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## Sensory projections from dorsal and ventral appendages in *Drosophila* grafted to the same site are different

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*Summary.* Owing to a new transplantation technique, we have been able to study the sensory projections of homologous and heterologous appendages grafted to the same abdominal site in *D. melanogaster*. Axons from homologous transplants exhibit similar terminal patterns, whereas those from heterologous transplants do not. It is suggested that ectopic sensory axons specifically recognize central areas and pathways occupied by axons from homologous appendages.
*Key words.* Neuronal specificity; sensory projections; serial homology; ectopic transplantation; *Drosophila melanogaster*.

The sensory system of insects has been widely used to study neuronal specificity by analyzing the pattern of sensory projections in the central nervous system (CNS) under various experimental conditions. Homoeotic mutants of *D. melanogaster* can help to assess how displacement of sensory cells affects their central projection[1-5]. Sensory axons from homoeotically transformed antennae and probosces project successfully into centers of serially homologous appendages (or less frequently into their proper center) but do not extend into other regions of the CNS[6,7]. This suggests that these centers are recognized because they are serially homologous themselves[7]. One might therefore expect that the sensory projections from heterologous structures would differ if they were placed at an identical site on the body surface. Owing to a new technique, we were able to transplant dorsal and ventral appendages to the same abdominal site and to examine the sensory projections. As predicted, we found consistent differences in the projection patterns of heterologous tissues, but similarities in the patterns from homologous structures.

*Materials and methods.* Supernumerary dorsal appendages (wings and halteres) and ventral appendages (fore-, mid-, hindlegs and antennae) were produced in the pleural membrane of the fifth abdominal segment by a modified technique of 'surface transplantation'[8,9] (fig. 1). A small incision was made with a fine steel blade into the lateral abdominal cuticle of a light brown prepupa; then the desired imaginal disk from a white prepupa was injected with a glass capillary underneath the integument. Under optimal conditions, the disk is incorporated by the surrounding host's epidermis, allowing the evagination of the supernumerary appendage during metamorphosis. According to our experience, abdominal forelegs and halteres are everted at a frequency of about 70% of eclosed flies, mid-, hindlegs, antennae and wings at approx. 20%. The sensory projections were visualized by peroxidase diffusion. Peroxidase (HRP, Sigma Type VI, 10% w/v) was applied for 3 h following excision of abdominal bristles or cutting parts of grafted appendages. After another 4–14 h of survival, flies were fixed in 2.5% glutaraldehyde. The histochemical reaction[16] was performed on the dissected thoracic CNS.

*Results.* As a reference, we studied the central projections of two sensilla situated close to the transplantation site, and close to where imaginary dorsal and ventral appendages should develop in an abdominal segment. We chose the mechanosensory bristles located in the posterior corners of the fourth abdominal tergites and sternites (fig. 2A and B). Their axons reach the thoracico-abdominal ganglion through the main abdominal nerve and terminate in the fused abdominal neuromeres. The arborizations of the dorsal bristle remain strictly ipsilateral (fig. 2A), whereas those of the ventral bristle form ipsi- and contralateral branches (fig. 2B).

The sensory fibers from all types of supernumerary appendages enter the CNS via the main abdominal nerve as well. Most of the axons terminate in the abdominal neuromeres, but others extend into thoracic regions. In the abdominal ganglion, wing and haltere terminals occupy predominantly the ipsilateral side, as do the terminals of dorsal abdominal bristles (fig. 3C). In contrast, sensory projections from the first, second and third legs, or from antennae form both ipsi- and contralateral branches, like those of ventral abdominal bristles (fig. 3E).

In thoracic neuromeres, the differences between the projection patterns from dorsal and ventral transplants are even more accentuated. In the majority of HRP-fills from grafted wings or halteres, nerve fibers pass from the abdominal termination area in anterolateral direction into the ipsilateral normal wing center ('lateral pathway'). Comparison with fills from normal wings reveals that the fibers in this 'lateral pathway' follow the course of normal wing fibers extending back from the wing center into the metathoracic neuromere (cf. figs 3C with 3A). From the wing center, axons of wing or haltere transplants turn back towards the midline and bifurcate into an anterior and a posterior pathway (fig. 3C). The former extends into the neck connective; its course appears to correspond to the massive ascending tract followed by normal haltere axons (fig. 3B). Fibers from campaniform sensilla on the supernumerary haltere frequently branch off the 'lateral pathway' in the third thoracic neuromere and terminate in the center of normal haltere campaniform sensilla, called 'medial tuft' by Palka et al.[10] (cf. figs 3C and 3B). Sensory fibers from leg or antenna transplants are not observed in the 'lateral pathway', but instead, single axons projecting along the thoracic midline and into the neck connective may be present (fig. 3E). Scattered fibers extending along the midline into the head occur in normal leg projections as well[6] (fig. 3D).

