Genetic Studies of Neuronal Development in *Drosophila melanogaster*

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ABSTRACT

The projections into the central nervous system (CNS) of several wild-type and genetically ectopic sensory structures were studied by cobalt filling or silver staining and compared for the purpose of determining what factors guide the growth of the sensory axons. The head bristles all arborize in a similar fashion in the subesophageal ganglion although they reach the target by three different routes depending on their position on the head. This arborization is L-shaped with a longitudinal branch and a medially directed branch that crosses the midline. The antennal projection consists of an olfactory lobe component, organized into glomeruli, and an antennal mechanosensory component which can be further subdivided into three branches, the anteriormost of which is identical to the head bristle projection. The tarsi all have similar U-shaped projections into their segment's neuromere with no ascending, descending or contralateral branches.

Axons of ectopic thoracic bristles on the head may enter the brain or the optic lobes. The routes into the brain taken by the ectopic bristles were initially like those of the normal head bristles but were followed for greater or lesser distances and the region of the subesophageal ganglion that is the target of the head bristles was seldom reached. The terminal arborizations of the ectopic bristle axons were generally irregular regardless of where they were: in the subesophageal ganglion, brain or optic lobes. They resembled neither their normal arborizations in the ventral ganglion nor those of the local head sensilla in the brain.

Axons from antennal legs have a pattern of projection grossly similar to that of wild-type antennae in that the same regions of neuropil were innervated. The non-olfactory lobe components of the antennal leg projection were like those of the antenna. However, the arborization in the olfactory lobe was chaotic and there were adventitious projections from the lobe into adjacent neuropil, particularly the

subesophageal ganglion. Some elements of these adventitious projections in the subesophageal ganglion were found consistently in almost every preparation. No element of the projection resembled the leg projection in the ventral ganglion.

The axons of ectopic sensilla can reach a normal target if the distance to it from the new location is sufficiently small: axons from abdominal legs in *bxd* mutants terminate in normal metathoracic leg sensory neuropil and the axons of antennae misplaced as a result of the mutation *ant* can enter normal antennal targets.

In summary, axons of ectopic sensilla can't reach their normal targets if they enter the CNS far from those targets which suggests that there are no long range cues for guidance of sensory axons. In the "foreign" part of the CNS the axons of ectopic sensilla do not make projections that resemble their normal ones. They initially take routes characteristic of sensilla in their new location but do not follow them consistently. The exception, antennal leg mechanosensory projections, is likely to be a result of a homology between antennal and leg mechanosensory sensilla. These results suggest the following: insect sensory neurons reach their targets mainly by following local and not long-range cues. The growth of these axons is constrained to specific tracts and it is by these that they are guided over long distances to their targets. Tracts recognized by the axon can be recognized at any point and, as the present study shows, this recognition is required not only at the point of entry but continuously, all along the tract, for guidance of the axon. Guidance by the tract appears to depend on an affinity between the axon and the tract that may also exist between axons and tracts of their segmental or functional homologues. Since axons in foreign neuropil have irregular arborizations characteristic neither of their normal ones nor of those of the local sensilla, the arborization pattern is not a result of an internal branching program alone nor of the axon's milieu directing the branching but must depend on a specific interaction between the axon and its target.

The leg motorneurons were identified and described after HRP backfilling from cut legs. The pattern of their positions differs from segment to segment. The bithorax mutations transform the metathoracic pattern into a mesothoracic pattern, paralleling their effect on the epidermis.

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Part I

Genetic Studies of Sensory Axon Projection Patterns in *Drosophila melanogaster*

I. Genetic Studies of Sensory Axon Projection Patterns in *Drosophila melanogaster*.

INTRODUCTION AND LITERATURE REVIEW

A. Mechanisms of axonal guidance.

Developmental neurobiology is a field that deals with the most complex phenomenon in science, the construction of the nervous system from the genetic information contained in a single cell. One of the central questions is how does a neuron reach its target. Observation of neuronal development, in vivo and in vitro, in normal and in experimentally altered conditions, has pointed to not one but several, somewhat overlapping, mechanisms which share in regulating the growth of the neurite in a complex interaction. The question now is what is the relationship of each mechanism to the others within a given experimental system.

Some of the mechanisms that have dominated the literature are:

(1) Predetermined or innate growth: This mechanism proposes that the neuron has the information necessary for producing specific axonal branching patterns built-in, and can do so relatively independently of the neuron's environment. This was first suggested by Harrison (1910) to explain the early outgrowth of neurites in cultured neurons and later received support from the observations that the axons of cortical pyramidal neurons (Van der Loos, 1965) and Mauthner neurons (Stefanelli, 1951; Hibbard, 1965) always originate from the same pole of the cell body even in rotated portions of nervous system primordia. More recently, the *in vitro* observations that cell migration patterns of sister 3T3 cells are very similar (Albrecht-Bühler, 1977) and that detailed axonal

branching patterns of sister neuroblastoma cells are very similar (Solomon, 1979) reinforce the notion that the branching patterns of axons may be strongly influenced by internal programs. A modification of this idea is that neuritic growth is innate but its direction is influenced by the position of the cell with respect to the body axes, e.g. anterior-posterior (Hibbard, 1965). A further modification along these lines is that influence on the growth is not only from general position in the body but from local cues; this suggests the second mechanism.

(2) Local guidance: This mechanism proposes that the neurite passively follows specific, recognizable, cues in the substrate on which it elongates. Also originally suggested by Harrison (1910), this mechanism may be purely mechanical, as Harrison thought, which means that the neurites are physically constrained to follow a certain path. More recently, Letourneau (1975) has shown that the preference of a neurite growth cone for a particular path may depend on the relative adhesivity of that path as compared with that of the surrounding substrate. Letourneau used artificial substrates but Adler and Varon (1981) have shown that neurites also show preference for substrates containing substances deposited by cultured ganglia. *In vivo*, these paths may be marked continuously, perhaps by special "pioneer" axons (Bate, 1976b), or they may be in the form of discrete markers spaced along the path, or they may be in the form of a concentration gradient along which the neurite can orient itself. If such a gradient is not of a material in the substrate but of a soluble material, we have a third mechanism.

(3) Chemotaxis: This mechanism proposes that target sites secrete substances forming a concentration gradient and the neurite follows the gradient toward its source. For example, if nerve growth factor (NGF) is injected into the cerebellum one finds invasion of the cerebellum by axons from the sympathetic gan-

glion (for which NGF is a trophic substance) which normally do not innervate the cerebellum (Menesini-Chen et al., 1978). NGF antibodies block the innervation of rat iris in culture (Johnson et al., 1972). Cultured sympathetic ganglion cells orient their neurites up NGF gradients (Letourneau, 1978) and their growth cones will change direction and turn toward an NGF source within twenty minutes (Gunderson & Barrett, 1979). Yet, if all potential targets are secreting various chemotactic stimuli the neurite must have to choose the correct one. In the previous mechanism, local guidance, the neurite must sometimes have to choose one of several available paths. This implies a fourth mechanism.

(4) Chemoaffinity: This mechanism proposes that the growing neurite is chemically "labelled" so that it has a specific affinity for a post-synaptic site with a complementary label. The mechanism was suggested to explain the orderly process of establishment of retino-tectal connections during reinnervation (Sperry, 1963). At least for retino-tectal connections, it too may be physically manifested as a difference in adhesivity between target and non-target cells (Gottlieb et al., 1976). Stated more generally, this mechanism could underlie the choosing of one out of several possible chemotactic signals, guidance paths, post-synaptic cells or even different post-synaptic sites on the same neuron as in hippocampal pyramidal cells (Gottlieb & Cowan, 1973). Of course, choice is not always necessary and the fifth mechanism suggests a way of simplifying choice.

(5) Spatiotemporal arrays: These propose that neuron and target are matched according to their position in spatial or temporal arrays. For example, Macagno (1978) found that an array of cartridges in the *Daphnia* lamina is formed according to the order in which retinula cell axons arrive and it is this timing which imposes the order on the array. A similar mechanism has been invoked to explain the occupation of different synaptic sites by different hippocampal

afferents (Gottlieb & Cowan, 1972).

One way of viewing the interaction between these mechanisms is to note that the process of target finding is not a single process. Consider the insect sensory neuron, the subject of this paper. It must:

(1) Get to the central nervous system.

(2) Once there, it must establish a route to the appropriate region of neuropil.

(3) It must innervate only the appropriate region of neuropil and no other.

(4) Within the neuropil it must find and synapse with only the correct neurons.

(5) Since the relative positions of synapses on dendrites determines how incoming signals are processed (Rall, 1964), the axon must form the correct terminal branching pattern so that its synapses are placed correctly.

Different mechanisms that shape neurite growth probably have different relative influences on each of these aspects of neurite growth. The discussion that follows the presentation of my results will therefore consider each aspect of neurite growth separately and assess the evidence for the involvement of the various mechanisms. My results, where applicable, will be discussed in the context of relevant studies of sensory neuron development in insects and some other related work. These studies will therefore be briefly summarized before a presentation and discussion of my results. It should be kept in mind that these studies may not all pertain to the same features of axon growth.

B. Descriptive studies of axonal guidance in insect nervous systems.

1. Guidance of sensory neurons to the CNS.

During the development of the nervous system each growing axon must locate those target neurons with which it will synapse. In many cases the distances traveled by these axons are very large. One extreme case is the arthropod sensory neuron. These neurons are generated in the epidermis and their axons must find their

way to the central nervous system (CNS). Once there, they, like neurons generated in the CNS, must find their way to appropriate targets in the neuropil and avoid inappropriate potential synaptic sites. The guidance of sensory neuron axons to their targets has been studied more intensively than that of any other type of neuron. One reason is that the problems faced by these neurons in finding their targets, while like those of the central neurons, are even more difficult because of the generally larger distances they must travel. Another reason is that their location in the periphery makes them much more amenable to experimental manipulation.

Wigglesworth (1953) showed, in *Rhodnius*, that if cuticle was burned and allowed to regenerate the new axons could not find the CNS. Instead, they ran irregularly between the cuticle and the basement membrane, often forming bundles or networks and occasionally rings of axons. Some of the axons, in the course of their random growth, did succeed in finding a nerve from the unburned area and would join it. This suggested that axons require the existence of a guidance path to reach the CNS and can't do so on their own. If so, how are these paths originally set up?

Recent work in Orthopteran antenna, cercus and tarsus (respectively Bate, 1976; Edwards & Chen, 1979; Keshishian, 1980) has resulted in the observation that several pairs of peripheral neurons, termed "pioneers", develop earlier than the rest of the peripheral neurons. Their axons reach the CNS and enter it before any others and the sensory axons that subsequently appear form bundles about the pioneer axons. The adult nerves and their major branches are laid down on the framework originally set up by the pioneer axons. These pioneer axons may be guided in their growth to the CNS by cues in the basement membrane or perhaps by no cues at all. There may be no need for specific guidance this early when the distances from the appendages to the CNS are so small. A group of neurons has been described in the antenna of *Manduca* that may perform the same function during metamorphosis in holometabolous insects (Sanes & Hildebrand, 1975).[1]

Eliminating these pioneer neurons by ablating the region in which they are generated with a laser microbeam prevents the normal appearance of adult nerves (Edwards et al., 1981), although it has not been conclusively demonstrated that the sensory neurons can't reach the CNS without the pioneers in early embryos. Other mechanical cues can apparently provide some guidance. For example, Clever (1959) has described axons in the wing buds of *Galleria* that follow the channels of nascent wing veins. Keshishian and Bentley (1981) have reported that in grasshopper, *Schistocerca nitans*, some tarsal sensory neurons travel along an apodeme to reach proximal pioneer neurons which they subsequently follow.

In summary, the results of observations of the growth of sensory axons in insect appendages suggest that they reach the CNS not by seeking it directly but by following preexisting paths. These paths are created very early in embryogenesis when the distance between the periphery and the CNS is still very small. These paths are created with the assistance of, if not absolute requirement for, the pioneer neurons.

2. Guidance within the central nervous system.

Within the CNS the situation is somewhat more complicated. Here, the sensory (and central) neurites must navigate through a complex network of fasciculi and neuropil and find their correct targets. It is not surprising that the underlying mechanisms are even less clear than in the periphery. The results of some recent studies of the growth of peripheral and central neurons within the CNS will be briefly reviewed to provide a background for the present study. These involve direct observations as well as two sorts of experimental procedures: the use of surgical manipulations and homeotic mutations.

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1. In general, the imaginal discs of holometabolous insects are connected to the CNS by nerves before metamorphosis (Bate, 1978) and this is true of *Drosophila* as well (Hertweck, 1931; Reinhardt et al., 1977). These apparently also serve as guidance pathways as their disruption can prevent the sensory axons of the disc from entering the CNS at the appropriate point (Ghysen & Deak, 1978).

a. Guidance of peripheral neuron axons in the central nervous system. The entry of peripheral neurons into the CNS during embryogenesis has recently come under close study in the Orthopteran cercal projection to the terminal ganglion (Shankland, 1981a,b). The vast majority of the cercal sensory axons form a glomerulus (cercal glomerulus) in the terminal ganglion (which is formed as a fusion of abdominal ganglia A_8-A_{11}). The glomerulus is formed close to the axon's point of entry to the terminal ganglion. Therefore, most of the axons do not travel long distances to find their targets. A few cercal sensory axons do pass anteriorly through the terminal ganglion and then the longitudinal connective to terminate in the ganglion of segment A_7, the first ganglion anterior to the terminal ganglion. The cercal glomerulus has the structure typical of most insect sensory glomeruli -- a hollow shell with interneuron dendrites inside.

The peripheral pioneers do enter the terminal ganglion but pass anteriorly through it and terminate in ganglion A_7 (Shankland, 1981a). They enter the terminal ganglion at a time when there is little central neuropil and the consolidation of $A_{8\text{-}11}$ into a group physically separate from A_7 has not yet been established. The pioneer axons have transient filopodia but exhibit no branching.

The sensory axons enter the terminal ganglion after the pioneers do. Their behavior has also been described in detail (Shankland, 1981b). Some of their branches follow the pioneer axon anteriorly into A_7 although most do not. By the time that the cercal sensory axons enter the terminal ganglion it has already come to resemble the postembryonic ganglion in two important respects. The neuropils of ganglia A_8-A_{11} have already fused and the terminal ganglion has already become separated from the more anterior ganglion A_7. The only connections are the longitudinal connectives in which the pioneer axons now have come to lie. It is an interesting question whether the sensory axons could find the longitudinal connective, which is on the opposite end of the ganglion from the cercal nerve, without the

pioneer axon. The cercal projection into the terminal ganglion does not appear to be a product of exploratory growth of the axons followed by retraction of incorrect branches. Inappropriate branches were only rarely seen. Rather, it appears that the proper pathways were chosen at the outset without collateral branching into alternatives, and that the morphology of the cercal glomerulus at the earliest time it was formed closely resembled that of the adult glomerulus. Shankland (1981b) has suggested that this strongly implicates specific guidance of the sensory axons by local CNS cues into the appropriate target area. The MGI dendrite is already present when the sensory axons enter the terminal ganglion and it could provide guidance to the cercal glomerulus. The potential guidance role of the peripheral pioneers within the CNS has already been mentioned. However, we can't simply assume that the sensory axons are passively guided to their targets. Choice of which path to follow (e.g. to the cercal glomerulus or to A_7) can't be easily explained by a passive guidance mechanism nor the fact that the terminal arborizations in A_7 and the cercal glomerulus are quite different. Furthermore, as shall be discussed more fully below, each sensory bristle has a different pattern of terminal branching within the cercal glomerulus (Murphey et al., 1980; Murphey, 1981) which is constant from animal to animal. Thus each axon must choose a different portion of the MGI dendritic tree on which to synapse and form a different pattern of branching. It should be concluded then that although guidance by local CNS cues is important in determining the projection pattern of sensory axons, there are internal programs of branching patterns within the sensory axons as well as specific affinities to particular regions of the target neuropil which may need to be invoked to explain these observations. These, operating together, can explain the fact that the cercal sensory projection immediately forms its adult gross morphology. Further experimentation should focus on the individual sensilla; do they go right to their targets or is there a sorting out of the sensory projection within the sensory axon population?

b. Guidance of central neurons within the CNS. Much recent work on insect neuroembryology has used Orthopterans because of their large size and ease of culture. In the locust, *Locusta migratoria*, a segmental ganglion is formed from 61 neuroblasts arranged in two symmetrical sets of 30, one set on each side, and a single unpaired cell on the ventral midline between them (Bate, 1976a). Each neuroblast buds off a series of ganglion mother cells which in turn divide once to form two neurons. Thus each neuroblast can give rise to 10-100 neurons before it dies. There are, additionally, seven cells, called midline precursors (MP), situated medially between the plates of neuroblasts, anterior to the unpaired medial neuroblast, which, like ganglion mother cells, divide only once to form two neurons. These 14 neurons mature and send out processes before the vast majority of neuroblast progeny neurons do. The anterior trio of MP progeny on either side send one axon anteriorly and two posteriorly along the basement membrane. These meet their homologues from the adjacent segments and the resulting continuous longitudinal array of axons provides the framework which will eventually become the longitudinal intersegmental connectives (Bate & Grunewald, 1981). Other MP progeny axons are the founders of the dorsal median fiber tract and are among the earliest in the anterior and posterior ganglionic commissures (Goodman et al, 1981). Most of the MP progeny die during development although at least one, the H cell, survives in some segments and changes its branching pattern and physiological properties to new and different adult forms (Goodman et al, 1981; Bate et al. 1981). Close contact between the growth cone, as well as the filopodia, of certain identified neurons with "guide" axons indicate that these axons are indeed used for guidance and show how the adult morphologies of these neurons come to be established (Raper & Goodman, 1981). These descriptive studies suggest that fiber tracts in the CNS may be analogous to peripheral nerves in the manner of their origin. That is, the job of establishing the pathways is done by special sets of precocious neurons which differ

in appearance or ontogeny from the neurons that mature later. Their axons form the framework of the CNS tracts and the axons of the other neurons follow the axons of these "central pioneers". How these pioneers choose their paths is still a matter of speculation. It is possible that there are guidance cues in the basement membrane which these axons follow or that they use global positional information to determine the direction in which to grow and branch. However, early in embryogenesis the CNS is small in size and in number of cells so it is feasible that neuronal networks are established by direct contact between all of the neurons.

