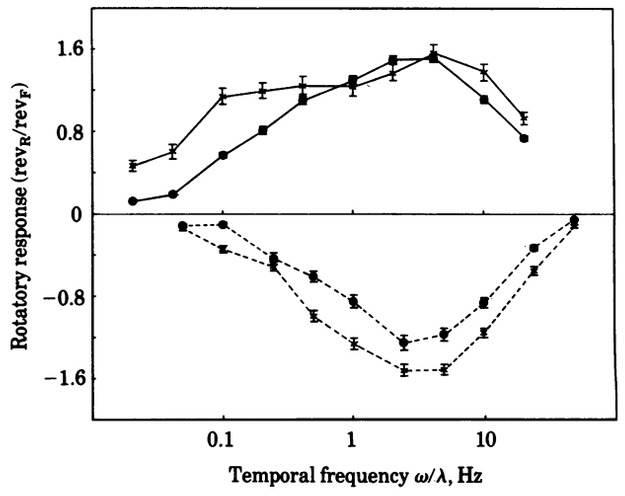

FIG. 7. Wild-type (×) and sol^{KS58} (●) optomotor yaw responses as functions of temporal frequency (ω/λ). Experimental conditions were as indicated in the legend to Fig. 6, except that the spatial wavelengths $\lambda = 18°$ (——) and $7.2°$ (– – –) were used. The negative reactions with $\lambda = 7.2°$ result from geometric interference between the pattern and the array of visual elements in the compound eye. Light intensity was log $I = 0$ in arbitrary units as given in Fig. 6. The error bars indicate the SEM of at least 66 repetitive measurements. Data were obtained from three flies per curve.

walking wild-type fly just after being released in the center of an arena heads towards a vertical black stripe, whereas the mutant tends to avoid dark patterns.

DISCUSSION

This paper contains a preliminary characterization of the *sol* mutants, which promise to facilitate the investigation of function, anatomy, and development in the visual system of flies. The mutants show that the complexity of the insect brain can be genetically reduced while leaving functionally valid subsystems. Halving the cell number of the medulla cortex eliminates certain cell classes and leaves others unaffected. Also, some pieces of visual behavior, such as the optomotor yaw response, are almost quantitatively retained. It seems reasonable to assume that the neural constituents of the EMDs are among the wild-type-like cells of the mutant medulla. Other pieces of visual behavior are severely altered. Freely flying and walking flies are obviously impaired.

Regarding development, it is interesting to note that the reduction of cell number in the medulla cortex is a result of extensive cell degeneration in the pupal stage. Therefore it seems that the sol^{KS58} mutation exerts its influence during cell differentiation. Our fate mapping experiment shows that this effect on the medulla cortex is not induced by mutant eyes. However, it is more difficult to decide whether sol^{KS58} acts autonomously in the degenerating cells or whether degeneration is induced by a primary gene action somewhere else in the brain. This could be answered by using in addition a histological cell marker (3).

The growth of lobula and lobula plate neurons into the medulla observed in sol^{KS58} seems to uncover a general developmental mechanism. We recently isolated a mutant on chromosome 2 called lobula plate-less (lop^{N684}), in which the homologues of the lobula plate giant neurons directly connect the medulla to the posterior slope. We would like to speculate that in the *sol* and in the *lop* mutants the respective cells grow into the medulla, because the right connections to presynaptic neurons in the lobula complex cannot be established. This would imply that for these cells contact with appropriate presynaptic neurons signals cessation of growth and that in wild type this happens in the lobula or lobula plate, whereas in the mutants suitable terminals are available only in the medulla. Whether the giant cells contact neurons of their wild-type functional pathway in the medulla is not yet known. If this were the case it would suggest a common cell label for neurons of a functional pathway, a hypothesis that has been discussed with respect to the proboscis extension reflex mediated by the ectopic tarsal sugar receptors in the aristapedia and antennapedia mutants (20, 21). Whatever the molecular mechanism may be, it would appear to be a good strategy to develop functional subsystems of the adult brain as units.

Although such a plastic developmental mechanism in some cases would complicate the interpretation of behavioral data of structural brain mutants, it may help to distinguish different functional pathways by anatomic means: of the lobula plate giant neurons in sol^{KS58} only V cells appear to send branches to the medulla, whereas H cells seem to have normal shape, in accordance with the almost quantitative retention of optomotor yaw responses.

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