In order to know whether afferents from supernumerary appendages are able to form functional connections in the CNS, we tested for an extension of the proboscis following gustatory stimulation of abdominal forelegs (for details see Stocker[11]). In 10 out of 33 flies, contact of the transplant with a 0.4 M sucrose solution yielded an extension almost as strong as in the normal

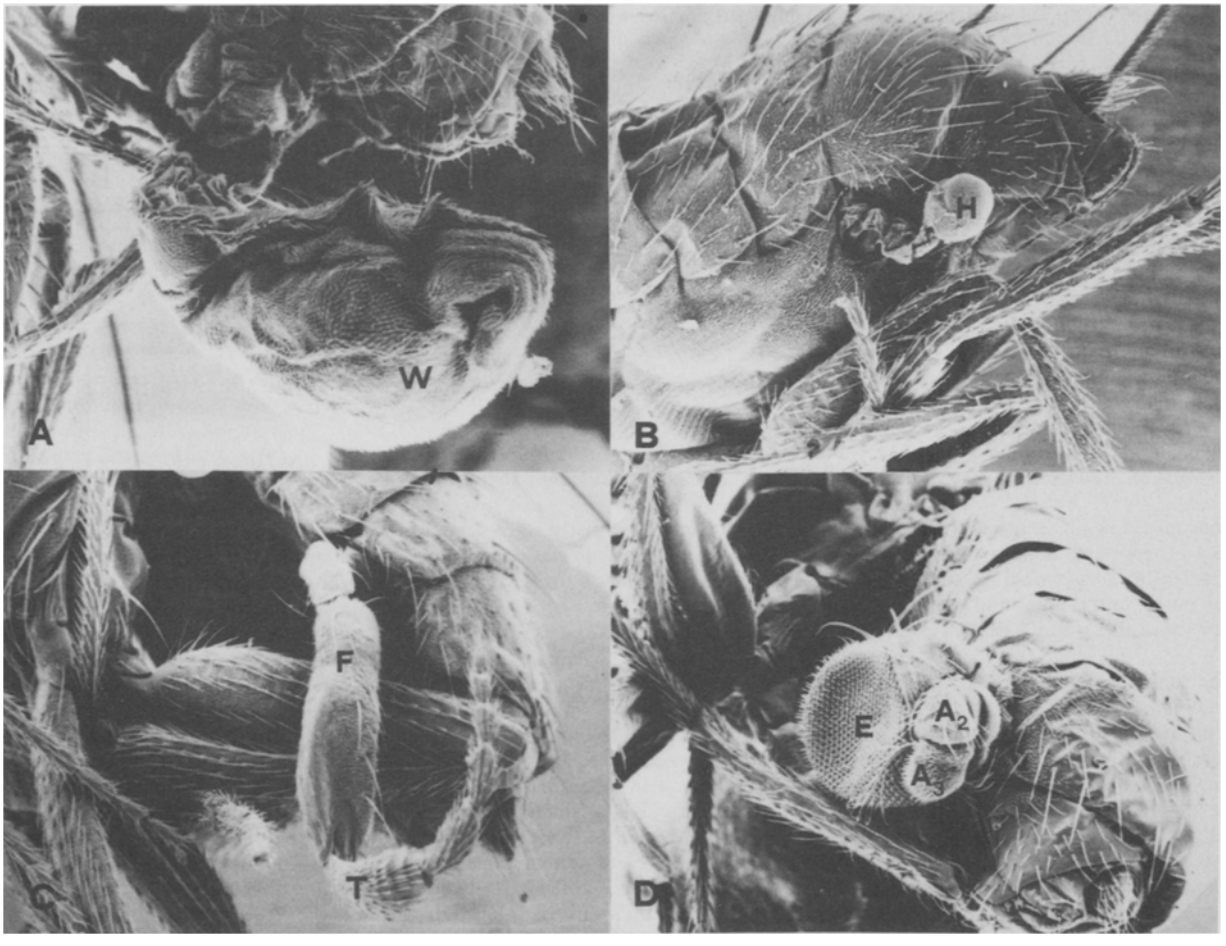


Figure 1. SEM micrographs of supernumerary wing (A), haltere (B), foreleg (C) and eye-antenna (D) created on the lateral side of the fifth abdominal segment of *D. melanogaster* by surface transplantation. In wing and haltere transplants, the central projections studied were those of campaniform and bristle sensilla, in leg transplants those of mechano- and chemosensory bristles and a few campaniform sensilla, whereas projections of antenna transplants included trichoid, basiconic and coeloconic sensilla. $A_{2,3}$ second/third antennal segments. E, eye; F, femur; H, haltere; T, tibia; W, wing. Approximately × 90.

foreleg control; eight flies were only partially responsive, and the remaining 15 were completely unresponsive. Hence, as in the case of homoeotic transformations[11], surgical transplants in *Drosophila* are able to establish functionally specific sensory projections.

*Discussion.* Using microsurgery, we have developed a straightforward approach for studying neuronal specificity in *Drosophila* that avoids problems inherent to homoeotic mutants, such as a possible genetic transformation of the CNS[10], or the uncertain identity of sensory neurons of