Recent evidence for the existence of guidance pathways in the vertebrate CNS has come from the results of Katz & Lasek (1979) using *Xenopus*. They reported that optic axons from optic primordia transplanted to positions adjacent to the spinal cord travelled preferentially in specific paths in the spinal cord, regardless of to where along the spinal cord they had been moved. Although this does not suggest that optic axons normally travel in the spinal cord it does show that spinal cord pathways are differentially labelled so that different kinds of axons show preferences for different spinal tracts. In a similar type of experiment, *Xenopus* Mauthner neurons were transplanted to different positions along the length of the spinal cord (Katz & Lasek, 1981). These too preferentially followed a specific tract in the cord but one different from that used by the optic axons.

In the salamander brain the situation is not quite the same. If eyes are transplanted to different positions on the head one of three possible projection patterns can be seen depending on where the eye is placed (Harris, 1980). This is not a result of influence by the normal eyes as the same results are obtained if the eyes are transplanted to genetically eyeless salamanders (Harris, 1981). The difference probably is a result of the fact that distances within the spinal cord are small and the appropriate tract can be easily located. In the brain, there are a larger number of tracts and therefore a greater chance that different acceptable ones exist. Since

the brain is larger than the spinal cord there is less chance that the most acceptable tract will be found.

Guidance fibers per se have not yet been identified in vertebrates, but channels in the ependymal germinal neuroepithelium of regenerating and embryonic amphibian spinal cord have been described that may have the analogous function (Singer et al, 1979). These channels form before most of the neurons mature and are subsequently invaded by neurites. It appears that they provide the route of the spinal cord longitudinal tracts. Thus, in vertebrates as well as invertebrates pathways laid down early in embryogenesis may form the general plan of the tracts of the adult CNS.

C. Experimental studies of axonal guidance in insect sensory neurons.

The experimental studies summarized here as well as the results to be presented below share a common feature. The experimental paradigm has been to "cut and paste", to take sensilla from one portion of the cuticle and place them elsewhere. Three kinds of projections are compared: the *normal* projection of the sensilla, that is, the projection it would have had it never been moved; the projection it has now, after transplantation; and the projection of the *local* sensilla, the sensilla (if any) that normally occupy the spot to which the experimental sensilla have been moved. The general intent of these studies is to investigate the behavior of neurons in a novel environment. The similarity or differences of the behavior of the neuron in its new environment to its normal behavior, or to the behavior of the local sensilla, can tell us what aspects of the neuron's behavior are intrinsically determined, and which are imposed on it by its local environment or position. In the simplest cases, this sort of experiment distinguishes between three possibilities. One is that the axon finds its target by chemotactic cues which means that they can always find their normal targets even from ectopic locations. Another is that the projection

pattern is built in, that it is made automatically, perhaps with reference to global cues such as the body's coordinate axes. This would imply a normally shaped projection pattern but in an abnormal part of the CNS, that part of the CNS innervated by the local sensilla. The final simple case is that the projection pattern is specified purely by local cues laid down in the CNS, perhaps during embryogenesis. This implies a projection pattern just like that of the local sensilla. Of course, these simple results rarely occur and the projections seen often have novel features unlike the normal or the local patterns. These patterns imply that more than one mechanism is at work and that the system must be carefully examined to tease apart the interactions between the different mechanisms.

Two methods have been used for producing ectopic sensilla for study. One is surgical, in which cuticle is physically grafted, the other is genetic, in which mutations are used to generate ectopic sensilla. Using surgical techniques, particularly interesting results have been recently obtained in the study of the Orthopteran cercal projection, the Orthopteran wind hair projection and the Dipteran retina-lamina projection.

1. Surgical manipulations.

a. Experimental manipulation of the Orthopteran cercal projection. The anatomy and physiology of the projection of the cercal sensory axon to the giant interneurons has been intensively investigated in Orthopterans (e.g. Edwards and Palka, 1974; Matsumoto and Murphey, 1977; Murphey et al., 1980). Rather detailed comparisons can be made then between normal animals and those with surgically transplanted or otherwise altered cerci.

Edwards and Sahota (1967) transplanted a cercus to the position of the mesothoracic leg (which had been removed) and allowed the cercal sensory axons to regenerate. The axons traveled along the leg nerve and entered the mesothora-

cic ganglion.[2] The normal cercal inputs to giant interneurons are in the terminal ganglion. Since the giant interneurons synapse on the leg motorneurons (Ritzmann & Camhi, 1978), the cercal sensory axons entering the mesothoracic ganglion could have access to part of the giant interneuron axons. Indeed, Edwards and Sahota found that the sensory axons of the transplanted cercus passed the vicinity of the giant interneurons and did establish functional synapses on the giant interneurons (which showed electrophysiological properties characteristic of normal cercal to giant interneuron synapses). Thus, it appears that specific connections can be established between neurons even at locations other than the normal location for synaptic interaction. This further suggests that the two sets of neurons are specifically marked for recognition and that this marker is not localized to a specific part of the cell. However, the presence of synapses on other interneurons available in the mesothoracic ganglion was not investigated. Therefore the possibility that all potential synaptic sites (including those denervated by the removal of the mesothoracic leg) were non-specifically innervated can't be ruled out. There is in fact ample evidence (Murphey et al., 1980) that individual cercal sensory axons can discriminate between different parts of the giant interneurons' dendritic arbor although this may not be the same as the more general kind of cell-cell specificity demonstrated by Edwards and Sahota.

Palka and Schubiger (1975) examined cercal sensory projections to the Lateral Giant Interneuron in cerci rotated so that the dorsal-ventral axis was exchanged for the lateral-medial. The cercal filiform hairs make specific connections with the giant interneurons such that only hairs that are on the dorsal or ventral sides of the cercus contact the Medial and Lateral Giant Interneurons. The filiform hairs on the rotated cerci innervated the Medial and Lateral Giant Interneurons according to

2. This experiment was done well before cobalt filling of axons came into vogue and the course of axons was followed with silver staining. Therefore the details of the arborization of the axons within the mesothoracic neuropil could not be determined.

their original position on the cercus and not according to their new one. This suggests that once a sensory neuron has obtained a position-dependent "label" which constrains it to synapse on a particular interneuron, the label is not lost if the cell receives a new position. However it is not possible to conclude that the sensory neuron's preference for a particular target is, in general, independent of its route into the CNS. The axons are not completely ordered within the nerve with respect to their original position (Murphey, 1981) so all of the axons presumably had been guided to the same sets of interneurons as if no rotation had been done.

Within the cercal glomerulus the projections of the cercal clavate (Murphey et al., 1980; Murphey, 1981) and filiform (Bacon & Murphey, 1981) hairs are extremely precise. It is organized so that the position of a hair within the array of hairs on the cercus determines the position of its terminal arborization in the cercal glomerulus. That is, all the hairs in a particular row (parallel to long axis of cercus) have similar projections in the cercal glomerulus although more distal (older) hairs have larger arborizations which reach farther anteriorly. Hairs in different rows project differently such that as one moves around the circumference of the cercus, the position of the arborization shifts around the glomerulus in a somatotopic fashion.

The precision of this projection pattern makes it an attractive system for experimental manipulations which have only recently been initiated (Murphey et al., 1981). Like the Palka and Schubiger (1975) study, this was a study of the consequences of cercal rotation. The axons generally could make normal projections to their original targets, occasionally by circuitous routes, in spite of the rotation. However, in a third of the cases the axons had abnormal arborizations. The conclusion reached was that the route of the axon and, presumably, to what part of the target neuropil it is delivered can have a effect on its arborization. Nevertheless the axon still has a preference for its original target and when it can compensate for

the altered route in some way and gain access to its preferred site, it will innervate it. Thus both the chemoaffinity and pathway guidance concepts of neuronal development are supported.

The youngest hairs in each row are the most proximal with age increasing distally. Also, the distal hairs in the row have larger terminal arborizations than the more proximal. If the cercus is cut distally and allowed to regenerate, then the newly formed distal arborizations are like those of normal distal receptors in spite of the fact that these distal hairs are the youngest. It is their position and not their birthdate that determines their central arbor. Therefore a temporal array model is unlikely.

There is one serious problem with this and the preceding study. Here, sensory axons had already established synapses before the cercus was cut and allowed to regenerate. Sanes et al. (1978) have shown that regenerating motorneurons preferentially innervate their original sites. It is possible that the mechanisms implicated or ruled out here may have quite different roles during normal development. Specifically, the affinity that axons have for their correct target may be achieved simply by finding their original target of the sensory neuron in that position on the cercal array (obviously the original neuron is gone). The fact that there is an affinity for sites originally occupied by a neuron in the same position on the cercus is interesting but does not imply that chemoaffinity is used during normal development.

b. Experimental manipulation of the Orthopteran wind hair projection. The locust head hairs communicate information about wind or airspeed to the CNS (Weis-Fogh, 1949). Tyrer et al. (1979) divided these hairs into three groups based on their projections in the CNS. Within the brain (tritocerebrum), the projections from fields A, B and C are all similar but in the subesophageal ganglion and prothoracic ganglion

only field B projections are exclusively ipsilateral. Fields A and C are distinguished by differences in the morphology of their arborizations in the prothoracic ganglion and by the fact that only field C axons project into the mesothoracic ganglion. Field A and C axons, but not those of field B, synapse on the tritocerebral commissure giant (TCG) interneuron (Bacon & Tyrer, 1978).

Anderson and Bacon (1979) surgically interchanged pieces of head cuticle bearing sensilla from two different fields, A and B, which have distinguishable central projections and investigated the projections of the bristles that developed on the grafts after the operation. They found that the axon entered the CNS through the nearest nerve, that is, the one normally used by bristles in that position on the head. Yet, the arborizations of the axons in the subesophageal ganglion were determined by the original location of the cuticle used in the graft. Like the sensilla on rotated or interchanged cerci (Palka & Schubiger, 1975; Murphey et al., 1981), the wind-hair sensilla did not appear to acquire a new identity as a result of their having been moved to a new position. The central arborization of the axon depended on its identity and not on the route it took to the target neuropil in the CNS. Since the bristles of all three fields project to the same general region of the subesophageal ganglion and those that descend do so in the same tract, it would seem that the decision of how to arborize in the neuropil or how far to descend along the tract must be a function of an internal program in the sensory neuron. This experiment shows that the program is a stable inheritable commitment and does not change with position on the head or route of entry after it is determined. More recently Anderson (1981) has reported that the connections made on the TCG also depend on the origin of the graft and not on its new location.

In these studies of ectopic wind-hair projections, as in the studies of Murphey et al. (1981) described above, prior innervation of the central targets had existed, albeit not by the same bristles actually studied. There is the possibility then that

these results are merely the product of the axons seeking out the original targets of their head-hair type.

Head wind-hairs transplanted to the thorax enter a thoracic ganglion and establish projections of variable length in the Median Ventral Tract (Anderson, 1981). This is the same tract used by wind hair axons which normally project from the subesophageal ganglion into the thoracic ganglia. This suggests that axons have specific preferences for particular tracts if more than one is available as has been shown for the transplanted *Xenopus* eyes and Mauthner neurons. Here the pathway chosen was not merely a preferred pathway but specifically the one normally taken.

The TCG also projects into the thoracic ganglia but in a different tract, the Dorsal Intermediate Tract. Wind hairs grafted to the thorax fail to synapse on the TCG. This may mean that only certain parts of a neuron are available as postsynaptic sites for particular inputs, in contrast to the results of Edwards and Sahota (1967). However there are at least two other possibilities: The guidance of the wind hair axons and the TCG axon into two different tracts may prevent the establishment of connections. Alternatively, the wind hair input to the TCG may be mediated through an as yet unidentified interneuron which is present only in the tritocerebrum where the connection is normally made. Tests for monosynapticity of the wind hair to TCG connection have never been done.

c. *Retinula cell projections into the lamina.* The sensilla that are the subject of the present study, like nearly all external sensilla, are bristles or bristle homologues. Retinula cells project in a highly ordered and specific manner onto the monopolar cells of the optic lobe lamina (Braitenberg, 1967; Trujillo-Cenóz & Melamed, 1966). Each ommatidium has six retinula cells that project to the lamina and for each of the six axons there is a different spatial relationship between its ommatidium of origin and target lamina cartridge which is the same for all such axons on the

retina. Thus each cartridge receives inputs from six retinula cells which all view the same point in space. The one qualification is that there is a discontinuity at the retinal equator such that the pattern of retinula cells and their projections is mirror-image reversed between the dorsal and ventral hemiretinas.

Maturation of retinula cells takes place in a posterior to anterior sequence in *Drosophila* (Gottschewski,1960; Ready et al., 1976) and other insects (e.g. Anderson, 1978a; Egelhaaf et al., 1975; Melamed & Trujillo-Cenóz, 1975). This occurs throughout the pre-adult life of hemimetabolous insects, with the addition of new ommatidia at the anterior edge of an already functional eye, and during metamorphosis in the eye discs of *Drosophila*. In both cases, new axons enter a lamina that has already received innervation from axons posterior to them and could use these axons for guidance in establishing connections.

The movement of the retinula cell axon growth cones during development has been described (Meinertzhagen, 1973) and these observations suggest that the projection is formed in two stages. The first is the movement of the growth cones along the stalk connecting the eye disc and the brain. The growth cone is small in this stage, without filopodia, and moves rapidly. By analogy with the other discs it would seem that guidance here is purely mechanical, provided by the stalk. Upon reaching the lamina the growth cones enlarge and put out long filopodia which form complex networks with those of other growth cones and surround the lamina cells. The growth cone eventually follows a filopodial projection in a straight line to the region where the correct target cartridge will form. It stops on the side of the cartridge closest to its ommatidium of origin.

How are the retinula cell axons guided to their targets? Experimental studies have ruled out some possibilities but the question is not yet answered. Although the optic lobe to lamina projection is as good an example of precision in neural connec-

tions as has ever been observed there are occasional mistakes. An analysis of 17 such mistakes (out of 500 axons), caused by a congenital dislocation in the retinal equator (Horridge and Meinertzhagen, 1970; Meinertzhagen, 1972) allows some statements to be made about the criteria used by the retinula cell axons in target selection. First, the fact that mistakes do occur suggests that there is no highly specific affinity between a retinula cell and its target lamina cells. Second, the mistakes were more frequent in axons 3 and 4, which travel farther than 1, 2, 5 and 6, and one of the major types of error (4 out of 17) was a reversal of target between 3 and 4 (which project to targets having a smaller angular separation than between any other pair). This, and the fact that the growth cones follow straight lines, suggests that the angle to be taken by the axon with respect to its ommatidium of origin is predetermined. Third, none of the 17 mistakes involved overshooting the target. Rather, cartridges closer than the correct one were innervated, suggesting that the distance to the target is also predetermined and the axon will not exceed it.[3] Fourth, the errors were seen around a dislocation in the equator and rarely elsewhere suggesting that continuity of the substrate on which the axons must travel is important. In summary, the errors never involve large changes of direction of projection nor increases in maximum distance travelled. This suggests that direction and distance are highly conserved and may indeed be the criteria used by the axon in finding its target.

Another means of studying the establishment of optic connections has been the surgical rotation of retina with respect to underlying lamina. Horridge (1968) reported normal optomotor responses following a 180° rotation of the retina in *Schistocerca* and concluded that the retina had established new connections in the same manner as the old. That is, each axon had innervated a cartridge with the same spatial relationship to its ommatidium as in a normal animal with no

3. Strausfeld (1971) reported axons in the lamina which did overshoot their targets or which even innervated two targets but these would appear to be much less common types of errors.

preference for original targets. However, it now seems likely (Anderson, 1978b) that none of the axons in of the excised retina had regenerated and that the response was due to retinula cells that had been born after the graft. Anderson (1978b) studied the projections from small grafts of retina taken from proliferation zones and could therefore still send new axons into the lamina. The grafts included rotated retina and retina taken from older or younger animals (which would correspond to more posterior or more anterior retina). In all cases the grafts innervated the lamina as normal ommatidia in that location would. This argues against a specific retinula cell to lamina cell matching.[4] Although Anderson (1978b) interpreted her results to imply a contact guidance mechanism, perhaps with newly generated axons using the posterior older axons as guidance cues, I do not feel that this is the only mechanism consistent with the results described above. Guidance by local cues is difficult to reconcile with the fact that axons project over the retinal equator in no different a fashion than any other axon in that hemiretina. Yet the guidance cues across the equator must be mirror image reversed as are the organization of the retinula cells in the ommatidia and the direction of the retinula cell projections. Furthermore, the retina and the lamina are homogenous structures, made up of identical repeating units. This makes it difficult to see how axons can be guided to specific lamina cartridges by local cues that distinguish each cartridge. The temporal pattern of axon arrival only gives information about the anterior-posterior axis on which a particular cell or growth cone is situated and none about the dorsoventral axis. Therefore, the timing of the posterior to anterior wave of maturation which sweeps over the retina and lamina cannot account for the specificity of the connections. It may simply be that the axons are guided by an internal program at the initiation of axon growth. The possible use of polar coordinates for orienting such a program is suggested by the relative invariance of angle

4. Of course, since the retinula cells were born and matured in a new position, they may have acquired new positional values and new chemoaffinity labels.

and maximum axon length mentioned above and the fact that positional information in regenerating discs appears to be specified by polar coordinates (French et al., 1976). While I think this model should be considered, as it does explain the experimental results of Meinertzhagen (1972) and Anderson (1979), from the evidence available, models based on contact guidance or interactions with other retinula cell axons can't be ruled out. The behavior of the growth cones and filopodia within the lamina does suggest that such interactions are occurring. However, the contacts between the retinula axon growth cones may occur for any of several other reasons, for example, as a means of ensuring that a complete set of retinula cell axons are present in each cartridge, or for recruiting lamina cells, or as an aid to the direction determining and distance measuring mechanisms of a growth cone. The behavior of individual retinula cells in suitable culture medium could perhaps be observed as a means of testing whether their axon growth is determinate or controlled by the environment.