transformed appendages[5,12]. Moreover, we have been able for the first time to follow sensory projections of heterologous appendages from the same ectopic location. The present results are in agreement with our observations of specific projections from homoeotic appendages into centers of serially homologous appendages[7], namely: a) fibers from abdominal wings or halteres terminate in the abdominal ganglion in a fashion similar to dorsal abdominal bristles; moreover, they extend into the wing and haltere centers. b) Axons from abdominal legs or antennae project into the abdominal neuromeres as well, but they show a pattern reminiscent of ventral abdominal bristles. The important new finding is that the sensory projections from heterologous ectopic appendages differ. These data suggest that fibers from ectopic appendages, rather than reproducing their characteristic projection pattern in another neuromere, recognize centers occupied by axons from homologous appendages (or from nearby sensilla in abdominal segments).

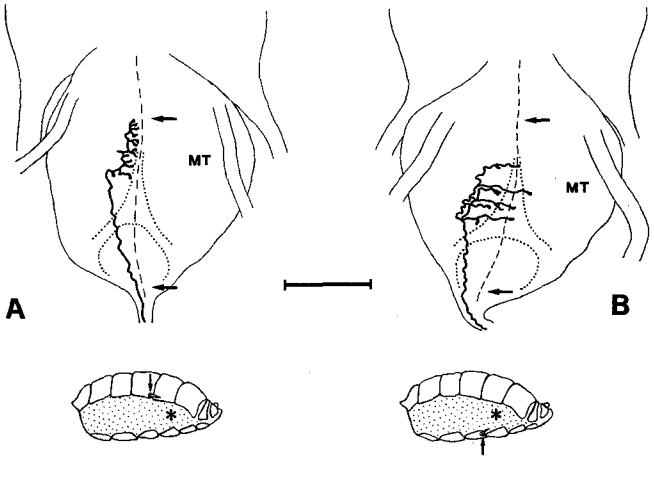


Figure 2. Camera lucida drawings of peroxidase-labeled sensory projections from bristles in the posterolateral corners of fourth abdominal tergite (A) and sternite (B). The bottom figures of the abdomen illustrate the precise localization of the two sensilla in relation to the transplantation site (asterisks). Terminals of the tergite bristle remain ipsilateral, whereas those of the sternite bristle occupy ipsi- and contralateral sides. Arrows indicate length of fused abdominal ganglion. MT, right metathoracic neuromere. Bar 100 µm.