2. Homeotic mutations.

Another way of transplanting sensilla-bearing cuticle to ectopic locations is by the use of homeotic mutations. The genes affect developmental pathways in such a way that particular appendages or regions of cuticle are transformed into others. These ectopic sensilla can be filled with cobalt chloride ($CoCl_2$) or horseradish peroxidase (HRP) so that their projections into the CNS can be determined. The advantages to this method are that surgical trauma can be avoided and one does not have to make the assumption that regenerating neurons behave as embryonic neurons do. However, there are problems peculiar to the use of "genetic surgery" that need to be considered:

(1) The identity of a particular patch of cuticle is established by checking its external appearance. Sometimes the detailed morphology of the sensilla needs to be checked by microscope. There is no way, however, at present of establishing the

identity of the neurons within the bristles. If the projection pattern from the sensilla of a transformed appendage resembles that of the local sensilla it may be a result of the mutation not having any effect on the sensory neuron. Although this possibility can't ever really be ruled out completely for a given mutation, there is some reason to assume, until evidence to the contrary is produced, that the sensory neuron is indeed transformed:

(a) The sensory neuron is derived from one of a small number of cells (3-8, see Lawrence, 1966, for a review) generated by two or three successive generations of cell divisions from a single cell initially indistinguishable from the surrounding epithelial cells and not apparently predetermined by ancestry (Lawrence, 1966). The rest of the progeny of the "bristle mother cell" make up the cuticular elements of the sensilla, a glial cell and the other sensory neurons (if any). The very close clonal relationship between the neural and cuticular cells of the sensilla strongly suggests that the mutation affects them all in a parallel fashion.

(b) The CNS is affected by hómeotic mutations of the bithorax complex (Green, 1981; Jiménez & Campos-Ortega, 1981) so it is reasonable to assume that the peripheral sensory neurons, closely related as they are to epithelial cells, are transformed as well.

(c) Sensilla on transformed cuticle can have physiological functions characteristic of their external appearance and not of the replaced cuticle (Deak, 1976; Stocker, 1977). The tarsus (and proboscis) bear taste sensilla which, when touched with sugar, initiate a proboscis extension reflex (PER). A PER is never elicited by touching the antenna with sugar. Yet if tarsal tissue replaces the antenna as a result of spineless-aristapedia or Antennapedia mutations then touching the ectopic tarsus with sugar elicites a PER.

Ultimately it will probably be necessary to control for this problem by produc-

ing similar types of ectopic cuticle by means of "forceps-and-razor surgery" as were produced by "genetic surgery".

(2) It could be said that projections unlike those of the local sensilla are a result of an effect on the CNS of the mutation. This problem can be controlled for by the use of genetically produced mosaic flies (Palka et al., 1979; Stocker & Lawrence, 1981) in which mutant sensilla project into wild-type CNS.

In spite of very different sources of artifacts, the results from surgical manipulation of the positions of sensilla are compatible with the results from genetic manipulations. Before the actual results are summarized and discussed a brief digression will be made to provide some background into the neuroanatomy of *Drosophila*.

a. Relevant aspects of the anatomy of the Drosophila CNS. The general plan of the arthropod nervous system is a supraesophageal ganglion, or brain, dorsal to the esophagus and a series of ganglia, segmentally organized, ventral to the gut. These are connected to each other by the longitudinal or intersegmental connectives and the anteriormost of them is connected to the brain by a pair of circumesophageal connectives lateral to the esophagus. In the generalized insect the first three segmental ganglia are fused to form the subesophageal ganglion which can be found with the supraesophageal ganglion in the head. A long cervical connective runs through the neck to connect the subesophageal ganglion with the anteriormost thoracic (prothoracic) ganglion and the circumesophageal connectives still connect the subesophageal ganglion with brain. In most insects fusions occur between two or more of the other segmental ganglia so the number of ganglionic masses in the ventral chain is smaller than the number of segments.

In *Drosophila* and other related Diptera this process of fusion has been carried out to an extreme. The ventral ganglion (sometimes, confusingly, called the thora-

cic ganglion) is the entire ventral chain (minus the subesophageal ganglion) fused into one mass (figure 9). Within it, though, the larger thoracic segmental ganglia are discernable as masses of neuropil termed neuromeres. The small abdominal ganglia are indistinguishable with general histological techniques.

Afferent sensory projections from the head macrochaetes, the antenna and the tarsus of the leg into the central nervous system (CNS). will be described. The projections of the first two are into the supra- and subesophageal ganglia. In *Drosophila* and other diptera these are fused into a single mass, often called the brain, through which the esophagus passes in the esophageal channel. The brain is therefore a rather complex structure and some of its elements that will be of particular concern to the following studies should be briefly mentioned.

The *Drosophila* brain is diagrammed in figure 1. Ventrally, below the esophageal channel, is the subesophageal ganglion which is the result of the fusion of the anterior three ganglia of the ventral segmental chain. The subesophageal ganglion is connected to the proboscis by the labellar nerves and to the ventral ganglion by the cervical connective. The supraesophageal ganglion is composed of three parts which, ventral to dorsal, are the tritocerebrum, deuterocerebrum and protocerebrum. These major subdivisions of the supraesophageal ganglion are easily distinguished in most insect species but in those, like *Drosophila*, in which considerable fusion of different parts of the CNS has occurred, this is not the case. Pertinent examples would be the difficulty in determining precisely the posterior border of the antennal mechanosensory area (between it and the protocerebrum) or the tritocerebrum (between it and the subesophageal ganglion).

The tritocerebrum is connected to parts of the proboscis and front of the head by the labrofrontal nerves which also branch to form the recurrent nerve which passes back through the esophageal channel to innervate the digestive system. The

deuterocerebrum is connected to the antenna by the antennal nerves and is subdivided into the olfactory lobe and the antennal mechanosensory area, which is lateral and posterior to the olfactory lobe. The protocerebrum contains the optic lobes laterally which have three layers of neuropil: the lamina, the medulla and the lobula in lateral to medial order. The term "central brain" will be used to refer to the brain medial to the optic lobes. The central protocerebrum is subdivided into a number of neuropil regions of which some are concerned with the antennal input as shall be demonstrated in the results of the present study. These, labelled in figure 1, are the ventrolateral protocerebrum, lateral to the olfactory lobe and the mushroom body. The latter is a complexly shaped structure with a calyx region in the extreme dorsoposterior part of the protocerebrum and a branching anterior protuberance (not shown in the figure) which extends to the anterior of the protocerebrum just dorsal to the olfactory lobe.

b. Mutations causing ectopic sensilla in the thorax. The projections of the thoracic sensilla in wild-type and several genetically and surgically altered conditions have been intensively investigated (Ghysen, 1978, 1980; Ghysen and Deak, 1978; Ghysen and Janson, 1980; Palka et al. 1979; Palka and Schubiger, 1980; Strausfeld and Singh, 1980). Most of the dorsal thorax, or notum, is the mesothoracic segment. On it are 13 pairs of macrochaetes and about 200 microchaetes. In addition, the wings have three kinds of sensilla: many small campaniform sensilla on the proximal wing blade, five large campaniform sensilla on the distal wing blade, and bristles on the wing margin. Dorsal metathoracic sensilla are restricted to the halteres and are of two kinds, both similar to the wing's. There are a large number of small campaniform sensilla proximally and a few bristles distally. The proximal campaniform sensilla of the wing and haltere can be distinguished from each other by scanning electron microscopy (Palka et al., 1979; Cole and Palka, 1980).

The notal bristles (Ghysen, 1980) all project into the ventral ganglion by way of the posterior dorsal mesothoracic nerve, except for the humeral bristles which go by way of the dorsal prothoracic nerve. Within the ventral ganglion they all project in the same tract which runs longitudinally through the ventral ganglion. Yet there are subtle differences between the projections of the different bristles. Whereas all of the bristle axons branch upon encountering the longitudinal tract, producing an anterior and a posterior branch, the relative distance covered by the two branches varies from bristle to bristle. Also the bristles differ in whether they take any of the four available crossbranches and, if so, which ones. Specifically, more posteriorly located bristles tend to have shorter anterior branches and longer posterior ones, and to project into more posteriorly situated crossbranches.

The small proximal campaniform sensilla of the wing and haltere have longitudinally oriented projections in the same dorsal tract. Like the tract taken by the notal bristles, this runs longitudinally through the ventral ganglion. The haltere projection enters by the haltere nerve, the wing projection by the anterior dorsal mesothoracic nerve. It is therefore not suprising that the haltere projection extends more posteriorly than that of the wing. Anterior to the entry of the proximal wing projection, the wing and haltere projections overlap closely although they are distinguishable by some differences in side branches. The anterior ends of both projections are in the brain and there too the haltere and wing projections can be distinguished (Strausfeld and Singh, 1980). Ghysen (1978) has shown that if the haltere projection is surgically misrouted so that it enters one of the wing nerves, it still has a characteristically haltere-like projection. That is, it fills out the same part of the tract that a haltere normally does and takes the same crossbranches. It does this even though it enters the tract at an incorrect location.

The bristles of the wing and haltere have short, medially directed projections close to their entry into their respective neuromeres (Ghysen, 1978, 1980; Palka et

al., 1979).

Finally, to complete the catalogue, the distal large campaniform sensilla are present only on the wing and have no haltere homologue. These also have a longitudinally oriented projection through the ventral ganglion but in a much more ventrally placed tract than the aforementioned.

Several mutations were used by Ghysen (1980) to study the effect on the projections of surviving bristles after removal of adjacent bristles and to study the projections of supernumerary bristles. The mutations Hairy-wing (*Hw*) and scute (*sc*) respectively add or delete bristles. The mutation Contrabithorax (*Cbx*) is a homeotic mutation which converts meso- to metathorax thereby suppressing the development of mesothoracic bristles. The mutation wingless (*wg*) causes duplications of the mesothoracic notum. In *sc* and *Cbx*, the projections of surviving bristles were normal. In *Hw* and *wg*, the projections of supernumerary bristles were like those of their normal homologues even in a few cases where they entered by way of a different nerve.

The bithorax-complex mutations bithorax and postbithorax, in combination, transform the metathorax to a second mesothorax in appearance. There is a second set of notal bristles and the haltere is replaced by a wing. The distal wing campaniform sensilla and the notal bristles have no counterpart in the cuticle they replace and so are completely novel structures to their new position. On the other hand, the proximal small campaniform sensilla and the wing margin bristles may be replacing segmentally homologous structures. At the very least they are replacing structures of roughly similar appearance and physiology. As with all genetically transformed sensilla to be discussed, this transformation will be referred to as if the sensilla had been physically moved to the new location, that is homeotic mutants will be regarded in the same way as surgically altered experimental

animals. This is done, in full cognizance of the potential hazards, in order to simplify the descriptions of the experiments.

The wing margin bristle projection of the homeotic metathoracic wing is identical to that of the normal mesothoracic wing except that it is shifted to the metathoracic neuromere instead of being in the mesothoracic (Palka et al., 1979). Three reasons can be found to explain this:

(1) The fibers are genetically programmed to make short medially directed projections.

(2) The metathoracic neuropil into which the homeotic axons project is segmentally homologous to the mesothoracic neuropil into which the normal wing margin bristles project and can therefore serve as a suitable postsynaptic site. Evidence for (1) and (2) above can be derived from the observation that the few existing bristles on the wild-type haltere make a short medially directed projection.

(3) The CNS is transformed by the mutation and therefore the metathoracic neuropil resembles the mesothoracic. Although this may be true it can't account for the similarity of the projections because homeotic wing margin bristles, generated on the haltere by small bithorax clones in mosaic flies have the same projection in presumably wild-type neuropil as do homeotic wing margin bristles in neuropil of completely mutant flies. (Palka et al., 1979).

In bithorax postbithorax flies the ectopic notal bristles possess a central projection much like that of the normal mesothoracic notal bristles (Ghysen, 1978, 1980). That is, it enters the ventral ganglion through the dorsal metathoracic nerve, arrives at the same tract, and sends branches anteriorly and posteriorly. However, since they enter in the metathoracic neuromere instead of the mesothoracic, the branch point and the posterior projection are shifted posteriorly with respect to the normal mesothoracic bristle projection although the projection is normal with

respect to the position of the bristle or the entry into the tract. The anterior branch generally reaches the region of arborization of the normal notal bristles and fills out part of it. The axon then can recognize its normal tract within the ventral ganglion even if it meets it in a abnormal place.

The projection of the distal campaniform sensilla on the homeotic metathoracic wing is essentially identical to the normal distal wing campaniform projection within the ventral ganglion except that it enters by a different nerve (Ghysen, 1978; Ghysen and Janson, 1980; Palka et al., 1979). That is, unlike the projection of the ectopic notal bristles, its branching pattern is like that of the normal wing distal sensilla with respect to the position within the ventral ganglion and is not shifted according to the position of the bristle or the entry into the tract.

In a bithorax postbithorax fly the projection from the ectopic (metathoracic) wing proximal sensilla resembles that of the haltere which it replaces, both in the ventral ganglion (Palka et al., 1979) and in the brain (Strausfeld and Singh, 1980). The wild-type projections of wing and haltere proximal sensilla are very similar but are not the same in detail, and the ectopic wing proximal sensilla projection is clearly identical to that of wild-type haltere proximal sensilla.[5] This is true even if the metathoracic wing nerve is misrouted so that it enters the mesothorax by way of the normal wing nerves. This rules out any effect of the metathoracic CNS on the projection. The reason for the similarity is likely to be the fact that the transformation of haltere to wing is incomplete in the region of the proximal sensilla by external morphological criteria (Cole and Palka, 1980).

In summary, the following types of ectopic sensilla can recognize and follow their normal pathway even if they enter it at an abnormal location: (1) notal bristles entering the metathoracic instead of the mesothoracic neuromere, (2) wing

5. Ghysen (1978) actually disregarded the differences between them and concluded that the projection of the ectopic wing proximal sensilla was the same as that of the normal wing proximal sensilla. This was probably because he emphasized the fact that the same tract is used.

proximal campaniform sensilla entering the metathoracic instead of the mesothoracic neuromere, (3) wing distal campaniform sensilla entering the metathoracic instead of the mesothoracic neuromere, and (4) haltere proximal campaniform sensilla entering the mesothoracic instead of the metathoracic neuromere. This suggests that there are "labelled pathways" in the central nervous system which can guide different sets of axons in different areas but be recognizable at any point, an idea which has been discussed previously with regard to guidance of central neurons in the grasshopper and the amphibian. It is important to note here that since this is not a regenerating system the "labels" must have been there before the sensory axons entered. Therefore, the labels are central in origin but are recognized by the sensory neurons. Furthermore, since the tracts containing sensory neurons also contain central neurons, the labels must also be recognized by more than one type of axon.

Within these pathways the axons behave differently but all show behavior typical of their normal behavior within the pathway. The notal bristles form a "correct" projection shifted posteriorly so that it remains correct with respect to their new point of entry to the nerve. The distal campaniform sensilla form a projection that is correct with respect to its position in the ganglion and its targets. Although these sensilla all form projections with some resemblance to their normal projections they differ in which elements of the normal projection are retained, branching pattern or target. These differences can be explained by assuming either that (1) The projection of a notal bristle is determined by its relative anterior-posterior position but not with respect to the whole animal; only with respect to the segment it is in. The projection pattern is generated actively by the sensory neuron. Since the branching pattern is pre-specified it will look the same no matter where in the tract it is made. It will however be shifted posteriorly. (2) The projection of a notal bristle is determined by its relative anterior-posterior position. If it is on the

metathorax then it will acquire a posterior positional value for its projection and choice of targets. By this hypothesis, the projection can't really be compared directly with that of the normal bristle as it is no longer really the same bristle as regards its internal program of development. Both of these ideas have interesting implications for the mechanisms of axonal growth but there is no way of deciding now what the cause of the difference really is. It should simply be noted that different sensilla may behave quite differently, even in their normal tracts, when they enter them at ectopic locations.

c. Mutations causing ectopic sensilla in the head; introduction to the present study. In the experiments described above the ectopic sensory axons were able to reach and enter familiar tracts (presumably because of the proximity). In all these cases elements of the normal behavior of the axons are preserved even though they entered the tract at an abnormal spot. That is, the tracts are either followed for distances characteristic of the normal distances traveled by these axons or to the normal targets. While this result is important, and has led to the idea of labelled guide pathways within the CNS, it does not provide information on the behavior of neurons in abnormal or "foreign" tracts. A different experimental system is required in which it is possible to place the ectopic cuticle where it would be difficult for its sensory axons to reach their normal tracts and targets. If they could reach them at all the inference could be made that there are long-range chemotactic cues which guide sensory axon growth. If not, the ectopic axons would project, abnormally, into foreign neuropil. We could then ask how an axon can be guided by a foreign tract. Can an axon enter foreign neuropil and synapse therein? Can an axon form normal branching patterns in foreign neuropil? The answers to these questions will allow us to assess the interaction of local guidance cues, chemoaffinity and internal programs in establishing each feature of the projection.

The system used here allows this kind of experiment to be performed. Mutations of the Antennapedia or spineless-aristapedia genes were used to produce ectopic leg cuticle on the head. Specifically, these mutations convert all or part of the antenna into leg. The first homeotic mutation used for studies of central projection was Antennapedia (Stocker et al., 1976). This mutation replaces most of the antenna with a small leg. Antennal mechanoreceptor sensory neurons are replaced with leg mechanoreceptor sensory neurons, the number of olfactory sensilla is reduced and taste sensilla not normally on the antenna are added. Stocker et al. used degeneration methods to map the projections of the wild-type antennae and antennal legs. This method reveals which region(s) of the CNS are innervated but details of the arborization within these regions cannot be seen. Their results, summarized, were:

wild-type antenna: heterolateral degeneration in the olfactory lobe, ipsilateral degeneration in the antennal mechanosensory area.

wild-type leg: generally ipsilateral degeneration in the ventral ganglion neuromere which the leg nerve entered.

homeotic antennal leg: ipsilateral degeneration in olfactory lobe with a small amount of contralateral degeneration in the region of the antennal commissure, ipsilateral degeneration in the antennal mechanosensory area.

Thus, with the exception of the contralateral olfactory lobe, the antennal leg projection resembled that of the antenna and does not enter any normal leg targets.

The spineless-aristapedia mutation transforms only the distal antenna (arista) to distal leg (tarsus). The antennal olfactory sensilla are still present (in slightly reduced numbers) and leg mechanosensory bristles and taste sensilla are added. The projection of the tarsal bristles were traced by cobalt filling, a technique better able to resolve details of the arborizations (Green, 1980; Stocker and Lawrence, 1981). These studies showed that the tarsal bristle axons arborize irregularly in the olfactory lobe neuropil and are not confined to the olfactory lobe but project out of

it into the subesophageal ganglion. Also, Stocker and Lawrence (1981) showed that the differences were not a result of a genetic change in the CNS by looking at the projection into wild-type brain of tarsal bristles on small clones of spineless-aristapedia cuticle in mosaics. The projection of these bristles into presumably wild-type brain was like that of non-mosaic mutants.

There are some additional problems which have motivated the present study. The antennal leg projection has some elements like and some elements unlike that of an antenna. Those elements like that of a wild-type antennal projection could come about in three ways: First, some of the antennal neurons might not be affected by the mutation even though the cuticular parts of the sensilla appear to be. Second, the mutations used in the previous studies do not completely transform the antenna into leg. There are proximal antennal olfactory sensilla remaining on the appendage. Since antennal pioneer axons are peripherally derived (Bate, 1976), the remaining untransformed antennal epithelium might produce pioneers that will establish a route to the olfactory lobe later to be passively followed by the antennal leg sensory axons. Third, the $CoCl_2$ can be taken up and transported by intact neurons (Strausfeld & Obermayer, 1976). Very often, $CoCl_2$ can diffuse through a cut appendage and be taken up by uncut neurons. If the mutations used do not completely remove the proximal antennal olfactory sensilla, they may take up and transport the $CoCl_2$ even though they have not been damaged. Thus, the elements of the antennal leg projection that resemble elements of the wild-type antennal projection may indeed be from untransformed antennal sensilla.

These problems were dealt with in the present study by using flies of the genotype $Antp^{73b}$ $Df(3R)sbd^{104}$ / Pc^{3} ss^{a} which produces a more complete antenna to leg transformation, which will be described more fully in the results section. No olfactory sensilla were present to interfere with the study. Although this does not completely solve the problem of transformation of the neuron, it does mitigate it.

The use of this fly also allows selective filling of only the mechanosensory bristles on the antennal leg, permitting discrimination between elements of the projection from mechanosensory neurons and elements from taste neurons.

I have also examined the projection into the brain of ectopic thoracic bristles on the head. This has the advantage that the ectopic bristles are large enough to be filled individually so that the projections of individual axons can be compared. In addition, other ectopic or supernumerary sensilla were studied to provide various controls which will be discussed below.

The studies summarized above suggest that much of the ability of the sensory neuron to reach its proper target is a result of guidance by a central pathway which has a specific chemoaffinity label all along its length. The present study will examine this hypothesis more closely and provide information on the following: How is the correct pathway chosen and what constrains the axon to follow it? How is the axon's arborization pattern in the target neuropil determined?

METHODS AND MATERIALS

A. Visualization of afferent axons with $CoCl_2$.

$CoCl_2$ was used for visualizing afferents from antennae, legs, antennal legs and individual head bristles. In the first three cases the appendage was immersed in a $CoCl_2$ solution in a small wax chamber and cut with a small iridectomy scissors. Neurons from individual bristles were cobalt filled by placing a drop of $CoCl_2$ on the bristle and plucking it out with forceps. Various $CoCl_2$ concentrations and times for filling were tried. The best fills were obtained with 1.5% $CoCl_2$ + 0.13 mg ml^{-1} bovine serum albumen in distilled water left on the cut for 20 to 40 minutes. Following this, the flies were rinsed with distilled water to remove the cobalt and then transferred to a dish of saline solution. The required tissue was dissected out, rinsed again in saline, left for 5 minutes in $(NH_4)_2S$ (one drop of a 40% stock solution in 5 ml saline), rinsed in saline, and transferred to Carnoy's fixative. As much as possible of the attached cuticle, trachea and fat body were removed and the tissue was fixed for about 3 hours. After transferring the tissue through descending ethanol concentrations to distilled water, it was intensified by the method of Bacon & Altman (1977). Twenty to thirty minutes in the developer was generally sufficient.

The brains or ventral ganglia were dehydrated through ethanol, cleared in methyl salicylate, mounted in immersion oil (Cargill) and photographed or traced with a drawing tube. Selected preparations were embedded in epon-araldite, sectioned at 2.5-3.5 microns, reintensified by the method of Tyrer and Bell (1974), and photographed or traced again for more detailed examination.

B. Visualization of afferent axons with horseradish peroxidase (HRP).

HRP was used only for filling afferent axons from the legs. No HRP method tried reliably filled antennal or antennal leg afferents. The legs were filled only from the tarsi. These were placed in a wax chamber, covered with a drop of distilled water

and cut. After 5 to 10 minutes the water was replaced with a 20% solution of HRP (Sigma type VI) in distilled water. 3% alpha-lysolecithin (Sigma) was added in some cases. After 10 to 14 hours in a humid chamber, the flies were washed with distilled water to remove the HRP and transferred to a dish of saline. Procedures adapted from those given by Hanker et al. (1977) or Mesulam (1978), detailed in Green (1981), were used for visualizing HRP-filled neurons.

C. Silver staining

Silver staining was used for determining the general structure of the neuropil and for observing the course of axons from noneverted antennae in *ant* mutants. The procedure, obtained from Don Ready (personal communication), was as follows:

— Fixed for several days in alcoholic Bouin's fluid.

— Washed for about 4 hours in at least 3 changes of saturated Li_2CO_3.

— 3 hours in fresh 2% NH_4OH in 95% ethanol.

— Rinsed in 0.5M $NaNO_3$.

— 24 hours in 10% $AgNO_3$.

— Rinsed in 0.5M $NaNO_3$.

— 9 hours in ammoniated silver: Mix 7 ml 0.5M $NaNO_3$, 30 μl 40% NaOH, 130 μl concentrated NH_4OH. 10% $AgNO_3$ added dropwise, with continuous stirring, until the solution remains just slightly cloudy and a drop of it turns filter paper dark brown within 20 seconds.

— 1 hour in developer: 10 ml 9% Na_2SO_3 (fresh), 1 ml 5.5% $AgNO_3$, 0.5 ml 0.5% hydroquinone (fresh).

— Washed in distilled water, dehydrated in ethanol, cleared in propylene oxide and embedded in epon-araldite. The blocks were sectioned at 2.5-3.5 microns.

D. Scanning electron microscopy

Flies were dehydrated in ethanol, critical point dried in carbon dioxide, coated with gold and viewed in an ETEC Autoscan scanning electron microscope.

E. Genetic techniques.

1. Ectopic head bristles.

Two methods were used to produce flies with ectopic bristles on the head. In the first, flies carrying the temperature-sensitive cell death mutation ecdysone (ecd^{ts}; 3:unmapped; Garen et al. 1977) were heat pulsed during development with the following regimen (Tim Sluyter, personal communication): Eggs were collected for 12 hours at 22°. The bottles were cleared of adults and the progeny were raised at 22° for 96 hours more and then subjected to a 29° heat pulse for 84 hours. They were returned to 22° and left to pupate and eclose. In the second method, appropriate flies were selected from stocks homozygous for the mutation eyeless-Ophthalmoptera (ey^{Opt}, 4:unmapped; Lindsley & Grell 1968), the phenotype of which is described in the results.

2. Antennal legs.

Three mutations and one deficiency were used in combination to transform antenna to leg. These are Antennapedia ($Antp^{73b}$, 3:48), Polycomb (Pc^{3}, 3:48), spineless-aristapedia (ss^{a}, 3:58.5) and a deficiency for spineless, $Df(3R)sbd^{104}$ (89B5;89C7-D1). The combinations $ss^{a}/Df(3R)sbd^{104}$ and $Antp^{73b}/Pc^{3}$ were used to produce antennal legs in some early studies but much better transformations were obtained with the genotype $Antp^{73b}\ Df(3R)sbd^{104}/Pc^{3}\ ss^{a}$ which was made by selecting D^{+} progeny of the cross $Antp^{73b}\ Df(3R)sbd^{104}/DcxF \times Pc^{3}\ ss^{a}/DcxF$. The phenotypes of all of these are described in the results.

RESULTS

A. Afferent projections in wild-type (Canton-S) flies.

1. Head bristles.

The head of Drosophila is shown in figure 2. Several sensory structures can be discerned. These include the eye, the antennae, and many large and small bristles (macrochaetes and microchaetes). The positions of the macrochaetes are identical in all *Drosophila melanogaster* and many related species (Sturtevant, 1970). The central projections of those bristles labelled in figure 2 have been determined by plucking out each bristle and applying cobalt chloride solution to the socket. Fifty-six successful cobalt fills were obtained.

Even for axons coming from the same bristle, the precise morphology of the projection varies slightly from animal to animal. The most noticeable variation is that the divergence of the two branches can occur at a variety of positions. The variation is from the ventral-posterior branch emerging ventrally as a ventral collateral of the medial-anterior branch to there being a branch-point dorsal to the level of the medial-anterior projection.

a. Terminal arborization of the head macrochaetes. All macrochaetes studied have essentially identical L-shaped arborizations in the same neuropil region of the subesophageal ganglion regardless of their position on the head. That is, the variation in form or position of projection between bristles is no greater than for the same bristle in different preparations. Figure 3 shows typical examples of the arborizations of the head macrochaetes. The arborization has two parts. One, about 65-70μ from the anterior surface of the brain, is a 55-60μ long medially directed branch. The other branch, somewhat ventral to the first, runs posteriorly and is about 40-45μ long. In many cases, the medial-anterior branch, after crossing the midline, can be seen to continue on and fill out the contralateral ventral-posterior

projection.

b. Routes taken by head macrochaete axons. Although the arborizations of all the large bristles studied are the same, the routes by which the axons reach the subesophageal ganglion are not. The antennal (figure 3a), orbital (figure 3b), ocellar and postvertical bristles (figure 4) all enter the CNS by way of the antennal nerve, the antennal bristles through the main trunk of the nerve and the latter three groups through a small dorsal branch. When the antennal nerve enters the brain, the axons are at the dorsal margin but they dive ventrally through the antennal tract to emerge from the ventral edge of the tract about 70μ into the brain. They continue in this ventromedial direction to reach the target neuropil in the subesophageal ganglion. The axons divide sending one branch medially to make the anterior-medial arborization and one branch ventrally and then posteriorly to make the ventral-posterior arborization.

The vibrissae axons (figure 3c) enter the CNS through the labellar nerve and run dorsoposteriorly to reach their target neuropil. The axons divide sending one branch directly back to make the ventral-posterior arborization and one medially to make the anterior-medial arborization.

The axons of the vertical bristles (figure 3d) enter the CNS through a small nerve, the posterior tegumental nerve, which lies on the posterior wall of the head (Duncan Byers, personal communication; Strausfeld, 1976, p. 44). This nerve loops around the cervical connective and enters it ventrolaterally. The vertical bristle axons run along the ventral margin of the cervical connective turning laterally when it enters the CNS, running anterolaterally on the ventral edge of subesophageal ganglion. About 30μ anterior to the entry into the subesophageal ganglion the axons turn sharply, leaving the ventral surface of the subesophageal ganglion, and run anteromedially to reach their target neuropil, making the ventral-posterior

arborization at this point and sending a branch forward to make the anterior-medial arborization.

$CoCl_2$ fills of different bristles on opposite sides were done simultaneously to make certain that the central arborizations were indeed the same regardless of the bristle's position and the route of its axon. Figures 4a and 5 show such multiple bristle fills and prove that the terminal arborizations are nearly identical and are in the same target neuropil.

c. *Eye interommatidial bristles and other small bristles.* The interommatidial bristle axons (figure 6) all enter the subesophageal ganglion through the labellar nerve and break into two streams which converge to form a dense glomerulus in a region of neuropil just lateral and ventral to the ventral-posterior branch of the large bristle projection. Detail within this glomerulus cannot be distinguished.

The other small bristles on the head were not studied in detail. However in a few preparations microchaetes were cobalt filled and they appear to have projections very similar to those of neighboring macrochaetes (figure 4b). This applies both to the route taken to the subesophageal ganglion and to the arborization within it. Their axons can be distinguished from those of the macrochaetes by the observation that they are much thinner, sometimes visible only as lines of small dots. Ghysen (1978) has reported that neighboring large and small bristles on the notum have similar projections as well.

2. Afferent neurons of the antennae.

The antenna is both an organ of olfaction and of mechanoreception. The antennal third segment is shown in figure 2. It is covered with hundreds of sensilla of several types serving both of these sensory modalities. In general, the chemosensory bristles have several neurons at their base (Slifer, 1970). One is mechanosensory and does not send a dendrite up into the bristle but only to its base while the

other neurons are chemosensory and their dendrites, projecting into the bristle, communicate with the environment through pores in the wall of the bristle. The projections of these sensilla into the CNS were studied by making small cuts in the third antennal segment and applying $CoCl_2$ solutions to the cuts. All of the antennal sensory axons are collected into one large nerve, the antennal nerve, which runs directly back to enter the CNS.

a. Projection to the olfactory lobe. Immediately after the antennal nerve enters the CNS the olfactory axons, which are in the dorsomedial edge of the nerve, leave it to enter a large region dorsal and medial to the nerve in the extreme anterior of the CNS, the olfactory lobe (figure 7a). The establishment of this region of neuropil as olfactory is a result of electrophysiological studies in several different insect species (e.g. Boeckh et al., 1975; Suzuki & Tateda, 1974). The olfactory lobe is a spherical structure approximately 65μ in diameter. The axons of the olfactory sensilla form about 32 roughly spherical glomeruli[1] which make up the outer shell of the lobe (the core is composed of the axons of olfactory lobe interneurons). The average diameter of the glomerulus is 13μ but the values vary greatly among the glomeruli from 8 to 17μ. The glomeruli appear to be arranged in a stereotyped fashion from fly to fly, as has been reported for the olfactory lobe of the cockroach *Blaberus craniifer* (Chambille et al., 1980), however a detailed study of their organization in *Drosophila* has not been undertaken.

The sensory axons enter the olfactory lobe at its ventrolateral corner at the extreme anterior of the brain, just after the nerve enters (figure 7b). From this corner of the lobe some of the axons enter the lobe to innervate adjacent ventrolateral glomeruli. Most of the axons, however, enter a superficial fiber layer on the surface of the lobe. This covers the entire surface of the lobe and the axons travel

1. This figure is not exact but was determined by counting all of the glomeruli in sections of five brains and then applying the counting correction formula of Abercrombie (1946).

within it to a point adjacent to their target glomeruli. The axons then penetrate the lobe near their targets, turning inwards and branching to form the glomeruli (figure 7c).

For each sensory axon, an additional branch usually exists which crosses contralaterally in the antennal commissure which is posterior and dorsal in the lobes, directly opposite to the point of entry of the axons from the nerve (figure 7d). Each commissural axon connects two identically situated glomeruli in the right and left olfactory lobes. Furthermore, the axons of a single glomerulus tend to stay together in a bundle. Thus, the antennal commissure, in its fine structure, is composed of small discrete bundles of axons each associating two symmetrically situated glomeruli. In general, the dorsoventral position of a glomerulus is reflected in the position of its axon bundle in the commissure; dorsolaterally situated glomeruli project dorsally in the commissure and ventromedially situated glomeruli project ventrally. Since (1) the commissure divides after crossing the esophagus, distributing axons into the superficial fiber layer medial or dorsal to the olfactory lobe, and (2) these regions of the fiber layer are associated, respectively, with ventromedial and dorsolateral glomeruli, this may represent a mechanism for providing a preliminary, rough guidance for the establishment of specific connections between bilaterally homologous glomeruli.

Figure 7e is a tracing of the projection of a single olfactory sensory axon and demonstrates the general features of such axons: it enters the olfactory lobe from the nerve, runs dorsally and posteriorly over the surface of the lobe, and sends one branch inwards to an adjacent glomerulus and one through the antennal commissure to the same glomerulus in the contralateral olfactory lobe. In addition, small branches can be seen entering an adjacent ipsilateral glomerulus, a feature commonly, although not always, seen in these preparations.

b. Antennal mechanosensory projections. The antennal nerve continues caudally after the olfactory axons have branched off. It breaks up into four major parts shown in figure 8. This distribution of the antennal axons to different targets becomes apparent soon after the nerve enters the brain. Examination of the section through the nerve shown in figure 7c shows that even 27μ into the brain the different elements of the nerve have begun to separate. Coarse fibers can be seen laterally and ventromedially while the central part of the nerve consists mainly of finer fibers. The lateral antennal nerve fibers separate further into two bundles, one dorsal and one ventral (figure 8a).