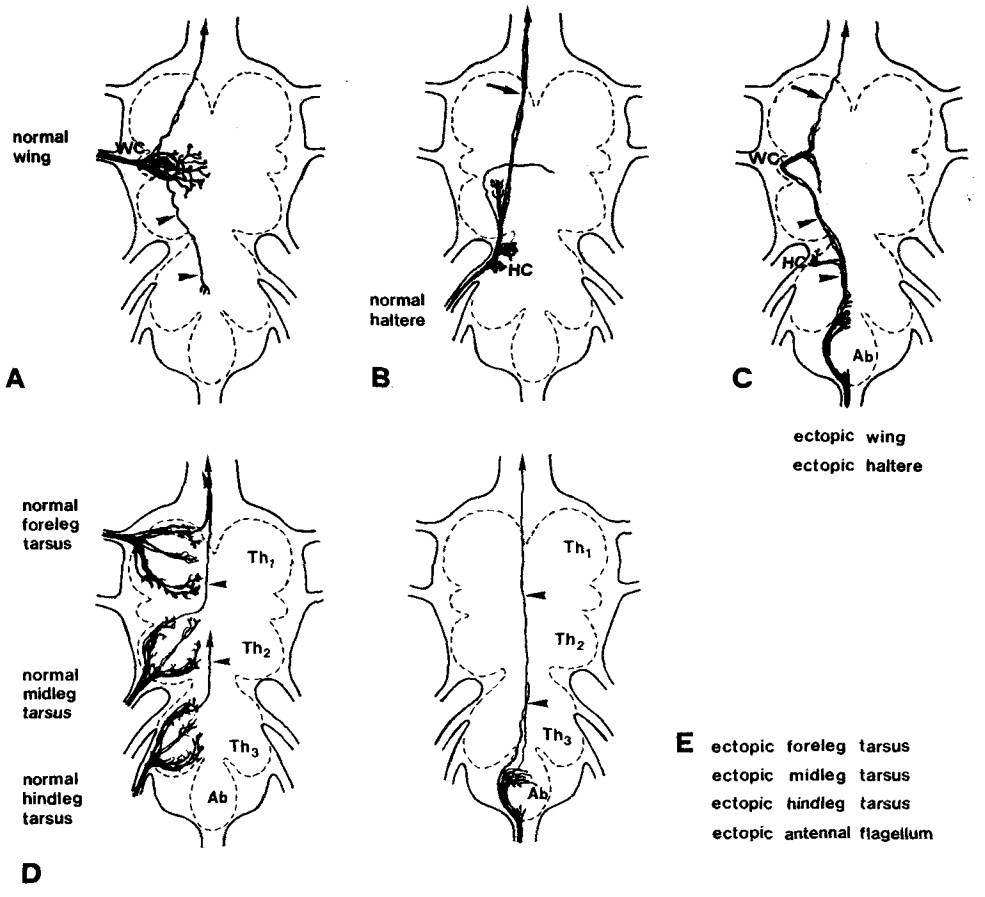


Figure 3. Sensory projection patterns of the various types of control and ectopic supernumerary appendages in the thoracico-abdominal CNS. Sensory fibers from wing or haltere transplants (C) remain ipsilateral in the abdominal neuromeres (Ab); in addition, they extend into the wing center (WC) following a pathway which is used by normal wing afferents (arrowheads, cf. with A). Graft fibers which pass into the neck connective appear to follow the massive ascending tract of the normal haltere projection (cf. C with B: arrows). Axons originating in campaniform sensilla of haltere transplants frequently terminate also in the normal haltere center (HC, cf. B). Fibers from supernumerary legs or antennae (E) exhibit bilateral arborizations in the abdominal neuromeres, and occasionally extend along the thoracic midline (arrowheads). Scattered midline axons occur in normal leg projections as well (D: arrowheads). $Th_{1-3}$, first, second and third thoracic neuromeres.

Wings and halteres or the three pairs of legs, share the same types of sensilla[5,13]; in contrast, the antenna and leg regions examined do not (see legend of fig. 1). Therefore, the similarities of projections from homologous transplants are not due to similarities of the sensilla patterns. We favor the following interpretation to account for the projection patterns of both genetic and surgical transplants: a) Sensory axons from serially homologous appendages (or from nearby sensilla in segments lacking appendages) possess biochemical surface properties in common. Thus, in addition to the individual neuronal labels demonstrated by studies of the developing nervous system[14,15], neurons might carry labels of a more general nature, such as dorsal vs ventral quality. b) Likewise, projection centers of serially homologous appendages (or central tracts associated with these centers) carry surface labels in common which are not shared by other regions of the CNS. c) Consequently, sensory fibers are able to recognize centers of serially homologous appendages.

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