The coarse ventromedial fibers of the antennal nerve leave the nerve in a thick bundle about 70μ from the anterior surface of the brain (figure 8b). These fibers project in a manner identical to that described above for the head macrochaetes. There is an anterior-medial arborization and a ventral-posterior arborization in the same location and having the same dimensions as those of the head macrochaete projection. This will be referred to as the antennal head bristle-like (or HB-like) projection. It is probably the same as the projection that Stocker & Lawrence (1979) have termed the L-fibers.

The dorsal bundle of lateral antennal nerve fibers projects directly dorsally, terminating about 20μ from the nerve (figures 8a-b). The ventral bundle runs ventroposteriorly, looping around the antennal nerve ventrally (figures 8a-b) and then running directly medially into the subesophageal ganglion about 95-100μ from the anterior surface of the brain (figure 8c). As the figure shows, this bundle remains distinct from, and somewhat dorsal to, the ventral-posterior arborization of the HB-like projection.

After the HB-like and lateral antennal nerve fibers leave the main bundle, some of the remaining axons continue projecting caudally and terminate in the antennal

mechanosensory area about 110μ from the anterior surface of the brain. Most of them, however, project medially just ventral to the esophagus (figure 8d) in a 12μ thick bundle that extends medially 90-95μ into the subesophageal ganglion about 94μ from the anterior surface of the brain. This element will be referred to as the medial fiber projection.

3. Afferent neurons of the tarsus.

Like the antenna, the tarsus of the leg has two sensory functions: mechanosensory and gustatory. However, the sensory projection from the tarsus is much simpler than that of the antenna, having only a single element, and gives no anatomical clue as to how these functions are separately processed. Furthermore, cobalt fills from the tarsus and the tibia show closely similar sensory projections even though the tibia does not appear to have gustatory sensilla (S. Green, unpublished observations).[2] The dipteran ventral ganglion is formed from the condensation of all of the thoracic and abdominal segmental ganglia into a single mass. The segmental organization is preserved within the ganglionic mass as each of the thoracic segments is represented by a discrete bilaterally symmetric region of neuropil termed a neuromere. The abdominal ganglia are not easily distinguishable from each other but they are represented by an abdominal neuromere at the caudal end of the ventral ganglion.

The projections of the tarsal sensory fibers into the thoracic neuromeres, from cuts made in the distal tarsus (3rd, 4th or 5th tarsal segment), are diagrammed in figure 9. A large leg nerve enters ventrally in each neuromere and from it the sensory axons project into the neuromere. The form of the projection is identical in

2. That there are no gustatory sensilla on the leg, other than those on the tarsus, was established by amputating the tarsus and touching the stump with sucrose solutions. Touching the tarsus with a 0.2M sucrose solution will always elicit a proboscis extension response in flies deprived of food overnight. This reflex is not obtained if legs without the tarsus are tested. Furthermore, observation of the legs with the scanning electron microscope did not reveal any chemosensory bristles on the tibia or femur.

each neuromere: immediately after entering the neuromere the projection branches to form a U-shaped pattern. It appears that most individual axons do not branch at the point at which the divergence begins so that this U-shaped pattern is a property of the population and not of the individual axons. Some axons do however contribute to both branches of the U but these collaterals appear slightly farther into the ganglion than the main divergence. It would be of some interest to know if differences in the central projection of the individual axons could be correlated with their position on the tarsus or their modality as is true, for example, for cricket cercal sensilla (Murphey et al., 1980) but this analysis has not yet been undertaken.

Although the U-shaped pattern is constant, the orientation differs in each of the three thoracic neuromeres. In the prothoracic neuromere, the nerve enters the anterolateral corner of the neuromere and the axonal branches extend medially. In the meso- and metathoracic neuromeres, the nerve enters posteriorly and the branches extend anteriorly. The branches do not completely fill the neuropil; the motorneuron arbor occupies the lateral neuropil in the meso- and metathoracic neuromeres and the anterodorsal neuropil in the prothoracic neuromere.

Tarsal sensory neurons do not project into neuromeres other than that into which they enter from the nerve nor do they project contralaterally or into the brain. All cases observed in these preparations of such elements were a result of transneuronal filling of central neurons by $CoCl_2$ or HRP. These features of the tarsal sensory neurons all markedly differ from the features of olfactory and mechanosensory antennal neurons which project contralaterally or into more than one region of neuropil.

B. Afferent projections in mutant flies.

1. Ectopic head bristles in ecd^{ts} and ey^{Opt} flies:

a. ecd^{ts} flies. Although *ecd^{ts}* was originally isolated as a mutation affecting ecdysone synthesis (Garen et al., 1977), it behaves as a temperature-sensitive cell death mutation (Tim Sluyter, personal communication). and as such can produce duplications of cuticular structures (Girton & Bryant, 1980). The mortality rate is extremely high following the heat pulse so only 10 successfully filled preparations could be obtained. All of these preparations had a greater or lesser portion of the anterodorsal eye missing and replaced with cuticle bearing large bristles. The wild-type head macrochaetes closest to these supernumerary bristles were the orbitals. In all 10 preparations the projection pattern resembled that of the orbital bristles in all respects, a conclusion reinforced in 4 preparations in which a fill was made from a contralateral wild-type bristle (figure 10).

b. ey^{Opt} flies. *ey^{Opt}* is a homeotic mutation which transforms eye to dorsal thoracic cuticle and wing (Lindsley & Grell, 1968). In effect, this mutation transplants bristles from the thorax to the head. The bristles, shown in figure 11a,b are of two types: large ones anterior to the eye, much like those resulting from the *ecd^{ts}* mutation, or small bristles on a knob growing out of the eye (shown in detail in figure 11b). These latter small bristles are similar to the triple row bristles on the anterior wing margin (figure 11c) and in many *ey^{Opt}* animals the knob resembled a small wing.

The bristles of the wing anterior triple row are of three types: doubly-curved bristles found in the dorsal row and interspersed among the sharp tipped bristles that comprise most of the bristles of the ventral row and stout blunt-tipped bristles of the middle row. The sensory neurons of these bristles were studied by Palka et al. (1979) who reported that the sharp-tipped and blunt-tipped bristles were purely mechanosensory while the doubly-curved bristles appeared to serve both mechano-

and chemosensory roles.

The results of cobalt fills of ectopic bristles on the head of 44 ey^{Opt} flies fall into three general patterns. In the first two, exemplified by the camera lucida tracings shown in figure 12, the axons did reach the target of the normal head macrochaete projection in the subesophageal ganglion. They did so by using any of the nerves (described above) used by the normal head macrochaetes, and in three of the preparations in which several adjacent bristles were removed, filled axons were seen in more than one nerve (e.g. figure 12d). The pattern of projection did not seem to be correlated with the type of ectopic bristles filled, triple row-like bristles or large ectopic bristles, with one exception mentioned below. In the first pattern, observed in 14 preparations, the arborization pattern was similar to that of the large head bristles (figure 12a). In all of these cases the anterior-medial branch was shortened, a phenomenon only rarely seen in the wild-type fly. This type of projection was never seen in cases where the fill was unambiguously made only from triple-row like bristles.

The second type of pattern, seen in 20 preparations, was a disorganized arborization in the subesophageal ganglion (figure 12b-d) and sometimes adjacent parts of the supraesophageal (figure 12d). Although these disorganized projections differed in detail, there were some general features: the initial part of each projection followed the same initial route as a normal head macrochaete. Compare, for example, the two bristles shown in figure 12c which both enter the CNS in the posterior tegumental nerve and which have identical projections initially until the region of normal head macrochaete arborization is reached. Furthermore, in most of the cases of this pattern, the projection entered the dorsal subesophageal ganglion directly or began in the ventral subesophageal ganglion, the region of normal head macrochaete arborization, and spread to the dorsal subesophageal ganglion, suggesting that the dorsal subesophageal ganglion may be a preferred target of these neurons.

In only a single case did axons enter the supraesophageal ganglion directly, in this case the ventrolateral protocerebrum, without being initially routed through the subesophageal ganglion (figure 12a).

The third type of pattern occurred in ten preparations and is exemplified by the camera lucida tracings shown in figure 13. In these cases the axons entered the optic lobe rather than the subesophageal ganglion. They entered the optic lobe initially running parallel with the retinula cell axons. In most cases they followed the external chiasm into the medulla. The thoracic bristle axons followed the general lateral to medial course of the intrinsic optic lobe neurons between the neuropil layers, sending out branches orthogonally which ramified through the lobula or the medulla. In two preparations the axons did not penetrate the optic lobe neuropils but diverged from the retinular axons and ran around the lobe anteriorly just under the cortex. In no case did the axons form a projection like that of head bristles or of retinula cell axons nor did they leave the optic lobe.

2. Antennal legs.

Figure 14 shows the (homeotically) transformed antennal legs in the three genotypes used in this study, and a wild-type (Canton-S) leg for comparison. In ss^{a}/sbd^{104} flies (figure 14a) the third segment (flagellum) is reduced in size and the distal three segments of a tarsus, with a full complement of tarsal bristles, emerges from the distal end. There is no arista but there are a large number of antennal sensilla still present on the flagellum. The Antennapedia phenotype is quite different from that of ss^{a}. Since *Pc* also transforms antenna to leg and enhances the *Antp* phenotype (Lindsley and Grell, 1968, p.186) the two mutations were used together. Figure 14b shows the phenotype of such an $Antp^{73b}/Pc^{3}$ fly. While ss^{a} affects only the distal antenna, *Antp* affects the entire appendage and nearby head cuticle. However, as Postlethwaite & Schneiderman (1971) reported, the transfor-

mation is weakest in the proximal and distal extremities of the antennal leg. Thus, there is visible on the coxa of the antennal leg a protuberance which bears antennal bristles, a small remnant of untransformed antenna. Also, the tarsus of the antennal leg is poorly transformed and terminates in an arista-like structure. Incomplete transformation at distal structures may explain why at least one *Antp* allele ($Antp^{B}$) fails to demonstrate a proboscis extension reflex when its antennal leg tarsus is touched with a a sucrose solution (Deak, 1976).[3]

The incomplete expression in the foregoing genotypes presents certain difficulties: First, I have found that $CoCl_2$ solutions can spread through an appendage when applied to a cut and be picked up and transported by intact neurons. Their axons will then appear to have been cobalt filled as if they had been cut. This type of filling can often be distinguished from the filling of cut axons by the fact that it is grainy, presumably because the uptake of the $CoCl_2$ resulted in its having been vesicularized and transported in that form, but it can be a cause of confusion nonetheless. It was therefore desirable to completely eliminate the antennal olfactory sensilla so that one could be sure that the projection pattern seen from an antennal leg had no real olfactory component. This is particularly important in interpreting results in which the mutant patterns are similar to those of the wild-type. Second, it is conceivable that any similarities between the wild-type and mutant projections could be a result of pathways laid down during development by wild-type antennal pioneer neurons should some untransformed antennal tissue remain in the appendage. Third, the tarsus and the antenna share one sensory modality, that of mechanoreception, while they each have one modality apparently not possessed by the other, olfaction in the antenna and taste in the leg. It was of interest to study the projections of a completely novel type of sensillum in these homeotic mutants, in this case the taste sensilla present on the transformed

3. The $Antp^{73b}$ allele used in this study and that of Stocker et al. (1976) does demonstrate a proboscis extension reflex when its proboscis is touched with a drop of sucrose solution (Stocker, 1977).

antennal leg. The fact that the tarsus is poorly transformed in $Antp^{73b}/Pc^{3}$ flies might mean that the taste sensilla are not present or are abnormal in some way so that the sensory projection from the antennal leg tarsus in these flies does not accurately portray their contribution. Finally, it is a tacit, and still unproven, assumption in this and other studies of sensory neurons in homeotic mutants that the neuron is affected by the gene in the same way as the surrounding cuticle. It is therefore desirable to have the best possible expression of the mutant phenotype so that even if the sensory neuron has a higher threshold to the mutation's effect than the surrounding cells it will still be affected in some way. The $Antp^{73b}$ $Df(3R)sbd^{104}/$ Pc^{3} ss^{a} genotype was constructed in an attempt to circumvent these problems and its phenotype is shown in figure 14c. The entire antenna has been transformed into a small leg and no olfactory receptors appear on the appendage.

In all cases the sensory axons are gathered into a single nerve which resembles a leg nerve in its ultrastructural details (Stocker, 1979). This runs back into the brain as does the wild-type antennal nerve. In the brain the projection resembles the wild-type antennal projection in that it is divided into two major parts, one innervating the olfactory lobe and the other to the antennal mechanosensory area. In its details, however, the first part, the projection to the olfactory lobe, is quite different in the mutants.

a. Projection to the olfactory lobe.

i. ss^{a}/sbd^{104} *flies.* Axons filled from cuts in the distal tarsal segment of the antennal leg formed an irregular arborization in the olfactory lobe, dense near the entry and becoming sparser with increasing distance into the lobe. (figure 15a). Stocker and Lawrence (1981) reported similar results from their studies of ss^{a} flies. If the cut includes part of the proximal antennal leg, a glomerular pattern of arborization

can be seen (figure 15b), superimposed on the previous pattern, presumably from the untransformed portion of the appendage. Since the remaining olfactory sensilla can still make correct arborization patterns in this mutant, the irregular pattern seen in this study and in that of Stocker and Lawrence is not a result of a putative central effect of the mutation.

There is one additional point to be made about this mutant combination. Unfortunately, the spineless locus, of which ss^a is an allele, is complex and has at least two additional effects on the fly of unknown relationship to the homeotic effect. One is the spineless phenotype itself, a reduction in the size of the bristles, which is not strong in ss^a homozygotes but is noticeable in ss^a/sbd^{104} and other combinations of ss^a with *ss* deficiencies and breakpoints (S. Green, unpublished observations). This can be seen in figure 14a. The second is an effect on tarsal development, affecting leg and antennal leg tarsi, which can result in distorted tarsi with swollen, reduced, missing or fused segments. Again, it is not a strong phenotype in ss^a/ss^a flies but it sometimes is in ss^a/sbd^{104} flies. To control for these pleiotropic effects of the mutation, fills were made from the distal tarsus of the legs of ss^a/ss^a, ss^a/sbd^{104} and $In(3R)ss/sbd^{104}$ flies. The sensory projections from these preparations differed in no way from those of wild-type flies (nor did the morphology of the motorneurons). Therefore the use of ss^a in combination with deficiencies should present no problems as a result of pleiotropic effects.

ii. $Antp^{73b}$ $Df(3R)sbd^{104}$/ Pc^3 ss^a flies. In flies of this genotype, all bristles filled from the transformed antennal leg are leglike so far as can be determined by scanning electron microscopy. Two types of fills were done from this appendage. For the first, fills were made from cuts in the distal tarsus (4th or 5th tarsal segment). For the second, the entire tarsus was removed and after allowing at least a week for the tarsal axons to degenerate, fills were made from the distal tibia. Since only the tarsus bears taste sensilla, these procedures should allow determination of which

component of the projection pattern in the mutant was a result of the tarsal taste sensilla and which of the mechanosensory sensilla. In fact, the rather surprising result was that there was no significant difference between the results.

The sensory projection into the olfactory lobe from the *Antp*^{73b} *Df(3R)sbd*^{104}/ *Pc*^{3} *ss*^{a} antennal leg is shown in figure 16. The first difference from wild-type encountered is in the way that the axons enter the olfactory lobe. Rather than entering only at the anterior surface of the lobe, they enter all along the ventrolateral edge wherever the nerve and optic lobe neuropil appear to be in contact.

As in the *ss*^{a}/*sbd*^{104} flies that were filled from the distal tarsus, there is no glomerular pattern visible. All of the axons appear to branch irregularly through the olfactory lobe. They ramify indiscriminately throughout the entire olfactory lobe, unlike antennal olfactory axons which are restricted to the glomeruli on the periphery of the lobe and do not penetrate into its core. The only similarity to the wild-type olfactory projection is that many axons do travel for some distance in a superficial fiber layer on the surface of the olfactory lobe. This, however, does not seem to apply to as high a fraction of the axons as it does in the wild-type and represents a tendency rather than a requirement. In the wild-type fly, all of the axons that do not enter glomeruli near the entry from the nerve are routed into the superficial fiber layer. In the mutants, most of the axons travel long distances within the olfactory lobe neuropil.

The number of axons and branches is highest near the entry to the lobe and gets smaller with increasing distance from it. The same applies to axons crossing in the antennal commissure posteriorly. Their axons tend to penetrate a only short distance into the contralateral olfactory lobe so there is a lower probability of finding them in the anterior or ventral parts of the olfactory lobe. The number of contralaterally projecting axons is much lower in *Antp*^{73b} *Df(3R)sbd*^{104}/ *Pc*^{3} *ss*^{a} flies than in

wild-type. This, presumably, is because the axons are projecting randomly so that fewer of them will get to the dorsal posterior region of the olfactory lobe where the commissure is, and fewer of those that do get there will actually enter the commissure. Furthermore, there are many fewer axons to begin with since the terminal two tarsal segments have many fewer bristles between them than the third antennal segment and antennal bristles are nearly all multiply innervated (Slifer, 1970). None of the fills from antennal leg tibial bristles showed a contralateral component to the antennal lobe projection. The significance of this is not clear. It probably results from the smaller number of fibers filled in these preparations and the consequently smaller chance of seeing an axon projecting contralaterally. It is conceivable, though, that it represents a real difference between tibial (or mechanosensory) and tarsal (or taste) neurons.

In addition to the fact that the antennal leg projection does not, in its details, resemble that of an antenna, it does not resemble that of a leg either. Contralateral projections and projections into adjacent regions of neuropil (see below) do exist and the axons do not branch in the U-shaped manner characteristic of tarsal sensory axons.

iii. Adventitious projections. There is another important difference between the antennal and antennal leg projections into the antennal lobe. Unlike the wild-type olfactory lobe projection, the projections of the antennal leg mutants send branches, adventitious projections, outside the limits of the olfactory lobe and into adjacent neuropil. These adventitious projections were observed in all of the three genotypes studied: $Antp^{73b}/Pc^{3}$, ss^{a}/sbd^{104}, or $Antp^{73b}\ Df(3R)sbd^{104}/\ Pc^{3}\ ss^{a}$, and are generally similar. The most thorough study, however, was done in the $Antp^{73b}\ Df(3R)sbd^{104}/\ Pc^{3}\ ss^{a}$ fly because of the superior phenotype and because it was possible to compare the projections of mechanosensory bristles alone to those of the full tarsal complement. The following description is therefore based largely

on *Antp*^{73b} *Df(3R)sbd*^{104} / *Pc*^{3} *ss*^{a} flies.

The most common of these adventitious projections is a stream of axons from the ventromedial edge of the olfactory lobe into the subesophageal ganglion (figure 16a,b). In detail, this stream appears to be made up of two components, anteroventral and posterodorsal, although there is overlap between them. The first component is a group of axons that leaves the olfactory lobe and projects in a ventromedial direction into the tritocerebrum. It is seen at the anteriormost point at which these neuropils are in contact, starting about 30-35μ from the anterior surface of the brain (figure 16a). These axons ramify irregularly in the anterior tritocerebrum and anteroventral subesophageal ganglion with many axons running in the neuropil immediately under the anterior and ventral cortex of these regions of the brain. Posteriorly they can merge dorsally with the axons of the posterodorsal bundle or even, in a few cases, merge more ventrally with the anteriormost part of the HB-like arborization. The second component comprises another diffuse bundle of axons, about 10μ posterior to the first component, that projects directly medially into the dorsal subesophageal ganglion (figure 16b). Although some of these axons ramify irregularly and there is certainly mingling of axons from the two bundles, most of the axons from this posterodorsal group remain in a discrete region of neuropil in the dorsal subesophageal ganglion about 50μ from the anterior surface that stretches between the ventromedial borders of the olfactory lobes of the two sides. In fact, in two preparations, axons could be found that ran across the antennal commissure to the contralateral olfactory lobe and then left it in the posterodorsal adventitious projection to mingle with the mass of axons that entered this region of the subesophageal ganglion neuropil from the other side. In no cases could axons be found that left the contralateral olfactory lobe in any other adventitious projection.

Adventitious projections into the tritocerebrum and subesophageal ganglion come from the olfactory lobe in all preparations observed. In some they were joined by a bundle of axons coming directly from the antennal nerve (figure 16c). These axons enter the subesophageal ganglion much closer to the anteroventral bundle from the olfactory lobe than to the posterodorsal bundle. It is not surprising then that the axons from this bundle tend to enter the same anterior tritocerebral targets as do the anteroventral axons. Some axons can be seen, though, to enter the posterodorsal target neuropil as well as can be seen in figure 16c.

The final adventitious projection from the olfactory lobe is dorsal, entering the protocerebrum. Unlike the projections into the subesophageal ganglion this does not occur in every preparation. Generally these axons extend only 10 or 20μ into the protocerebrum but in a few preparations they may enter any fiber tract and follow it for fairly long distances.

These adventitious projections do not appear to be the result of differences between different types of axons. First, all four types of adventitious projections occurred in flies from which the fills were made from tibial bristles when the tarsus had previously been removed. In fact no differences in the relative fraction of fibers entering any part of the total projection pattern were seen when comparing fills made from tarsus and fills made from the tibia. Therefore choice of pathway does not appear to be modality-specific for these ectopic fibers. Second, in many suitable preparations, individual axons could be followed from the nerve and into the CNS. Many of them could be seen to branch and send collaterals into the olfactory lobe and into the subesophageal ganglion.

b. Antennal leg projections into the antennal mechanosensory area. Figure 17 shows the various components of the projection into the antennal mechanosensory area in $Antp^{73b}$ $Df(3R)sbd^{104}$ / Pc^{3} ss^{a} flies. Comparison with the wild-type shows

that the projections are nearly identical although the number of axons is generally smaller in this mutant. This is probably because the distal tarsal segments have many fewer sensilla than does the flagellum and is compounded by the fact that a much larger proportion of the axons enter the olfactory lobe in the mutant than do in the wild type.

There is no definite answer to the question of whether only mechanosensory neurons project into the antennal mechanosensory neuropil. Clearly mechanosensory neurons of the tibia can enter the olfactory lobe and the antennal mechanosensory area. It is not clear whether tarsal chemoreceptors can enter both areas as well. Since the majority of sensory neurons on the tarsus are chemosensory (Grabowski and Dethier, 1954), it might be that the appearance of the antennal leg tarsal projection reflects their contribution but this needs to be shown by filling individual chemoreceptor bristles.

3. Misplaced antennae.

The mutation *ant* (Lindsley & Grell, 1968, p. 15; Begg & Sang, 1945) can prevent the development of one or both antennae. Although Begg & Sang (1945) reported that the development of the antenna was completely blocked in affected discs, this no longer appears to be the case. *ant* homozygotes can be found with a range of phenotypes from no antennal development at all to wild-type antennae. This includes small, noneverted or partially everted antennae with no correlation in phenotype observed between the two sides of the head. Three animals that were being used for another investigation had an antenna that had failed to evert and appeared as an inside-out vesicle within the head. The axons could be followed in silver-stained preparations (figure 18). They coursed over the cortex or through the cortex and neuropil to reach the approximate site of the antennal nerve. From there axons could be seen entering normal antennal targets although a detailed

study could not be made without cobalt fills.

4. Abdominal legs.

In *Drosophila*, the genes of the bithorax complex control segmental determination in the thorax and abdomen (Lewis, 1978). Mutations of the bithoraxoid gene, a member of this complex, result in a transformation of the first abdominal segment to a metathoracic segment. In many cases the results in flies having four pairs of legs in which the first abdominal pair bear a full complement of normal leg sensilla (figure 19a). The central projections of the tarsal sensilla of these supernumerary legs were filled with HRP in the same manner as those of the thoracic legs. The genotype of the flies used was bxd^{51j} / *Df(3)P2*, which reliably provides flies with eight complete legs.

The abdominal neuromere in *Drosophila* has two bilateral pairs of thin nerves for the first two abdominal segments and a fifth nerve, emerging from the posterior tip of the neuromere, which branches into the other abdominal segments. In bxd^{51j} / *Df(3)P2* flies, the first abdominal nerve is considerably thicker than in the wild-type fly and resembles a leg nerve. The sensory axons enter in this transformed first abdominal nerve, bypass the abdominal neuromere completely, and project into the metathoracic leg neuropil (figure 19b,c). The form of the projection is generally like that of a metathoracic leg (figure 19d), however in 17 of the 51 preparations studied, the projection did not form a complete U-shaped arborization but had only the bundle of axons on the medial side of the normal projection represented (figure 19c). Comparison of these preparations with reduced silver stained ganglia suggests that in these cases the axons were restricted to the dorsal cervical fasciculus.[4] The first abdominal nerve, in which these axons enter, is medial to the metathoracic leg nerve. It is likely that the axons from the supernumerary abdominal leg can

4. This is the *Drosophila* analogue of the insect longitudinal connectives which run from anterior to posterior and associate the ventral chain of segmental ganglia. The *Drosophila* terminology is from Power (1948).

become routed into the medially located dorsal fasciculus and are not able to make the appropriate leglike arborization pattern in the metathoracic leg neuropil. Nevertheless, in the majority of the preparations studied this is not the case and the axons do enter and branch properly in the metathoracic leg neuropil.

C. Transneuronally filled central neurons.

Many of the cobalt-filled preparations had arrays of central neurons that had been stained by the cobalt in addition to the sensory axons. Presumably this occurred as a result of the uptake of cobalt leaked from the sensory axons. This phenomenon, first described by Strausfeld and Obermayer (1976), is a serious problem in the interpretation of data obtained by the cobalt method. Unless extreme care is taken it may be possible to confuse these transneuronally stained axons with the axons that were originally cut. The data and conclusions presented here on the projection patterns of sensory neurons is based only on preparations in which it was possible to exclude the possibility of the presence of transneuronally filled neurons. Any one of the following three criteria were used for their exclusion: First, any axons with visible cell bodies are clearly central since the cell bodies of the sensory neurons are peripheral and there are no muscles in the antennal third segment or the antennal leg for motorneurons to innervate. This was the primary criterion and the other two were subsequently used when their correlation with it was established. Second, cobalt taken up transneuronally tends to be vesicularized so the stained central neurons have a grainy appearance in sections unlike the unbroken appearance of the sensory axons. Third, transneuronally stained axons and cells are often brown instead of black in sections.

The transneuronally filled cells are of some interest in themselves after it can be established which axons are sensory and which are not. This is because of their reproducibility. Figures 20 and 21 are diagrams of the transneuronally filled cells

commonly observed in antennal or antennal leg preparations. Most of these are seen in all of the preparations in which transneuronally filled cells are seen at all and have little variability in their appearance between preparations. They are briefly described here for two reasons: first, their regularity of appearance in antennal cobalt fill preparations suggests that they are associated with the central processing of the antennal inputs.[5] Their anatomy then may be of some value to the understanding of the antennal sensory apparatus. Second, the same sets of transneuronally filled central neurons appear in cobalt filled antennal and antennal leg preparations. Even if they are not functionally associated with the primary sensory antennal axons, they are certainly spatially associated. They serve as a precise marker of the location of the sensory axons within the neuropil and therefore confirm that not only do antennal and antennal leg sensory neurons project into the same neuropil but that they are even in close proximity to the same central neurons.

The olfactory lobe projection from the antenna is associated with a thick bundle of central axons that ascend the antennoglomerular tract to the dorsal posterior protocerebrum (figure 20). The cell bodies of these neurons lie in the cellular cortex lateral to the olfactory lobes. Their dendritic arbors within the olfactory lobe are obscured by the sensory axons. The central axons terminate in a dorsal lateral area but, just medial to this, they send a mass of collaterals into the calyx of the mushroom body. Small numbers of axons can sometimes be seen running through a small commissure to the contralateral mushroom body calyx. The precise function of these protocerebral areas is unknown but anatomical evidence derived from reduced silver (Weiss, 1974) as well as electrophysiological evidence (Suzuki &

5. I do not mean to imply that the transneuronally filled cells are actually postsynaptic to the primary sensory neurons. The reasons for this phenomenon are not yet known and I am aware of no evidence that synapses are required for transneuronal transport of $CoCl_2$. It is only their presence in close proximity to the primary sensory axons and their arborization in the same neuropil that suggests they are functionally related.

Tateda, 1974) suggests that the mushroom bodies are responsible for processing olfactory information. A similar projection from the olfactory lobe to the two different regions of the protocerebrum has been described in the cockroach (Boeckh et al., 1977) and is suggested by the electrophysiological study by Suzuki & Tateda (1974). In addition to this, a small number of thin axons ascend laterally from the antennal nerve to the dorsal lateral protocerebral area in which the antennoglomerular tract axons terminate. Their cell bodies have not been determined.

In nearly every transneuronal preparation a small cluster of cell bodies (usually 6) can be seen in the anteroventral cellular cortex (figure 20). Their axons project dorsoposteriorly and then divide with one branch running medially into the dorsal bundle of lateral antennal nerve fibers and the other dorsoposteriorly into the ventrolateral protocerebrum.

Figure 21 is a diagram of several groups of transneuronally filled axons that cross in various commissures and associate the antennal mechanosensory areas of the two sides. These will be discussed in a ventral to dorsal order. Just posterior to the ventral bundle of lateral antennal nerve fibers, and paralleling their course closely, are two thick bundles of axons associating the ventral portions of the antennal mechanosensory areas of the two sides. They are not discrete, nor are there always two clearly visible bundles; axons can be seen to cross from one to the other. Branches turn ventroposteriorly to the cell bodies in the cortex. Some cells seen in the cortex lateral to the antennal nerve may also contribute axons to these bundles.

A large axon crosses these bundles dorsally, paralleling somewhat the route of the medial fiber projection from the antennal nerve. This axon branches after crossing the midline, one branch entering and ramifying in the ventral antennal

mechanosensory neuropil, and the other entering and ramifying in the dorsal antennal mechanosensory neuropil. Just ventral to the esophagus is the last and dorsalmost of these ventral commissural axons. It is a large axon that associates the antennal mechanosensory neuropils of the two sides. These last mentioned large axons probably have cell bodies in the group shown in the cortex lateral to the antennal nerve. This is not certain because the dendritic arborization of these cells within the antennal nerve is obscured by the filled sensory axons.

The transneuronally filled axons that cross dorsal to the esophagus originate from 4-5 very large ($\simeq 10\mu$ in diameter) cell bodies and one cell body about 20μ in diameter in the cortex at the posterior surface of the brain. They each send an axon forward which arborizes in a region dorsal to the antennal mechanosensory area. The 20μ cell body is the only one of this size in the posterior cortex which is also true of the cell body of the giant fiber (Mark Tanouye, personal communication). This, and the morphological similarity of the axon and branches within the brain and the ventral ganglion (the latter not shown here) of this transneuronally filled cell and of the giant fiber (described in Koto et al., 1981 on the basis of fluorescent dye injections) suggest that they are the same cell. This is of some practical value because the giant fiber can be impaled by microelectrodes. If the antennal inputs actually do synapse on the giant fiber, differences in between wild-type flies and those with antennal legs can be determined in terms of electrophysiological properties of the synapses. Usually the giant fibers of both sides can be seen regardless of which antenna was cut. This is probably a result of the electrical coupling between the two fibers (Koto et al., 1981) but may mean that both fibers arborize in the antennal mechanosensory areas of the two sides. From the dorsal arborization, the giant fiber branch destined for the ventral ganglion descends ventroposteriorly to the cervical connective. In a few preparations a process can be seen running laterally from the dorsal area to the lobula in which it branches.

Although it appears to come from the giant fiber, Koto et al. do not report such a process. Therefore, unless lucifer yellow does not enter this branch, it must be part of one of the other cells that arborize in the dorsal area.

There are two contacts between this dorsal arborization and the antennal mechanosensory area, both of them just posterior to the olfactory lobes. Laterally, a mass of fine branches extends ventrally to associate the two regions. This is probably part of the giant fiber arborization. More medially, a thick bundle of axons run anteroventrally to enter the antennal mechanosensory area with branches that run dorsally to enter the central commissure. Giant fibers axons and the other axons all cross in the central commissure and arborize in the same dorsal region on the other side of the brain that they do ipsilateral to the cut antenna.

DISCUSSION

A. Anatomy of the projections studied.

1. Wild-type projections.

a. Head bristle projections. I have found that the head macrochaetes in *Drosophila* enter the brain by a variety of routes yet they all have an identical branching pattern in the subesophageal ganglion. This consists of an ipsilateral posteriorly directed branch and a medially directed branch which usually runs contralaterally. The interommatidial bristles all terminate in a single ipsilateral glomerulus in the subesophageal ganglion.

In the locust, head bristles have been shown to be mechanosensory by ultrastructural criteria (Guthrie, 1964) and, more specifically, to function as wind detectors for flight (Weis-Fogh, 1949). The interommatidial microchaetes in the housefly, *Musca*, (Chi & Carlson, 1976) and in *Drosophila* (Perry, 1968), also appear to be mechanosensory. Stimulation of these bristles is necessary for triggering an eye cleaning reflex in the cricket (Honegger et al, 1979) and praying mantis (Zack & Bacon, 1981). They also serve as wind detectors for flight in the honeybee (Neese, 1965). The projection of these various head bristles have only been studied in a few species. The central projections of the wind hairs have been investigated in the locust, *Schistocerca gregaria*, (Bacon & Tyrer, 1979; Tyrer et al., 1979). Three different projection patterns were found and the head hairs could be divided into three fields on the head according to their central projection, in contrast to *Drosophila* in which all head hairs have identical central projections. In locust, axons of all three fields enter by the dorsal tegumental nerve except for some field B axons which enter by the ventral tegumental nerve. As in *Drosophila*, there is no difference in the central projection between axons that have entered through different nerves. Unlike *Drosophila*, all *Schistocerca* head hair axons have a small branch in the trito-

cerebrum before arborizing in the subesophageal ganglion and many axons in locust continue on and arborize in the prothoracic ganglion and those of field C reach the mesothoracic ganglion. This does not necessarily argue against the homology of the projections. In locust, the head hair axons synapse on interneurons in the brain which project into the thoracic ganglion (Camhi, 1969). In *Drosophila* the interneurons may obviate the need for descending collaterals of the primary sensory neurons. Within the subesophageal ganglion and the prothoracic ganglion, field A and field C axons have a medial branch which crosses the midline, as well as a posteriorly directed branch. Only field B axons have an entirely ipsilateral arborization. Within the subesophageal ganglion then there is a rough similarity between *Drosophila* and locust head hairs.

The interommatidial bristle projections have been studied in the praying mantis, *Sphodromantis lineola*, (Zack & Bacon, 1981) and the cricket, *Gryllus campestris*, (Honegger, 1977). The projections in these two species are roughly similar: a tuft in the tritocerebrum; a dense, ipsilateral projection in the subesophageal ganglion (a few medially directed fibers in the mantis cross the midline); and some fibers continuing on to the prothoracic ganglion. This pattern is quite unlike that in *Drosophila* and if they are functionally homologous it must again be supposed that the function of the descending projection is, in *Drosophila*, assumed by interneurons. Another difference between *Drosophila* and the cricket is that in the latter the interommatidial bristle projection is apparently the same as that of the wind-hairs while in *Drosophila* it is not.

b. Antennal projections. The projection of the antenna is composed of the axons of both olfactory and mechanosensory sensilla and is summarized in figures 22, 24 and 25. It consists of a bilateral projection in the olfactory lobe organized into glomeruli (figure 22), and a mainly ipsilateral projection into the antennal mechanosensory area. This in turn can be divided into three parts, the most

anterior is identical to the projection of the head macrochaetes (figure 24), the next consists of coarse lateral antennal nerve fibers which project dorsally or ventromedially (figure 25) and most posteriorly there is a medially directed projection of the fine medial antennal nerve fibers (figure 25).

There is some disagreement between this study and that of Stocker and Lawrence (1981) who do not include the projection of the lateral antennal nerve fibers in their figures; they reported neither the dorsal nor the ventral branches. This discrepancy cannot be readily explained. They may have seen the projection but considered it a transneuronally filled group of axons and not a primary projection. However, I have concluded that they are primary sensory projections by using the criteria established above and in addition, in a few suitable preparations, it was possible to trace individual axons from the lateral fiber group into the antennal nerve.

The projections of the antenna into the brain and particularly the olfactory lobe have received a great deal of attention in many species. Studies done with reduced silver are useful for establishing the general anatomy of the central neuropil. However, reduced silver is not generally useful for establishing the precise morphology of specific sensory projections or central connections because of the difficulty in following individual fibers within a dense neuropil through several successive sections. There are several studies available on the sensory projections from the antenna of various insects that have been done with Golgi, degeneration, or cobalt methods which permit the tracing of fibers or tracts of fibers through the neuropil. Some of these are that of Pareto (1972) in the honeybee, *Apis mellifera*, Boeckh et al. (1977) in the cockroach, *Periplaneta americana*, Ernst et al. (1977) in *Periplaneta americana*, and the locust, *Locusta migratoria*, Gewecke (1979) in *Locusta migratoria*, Strausfeld (1976) in the housefly, *Musca domestica*, Stocker et al. (1976) and Stocker and Lawrence (1981) in *Drosophila*.

These studies have had results rather similar to those described above for *Drosophila* as regards the gross organization of the projection. The olfactory lobe projection in all insects studied is essentially the same except that in the non-Dipteran insects the glomeruli are better defined as they tend to be separated by central neuropil. The antennal mechanosensory projections have been investigated in detail in *Apis* (Pareto, 1972), *Locusta* (Gewecke, 1979) and *Drosophila* (Stocker et al., 1976; Stocker & Lawrence, 1981). The non-*Drosophila* studies both show a branch of the projection entering the subesophageal ganglion from the antennal mechanosensory area and a branch entering the posterior protocerebrum from the antennal mechanosensory area. Gewecke has tentatively concluded that the two branches are homologous in the two species. This may be true although the evidence is not compelling. Nevertheless, the subesophageal projection described by Gewecke in *Locusta* is identical with the field B head hair projection of the closely related *Schistocerca*. Since the head hairs are wind receptors and antenna have also been shown to be wind receptor organs (Gewecke, 1972), it is tempting to speculate that this region of the subesophageal ganglion functions in the processing of airspeed information for the control of flight. In *Drosophila* I have found two projections into the ventral subesophageal ganglion of which one, the HB-like projection (figure 24), is identical to the head bristle projection (and, incidentally, is similar in appearance to the subesophageal ganglion projection from the antenna that Pareto described, in that both an ipsilateral and a contralateral component are seen). This projection then is probably homologous to the subesophageal ganglion projection in *Locusta* and therefore suggests that this region of neuropil processes airspeed information. The medial fiber projection and the projection of the lateral antennal nerve fibers of *Drosophila* (figure 25) have no obvious homologies in other insects. In addition, no protocerebral projection was found in *Drosophila*.

c. Tarsal projections. The tarsal projection appears to be a U-shaped projection into the medioventral part of each neuromere. The results reported here are in agreement with the degeneration studies of Geisert and Altner (1974) on the tarsus of the blowfly, *Phormia terraenovae*, and of Lamparter et al. (1969) on the prothoracic leg of the wood ant, *Formica lugubris*, in spite of the difference in the organization of the ganglia between Diptera (fused thoracic ganglia), on one hand, and the ant (unfused), on the other. Both studies reported primarily ipsilateral and ventral arborizations of the afferents. Neither saw any signs of afferent projections to the brain or other segments. Lamparter et al. (1969) did report a few contralateral fibers; however, these are likely to be a result of either secondary degeneration of post-synaptic neurons, a phenomenon reported by Geisert and Altner (1974), or retrograde degeneration of motorneuron collaterals.

Stocker and Lawrence (1981) reported axons from tarsal sensilla ascending in the longitudinal fasciculus. While I have occasionally seen such fibers in HRP filled preparations, although never in cobalt filled preparations, they are accompanied by medially located cell bodies in the ventral posterior rind. I therefore did not include them on the figure because of my suspicion that they are central transneuronally filled neurons. Otherwise, there is general agreement between my results and those of Stocker and Lawrence in that the sensory neurons make a two-part projection in the ipsilateral neuromere: a medial branch and a somewhat smaller lateral branch.

2. Projections in the mutants.

It was the purpose here to further investigate the interaction between the sensory neuron's intrinsic determinants and its milieu. This was done by using two genotypes which generate ectopic cuticle on the head. The first was $Antp^{73b}$ $Df(3R)sbd^{104}$ / Pc^{3} ss^{a} which transforms the antennae to legs, completely eliminating antennal sensilla. The second was ey^{Opt} which transforms part of the

eye to dorsal thorax and wing. The central projections of the ectopic sensilla into their brain can be compared to (1) the projection of these sensilla into their normal targets in the ventral ganglion and (2) the projections of all of the "local" sensilla (those that normally occupy the spot to which the experimental sensilla have been moved) which project into the brain -- the eyes, the antennae, the head bristles and the interommatidial bristles. These results taken as a whole can then be compared to the results from other studies of the projections of normal and ectopic sensilla.

a. Ectopic and supernumerary bristles. The projection of thoracic and wing margin bristles genetically transplanted to the head by the ey^{Opt} mutation was studied. The normal projections of these bristles has been described previously (Ghysen, 1978, 1980; Palka et al., 1979). For comparison, the projections of the bristles normally present on the head and eye ("local" bristles) have been described here. The projection of the retinula cells of the eye has also been described (Braitenberg, 1967; Trujillo-Cenóz & Melamed, 1966). The projections of the ectopic thoracic bristles are quite unlike those of the local head macrochaetes and interommatidial bristles. This could be a result of the fact that they are thoracic bristles or of the fact that they are present in unusual locations on the head or of the fact that they are supernumerary bristles. To control for these latter two possibilities supernumerary bristles at the same location as the ectopic thoracic bristles were produced by the mutation ecd^{ts} which duplicates the normal bristles and does not result in a homeotic change. These bristles had projections that were in all respects like those of wild-type head bristles. Therefore, abnormal projections from the ectopic thoracic bristles are a result of their character and not of their position.

The ectopic thoracic sensilla entered the CNS through nerves used by the local sensilla and initially used the same routes within the CNS used by the local sensilla. This included both projections into the optic lobe, in which they followed the

retinula and lamina cell axons for at least some distance, and projections into the central brain, in which they followed, for at least some distance, the routes of the axons of the head macrochaetes. In no case could fibers be seen projecting caudally through the cervical connective into the ventral ganglion in spite of the fact that in many cases axons entered the cervical connective by way of the posterior tegumental nerve and therefore had access to descending pathways. Therefore, there seem to be no long-range cues attracting thoracic sensilla into the ventral ganglion. On the contrary, the ectopic sensory axons join with nearby axons and run in local nerves to the CNS. This is in complete agreement with all other studies of ectopic sensilla.

After entering the CNS, the ectopic thoracic sensory axons have a substantially different fate from that of the local axons. Within the optic lobe there does not appear to be any particular target. Every preparation has fibers terminating at different places. It seems that there is no particular choice made by the axons, but that they merely grow to a certain length and then stop. In the central brain there also appears to be substantial variability in the projections. One dorsal region in the subesophageal ganglion (figures 12c and 12d) does appear to receive axons from ectopic thoracic bristles in a large fraction of preparations in which axons entered the central brain. This may indicate some affinity of thoracic axons for that area (it also receives adventitious projections from antennal legs) but it does not seem to indicate any guidance to that area by the path since the normal head bristles do not leave the path at that point.

The ectopic bristle axons do not necessarily stop in the same neuropil as the local fibers. They often go beyond it (e.g. figures 12b, 12d, 13a and 13b) or they can leave the pathway before it arrives at the target neuropil of the local axons (e.g. figures 12a and 12c). They often have bizarre arborizations completely unlike those of the axons of local sensilla. These arborizations are also different from the

normal pattern, the pattern exhibited by thoracic bristles or wing margin bristles in the ventral ganglion (e.g. Ghysen, 1978, 1980; Ghysen & Janson, 1980; Palka et al., 1979). In addition, wing (and haltere) proximal campaniform sensilla project into the subesophageal ganglion in a characteristic manner (Strausfeld & Singh, 1980). None of the ectopic thoracic sensilla projected to the same part of the subesophageal ganglion nor did they exhibit the same pattern. [1]

While the projection into the brain of the wing margin bristles was unlike the normal projection in all respects, the projection of these sensilla (which normally project into the mesothoracic neuromere) into the metathoracic neuromere in bithorax postbithorax mutants had the appearance of their normal projection (Ghysen, 1978; Palka et al., 1979). Therefore the appearance of a normally shaped projection from these bristles is dependent on the type of neuropil into which the projection is made. The subesophageal ganglion and brain are not suitable while the metathoracic neuromere (and the mesothoracic, obviously) are suitable. There can be two reasons. One is that the mesothoracic and metathoracic neuromeres are homologous in some ways; the other is that the effects of the mutation on the metathoracic neuromere make it like the mesothoracic. However, Palka et al. also found normal arborizations of wing margin bristles into presumably wild-type neuropil in mosaic flies. Therefore it is likely that the homology between the two regions of neuropil is responsible for the similarity of projection.

The following conclusions can be made from these results:

(1) The fact that the thoracic ganglion targets were not reached suggests that long-range cues do not serve an important role in the guidance of axons.

(2) With regard to guidance by central tracts, it appears that the axon is not passively guided. Rather, the axon must be specifically matched to the tract in

1. This was not entirely unexpected since I filled preferentially from bristles and not from campaniform sensilla.

order to be guided by it. Just as the axon can recognize the pathway at any point and enter and follow it, so it must continue to recognize it in order to continue following it. If the axon is in a foreign tract and its affinity to the tract is weak then it can leave the tract at any point. Since the axon is not mechanically constrained to follow the tract it can also leave it when it is adjacent to its target neuropil in any case.

(3) The arborization pattern of the axon appears to depend on an interaction between the axon and its immediate environment. Neither the axons internal determinants nor the neuropil can induce a characteristic arborization. This is suggested by the fact that arborizations characteristic of the normal thoracic bristle in the thoracic ganglion were never seen and arborizations characteristic of local head sensilla were not commonly seen. However, wing margin bristles have a normal arborization if they project into the metathoracic neuromere so presumably homologous neuropil can permit the expression of a normal arborization.

b. Antennal legs. The projection of the antennal legs into the brain is summarized in figures 23, 24 and 25. The axons enter through the antennal nerve into the deuterocerebrum along the same route as the wild-type antennal axons do. No wild-type antennal leg axons enter the cervical connective. The projection into the antennal mechanosensory area (figures 24 and 25) is identical in the wild-type and the antennal leg mutant. The projection into the olfactory lobe is substantially different. The arborization of the axons appears to be random and irregular. It is not confined to the olfactory lobe either. In a few preparations axons can be seen running from the dorsal olfactory lobe into the protocerebrum. Far more common were adventitious axons entering the subesophageal ganglion, sometimes directly from the antennal nerve, but always from the olfactory lobe. There is a tendency for the adventitious projections to enter specific regions of subesophageal ganglion

and tritocerebral neuropil. A region of the dorsal subesophageal ganglion, between the ventromedial borders of the olfactory lobes, is one such area. Axons are also always seen in the anteroventral tritocerebrum, although there they ramify in a rather diffuse and random manner often forming bundles just under the cortex. These semi-regular features of the adventitious projection suggest that there is in fact an affinity to these regions of the subesophageal ganglion and tritocerebrum. Since this region of the subesophageal ganglion receives input from the proboscis (Stocker & Schorderet, 1981) perhaps the close phylogenetic relationship between the proboscis and the legs makes them somewhat homologous and the homology permits leg axons to make the same connections. An alternative explanation is that there is no affinity but as the tarsal axons leave the olfactory lobe they tend to remain in nearby central fasciculi because of passive mechanical guidance. Although there does not appear to be a commissure in wild-type flies at this position in silver-stained preparations, the fibers may not be sufficiently numerous or compact to be readily visible. So, although it seems that the tarsal sensory neurons (and this includes mechanosensory) have found a region of neuropil in the brain to which they have a specific affinity, this cannot yet be determined for certain.

The arborization of the axons in the olfactory lobe and adjacent neuropil is irregular and apparently random. It resembles neither the arborization of normal tarsal sensory neurons in the ventral ganglion nor the arborization of the normal antennal sensory neurons even if some of them are still present (as in ss^{a}/sbd^{104} flies). In spite of the novel features shown the antennal leg projection still bears a strong resemblance to that of the antenna. The antennal projection enters two regions of neuropil, the olfactory lobe and the antennal mechanosensory area. The same two areas are innervated by the antennal leg axons with the exception that the branch into the olfactory lobe is not restricted to the olfactory lobe. Even this exception is qualified by the fact that the adventitious fibers are restricted to

specific regions. This similarity to the antennal projection is seen in $Antp^{73b}$ $Df(3R)sbd^{104}$/ Pc^{3} ss^{a} flies so it can't be a result of $CoCl_2$ leakage into remaining antennal sensilla nor of remaining antennal sensilla providing a guide path for the antennal leg axons.

Not only is the antennal leg projection directed to the same targets as the antennal projection, but part of it, the projection into the antennal mechanosensory area, is actually identical to that part of the antennal projection. Only the axons entering the olfactory lobe make a disorganized projection. Those in the antennal mechanosensory area behave as antennal axons do. This can't be a result of a failure of the mutation to affect these sensilla, because if that were so, then why is the olfactory lobe projection disorganized? It can't be a result of an effect of the mutation on the olfactory lobe itself because surviving, nontransformed, sensilla on the ss^{a}/sbd^{104} antennal leg have a normal olfactory projection.

It seems likely that the tracts taken by the antennal leg axons can provide some guidance, possibly mechanical, for short distances. As I have observed for the projections of the ectopic thoracic bristles on the head, foreign tracts provide poor guidance through the CNS. The ectopic axons left them after following them for varying distances. However there is a good chance that few axons would leave even a poorly recognizable tract within the short distance to the olfactory lobe (although sometimes a few do). This explains why the general morphology of the antennal leg projection resembles that of the antenna. The local tracts can serve to direct the incoming axons into nearby regions of neuropil. But why should the axons follow the antennal mechanosensory projection so faithfully? Perhaps the fact that the tracts are labelled does not completely restrict them to specific classes of neurons. Classes of neurons that normally do not use these tracts, but share certain features with the axons that do, may be able to follow the tracts if routed into them as the result of an experimental situation. These shared features might be sensory

modality or phylogenetic or ontogenic homology. For example, it is possible that mechanosensory bristles are fairly generalized throughout the fly and can recognize each other's guidance cues. This could also explain why the adventitious projections can enter putatively proboscideal target neuropil. As a counterargument it could be pointed out that there is quite a number of very specific mechanosensory projections such as the thoracic bristles of *Drosophila* or the head hair or cercal bristles of crickets. These imply that mechanosensory projections are not generalized and follow only their own guidance cues. Also, it is clear that antennal mechanoreceptor bristles in the wild-type fly do not invade proboscideal neuropil. Nevertheless, it is at least conceivable that failing to find a better pathway, the antennal leg mechanosensory axons can accept the antennal mechanosensory pathway as a "second-best choice" and then follow its guidance cues.

A second possibility is that the guidance of the antennal leg mechanosensory axons in the antennal mechanosensory pathway is mechanical only. That is, the axons do not leave the tract because they are physically constrained to stay within it. The morphology of the antennal tract, however, shows it to be a rather loose aggregate of fibers, especially posterior to the olfactory lobe, and argues against physical constraint of the axons. Furthermore, the results of the observations of ectopic thoracic bristles suggest that mechanical constraints do not play a major role in long-range guidance, rather, axons probably stay in particular tracts because of specific affinities.

These results support the conclusions reached above following the consideration of the observations of the ectopic wing and dorsal thoracic bristles but imply a modification of the second conclusion.

(1) Long range cues do not appear to be involved in the guidance of these axons to their targets.

(2) Guidance by foreign tracts appears to be possible if there is a homology, in

ontogeny or phylogeny or sensory modality, between the axons that normally follow the tract and the ectopic axons. This may be analogous to the situation described above in bithorax postbithorax flies where the wing margin bristles make a normal appearing projection in the metathoracic neuropil.

(3) The arborization of the antennal leg axons in the foreign neuropil was irregular and unlike either the normal projection of leg axons in the thoracic ganglion or the projection of the local, antennal, sensilla. This implies that the arborization pattern is neither a property of the axon alone, nor a property of the axon's milieu alone, but a result of a specific interaction between them.

c. *Abdominal legs and misplaced antennae.* In both of these cases the axons of the ectopic sensilla entered their "normal" targets, that is, the neuropil normally innervated by these sensilla. This neuropil was reached by a slightly unusual route in that the axons entered in a more medially placed nerve than the metathoracic leg nerve. Nevertheless, in most cases they arborized normally. Thus ectopic sensilla can still reach their targets if the distance is small, even if they must take unusual routes.

The evidence from the homeotic mutants discussed above indicates that long range cues do not exist in the nervous system but ectopic sensilla can reach their targets only if the new location is rather close to the normal location. Since Shankland (1981b) reported that sensory axons do not send out exploratory branches, but immediately innervate the correct target, it would seem that the furthest distance that an ectopic sensillum can compensate for (that is, still reach its normal target) must be the length of the filopodia on the growth cone. To establish this it will be necessary to in fact examine ectopic cuticle transplanted to various distances from their targets and see what the maximum distance is from which it can still reach its target. Also, it must be ascertained whether or not ectopic sensilla send out exploratory branches. Perhaps the presence of suitable target dendrites

suppresses exploratory branches.

One other point is that even though the abdominal leg generally innervated its target correctly there was some influence of the abnormal route as evidenced by the loss of the lateral branch of the projection in several preparations.

The results from the observations of the misplaced antenna reinforce the notion that within a certain distance neurons can compensate for their being misplaced. Here, too, an abnormally positioned antennal nerve associated the misplaced antenna with the antennal neuropil; however, the minimum distance was less than 50μ to the antennal neuropil.

B. General considerations.

Previous studies of sensory projections from ectopic cuticle resulting from surgical manipulations or bithorax-complex mutations generally succeeded in routing axons into abnormal parts of pathways that they normally use. These studies established the fact that these tracts are "labelled" and are recognizable at any point and that axons have preferences for some labels over others, preferring their normal pathway above all. Within these tracts different types of ectopic sensilla display different behaviors which suggests (and this is more speculative) that behavior within a tract is not entirely determined by the tract itself but that the neuron has intrinsic determinants of direction of growth, distance to grow, branching pattern and affinity for target neuropil

These experiments were done in order to investigate the behavior of axons that have been forced to enter pathways or regions of neuropil that they normally do not enter. Axons entering familiar pathways from ectopic locations seem to be able to follow them. Similarly, axons entering familiar neuropil from ectopic locations seem to be able to establish normal-looking arborizations in the neuropil. The results presented here show that this is generally not the case with axons entering

"foreign" tracts or neuropil.

There are some similarities between the results of the two kinds of experimental situations and these will be mentioned first. In both cases axons are conveyed to the CNS by local nerves and adhere, within the CNS, to the available tracts in the vicinity. The differences are that the level of adherence is not as stringent in the case where the axons are in unfamiliar tracts. Within familiar tracts ectopic axons display properties that they do when they have entered these tracts at normal points. They either take the tracts to their normal targets, following the tracts sometimes for long distances (e.g. distal wing sensilla in study of Palka, 1979), or they leave the tract by branching with their normal geometry but with the positions of the branches displaced to conform with the new place of entry (e.g. notal bristles in study of Ghysen, 1980), or they follow the tract for variable distances without leaving it (Anderson, 1981).

The results presented here show that axons in unfamiliar tracts display behavior quite different from the above. They did not display any properties characteristic of their normal projections. They entered the tracts to which they were conveyed by the nerve and followed them, at least initially, toward the regions of neuropil normally innervated by the local neurons. Therefore, their projections tended to have a greater resemblance to the projections of the local sensilla than to their normal projections. However they did not (with one exception) follow the local tracts consistently for very long distances. Axons from the antennal leg generally followed the antennal tract into the olfactory lobe, which is a very short distance; even so some axons left the tract before they reached the antennal lobe and entered the subesophageal ganglion. The axons of the ectopic thoracic bristles followed the tracts of the local axons for variable distances, sometimes reaching the target neuropil of the local axons, sometimes leaving the tract before reaching it, and sometimes going beyond it. The exception was the antennal leg projection to the

mechanosensory area where the ectopic axons did follow the route of the local axons in a precise manner.

It seems then that it is possible to add to the previous conclusions regarding the existence of labelled pathways that guide the growth of axons in the CNS. Guidance along the pathway is a function of the corresponding affinity of the axons for the pathway. Axons within foreign tracts have little affinity for the tracts. They will be associated with the tracts for variable distances and will follow them consistently only for short distances. It seems though that axons can have "next best" choices and will remain longer with tracts that are more similar (in whatever way the "labelling" is done) to their normal tracts. This is suggested by the consistent projection of the antennal leg neurons within the antennal mechanosensory tract. Perhaps axons which share ontogenic or phylogenetic homologies have a greater chance of recognizing the same labels on a particular pathway, as was suggested before to explain the apparent affinity of antennal leg axons for proboscideal targets.

At some point the axon leaves the tract that it is in to establish synapses in the appropriate neuropil. Since this region can be found from short distances away by ectopic sensilla that have not taken an appropriate route, it seems that recognition of the neuropil is by a chemoaffinity mechanism and not merely by passive guidance to it. If the branches of ectopic sensilla axons are made within the tract but are shifted in position so that they are never near the target site they do not innervate it. This suggests that internal determinants of branch length have importance in matching the axon to the position of its target and that axons do not merely proceed along a tract until they reach the target neuropil.

Within the appropriate target neuropil axons produce characteristic patterns of arborization. Ectopic sensilla that innervate familiar neuropil also branch nor-

mally even though the initial parts of their routes are disorganized. Ectopic sensilla described here that innervate unfamiliar neuropil do not have normal patterns of terminal arborization. *In vitro* studies, such as that of Solomon (1979), show that arborization patterns can be generated by internal programs. However the evidence from the observations presented here of the disorganized arborization of ectopic sensilla in foreign neuropil suggests that if such internal programs exist they can only be expressed in the appropriate neuropil.

Some insight into the manner by which the neuropil participates in the shaping of axonal branching patterns can come from looking at the variability of projections of identical neurons. *Drosophila* head bristles never vary the positions of the two main branches of their axon; however, the precise branching that gives rise to the two elements can vary somewhat. This suggests that it is not the precise morphology of the branching that is important for these axons but the positions of their terminals. Altman and Tyrer (1977) found a similar result in their observation of variations in the central projection of wing hinge stretch receptors in locust. In an occasional animal, the receptor cell axon would run in a tract parallel to but displaced somewhat from its normal tract. Nevertheless, the side branches compensated for the displacement by being longer on one side and shorter on the other. This put the terminal "twigs" in precisely the same locations as in the normal situation. It was of some interest that the morphology of some of the fine terminal twigs was nearly identical from animal to animal.

The observation that ectopic sensilla do not arborize correctly in foreign neuropil and the observations just noted point to a role of the neuropil in organizing the branching pattern. I would like to suggest that the observed consistency in branching patterns of identified neurons comes about in two steps. The first is the establishment of a generalized branching pattern by the pre- and postsynaptic cells. Even this may depend, at least for the sensory axon, on environmental cues for

appropriate expression, since branching patterns in the ectopic sensilla share few characteristics even on a gross level with their counterparts that project into familiar neuropil. In the second step, the axon growth cone filopodia contact all potential postsynaptic sites and growth of branches is directed towards the appropriate ones only. It is then the spatial arrangement of terminals that determines the details of the branching pattern and not vice versa. Further observations of the behavior of growth cones and filopodia in the establishment of synapses by identified neurons may serve to clarify this process.

C. Conclusions.

In summary, I have provided evidence that long range cues are not important in the guidance of sensory neurons over long distances to their targets. Rather, local cues serve that purpose by keeping the axon in central pathways that guide them through the complex fiber networks of the CNS. However, such guidance of sensory axons by central tracts is not passive but depends on point by point matching of the axon to the tract. The axon can leave the tract at nearly any point, presumably to enter its target neuropil in the normal case. Within the target neuropil an interaction between the axon and its local environment induces the appearance of a characteristic terminal arborization.

The conclusions arrived at from the consideration of the results presented here, considered together with the results of other studies on sensory neuron development, can best be summarized by going through the various features of axonal development and considering each feature in the light of available results. The growth of the axon will be divided into four stages: finding and establishing a route to the CNS; within the CNS, finding a route to the target neuropil; entering only the correct region of neuropil and no other and establishing synapses on the correct target dendrites; producing the appropriate terminal arborization geometry in the

target neuropil.

(1) Routes to the CNS: There is general agreement that the first step of the sensory axon's projection, finding the CNS, is mediated by mechanical cues. A continuous physical pathway appears to be required (e.g. Wigglesworth, 1953). Since there is only one route, that which appears to be set up by the pioneer fibers (Bate, 1976b), there is little need to invoke chemoaffinity for choosing between alternative routes. If a simple physical guide to the CNS from the cuticle is all that is required how is the direction to the nervous system determined? One simple way would be a chemotactic signal released by the nervous system, a mechanism suggested by some *in vitro* experiments (e.g. Johnson et al., 1972). Alternatively, the neuron may be able to refer to a more general system of body coordinates or simply a mediolateral gradient in the appendage. Keshishian (1980) has suggested that the pioneer axon may follow "guideposts" in the epithelium of the appendage. In the early embryo the distance to the nervous system is not far and perhaps a very precise mechanism is not necessary.

(2) Routes within the CNS: There is some evidence for the role of peripheral pioneer axons in guidance within the CNS (Shankland, 1981b) but that is an inference from observations that the course of some (but not most) of incoming sensory axons in the CNS follows that of the pioneer axon that arrived previously. There is no experimental evidence that the pioneer axon is required. Furthermore, that observation does not provide a satisfying answer to the question of how sensory axons are guided within the CNS but merely forces us to rephrase it to how does the pioneer axon find the correct targets in the CNS. Of course, there is an advantage to this in that the pioneer fibers enter when the CNS is much smaller and might be able to find the correct targets simply by directly contacting all or most of the potential targets. Nevertheless, until there is more experimental evidence for this mechanism others have to be considered.

The route to the appropriate region of neuropil may be defined by a complex set of factors. Although chemotaxis has been suggested as a potential mechanism by *in vitro* experiments, the results presented here strongly argue against this mechanism operating *in vivo* in *Drosophila*. Ectopic axons in my studies could find appropriate targets only if their new position was close to the normal position. This suggests that axons entering the CNS close to their target neuropils can enter them directly without actual guidance by central pathways. In fact, as can be seen from the results of the studies of the projections from abdominal legs and misplaced antennae, appropriate target neuropil can be found even if the local guidance cues would actually tend to direct the sensory axons in another direction. Axons then have affinities for their targets and these can serve as short range guidance cues. The most likely explanation is that growth will be directed toward a target within range of the growth cone filopodia, other guidance cues notwithstanding.

In the studies of Ghysen (1978,1980), Ghysen and Janson (1980) and Palka et al. (1979) of sensory projections in bithorax mutants ectopic axons found appropriate targets after entering suitable tracts. These were tracts which the axons normally used in the wild-type. The significance of guidance by pathway is made even stronger by the observation of Anderson (1981) that ectopic axons can't reach a normal target if they are in a different tract. This means that if an axon is in a familiar tract it is very much restricted to that tract. The important thing then, for axons that need to reach targets long distances away in the CNS, seems to be the choice of a fiber tract which runs to or by the target region of neuropil.

It has been shown here that matching of axon to tract can vary. Some axons are able to follow tracts that they normally do not use, possibly because of some similarity with the axons that do use, and can recognize the tract.

Nevertheless, neurons are not generally reliably guided by foreign tracts and therefore can only use these pre-established pathways if they can identify the proper tract and recognize it at all points along its route. How is the appropriate tract identified? The studies of the ectopic sensilla of bithorax-complex mutants, and that of Anderson and Bacon (1979) of ectopic cricket wind-hairs, and those of Katz & Lasek (1979,1981) of transplanted eyes and Mauthner neurons in the frog all suggest that what is important to the identification of the correct tract by an axon is an appropriate label on that tract. The major pathways have distinctive labels which are the same all along their length. These labels must precede the arrival of sensory axons because they could be recognized by ectopic sensory axons in bithorax mutants where there were no previous sensory axons of that type. The observations of Bate & Grunewald (1981) and of Goodman et al. (1981) suggest that the labels on these routes are defined by special central pioneer neurons, often with a slightly different ontogeny, whose axons precede the axons of all of the other neurons in a tract.

(3) Choice of the correct target: By analogy with choice of the correct tract it would seem that affinity between the target cell and the axon determines this. The study of Edwards & Sahota (1967) extended the analogy by showing that the affinity label is present along the length of the postsynaptic cell as it is in the tract. The importance of affinity between cell and target is supported by the observations of Murphey et al. (1981) and Palka & Schubiger (1975) that receptors on surgically rotated cerci innervate sites specific to the axons of that original region on the cercus. This argued against alternatives such as programmed geometry of branching or guidance cues. Further evidence comes from the results presented here on the projections of abdominal legs and misplaced antennae. In these cases it was possible, given the short distance to the appropriate target, to reach it in spite of the lack of local guidance cues. Then

for finding targets that are not far away guidance does not seem to be necessary.

The only system in which specific affinity between axon and target may not be involved is the optic lobe (Anderson, 1979; Meinertzhagen, 1972, 1973). If that is so, it may be because only in the optic lobe, which is essentially a system of identical repeated subunits can geometry be relied on to find the target.

In view of the fact that guidance of the sensory axon by the tract is not a passive process but involves specific matching between the axon and the pathway at all points, it would seem that the growing axon constantly checks its milieu all along its route. If the match between the axon and its path is good, as is normally the case, then it will adhere to the path. If it encounters its target neuropil presumably it will enter it.

(4) Terminal branches: *In vitro* experiments (Albrecht-Buhler, 1977; Solomon, 1979) have shown that innate programs for determining branching patterns do exist. Murphey et al. (1981) have further shown that the terminal arborization made by cercal afferents is a function of their proximodistal position along the cercus and that newly formed sensilla at that position adapt the same pattern. This would suggest that neurons form branching patterns according to internal programs and that these programs are in turn determined by the position of the bristle. Similarly, branching patterns of *Drosophila* notal bristles in the ventral ganglion seem to vary according to the anterior-posterior position of the bristle (Ghysen, 1980).

Tyrer et al. (1979) showed that the head hairs all project into the same neuropil but those coming from different hair fields on the head differed in their branching patterns. This suggests that the different branching patterns are not determined by the central neuropil but by the sensory neuron itself. Further-

more, the experiments of Anderson and Bacon (1979) show that the branching pattern of an individual hair is a stable commitment since moving the cuticle that will give rise to hairs to a different field does not alter the final branching pattern of those hairs.

In none of these cases could the role of the CNS be properly assessed since in all experimental conditions the hairs projected into the same neuropil. If, however, the sensilla are made to project into foreign neuropil as was done in the experiments with ey^{Opt} flies, we find abnormal branching patterns. The same result was obtained by looking at the arborization of the leg axons in the olfactory lobe. This means that although the program for branching may be internal, it can only be triggered by the appropriate milieu.

It seems then that the terminal branching pattern of the axon may be an environmentally triggered internal program which is chosen early in development according to the position of the epithelium that will give rise to the neuron. The "environmental trigger" may be the correct set of postsynaptic sites and it is possible that the branching program is defined not as a geometry of branches but as a set of positions of synaptic terminals. This implies that the postsynaptic cell may have a role since it must appropriately label its dendrites so that the various axons will put their synapses in the right places.

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FIGURES

Figure 1. Diagram of the *Drosophila* brain (frontal view) showing features of significance to the central projections of the head sensilla referred to in this study.

a-g: antennal-glomerular tract

a n: antennal nerve

La: lamina

Lo: lobula

Me: medulla (serpentine layer is shown in gray)

Mu B: calyx of the mushroom body

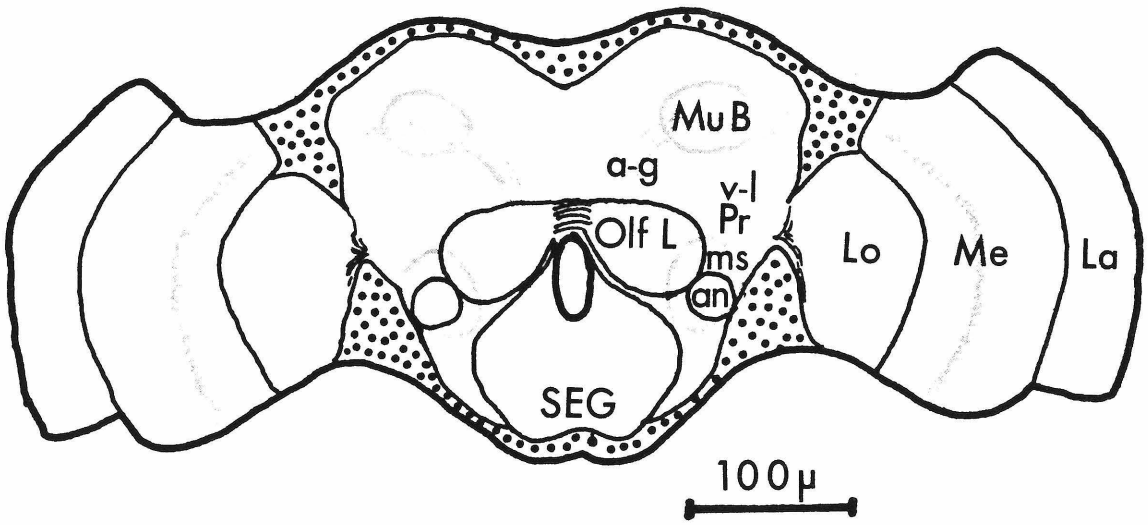
m s: antennal mechanosensory region (posterior and lateral to the olfactory lobe)

Olf L: olfactory lobe

SEG: subesophageal ganglion

v-l Pr: ventrolateral protocerebrum

The cellular cortex or rind is indicated by dots. Frontal view, dorsal at top. Scale bar = 100μ



1.

Figure 2. The head of a wild-type *Drosophila melanogaster* (Canton-S) with all sensilla referred to in this study labelled.

A: bristles of the second antennal segment

A_3: third antennal segment (flagellum)

Eye: eye with interommatidial bristles

Oc: ocellar bristle

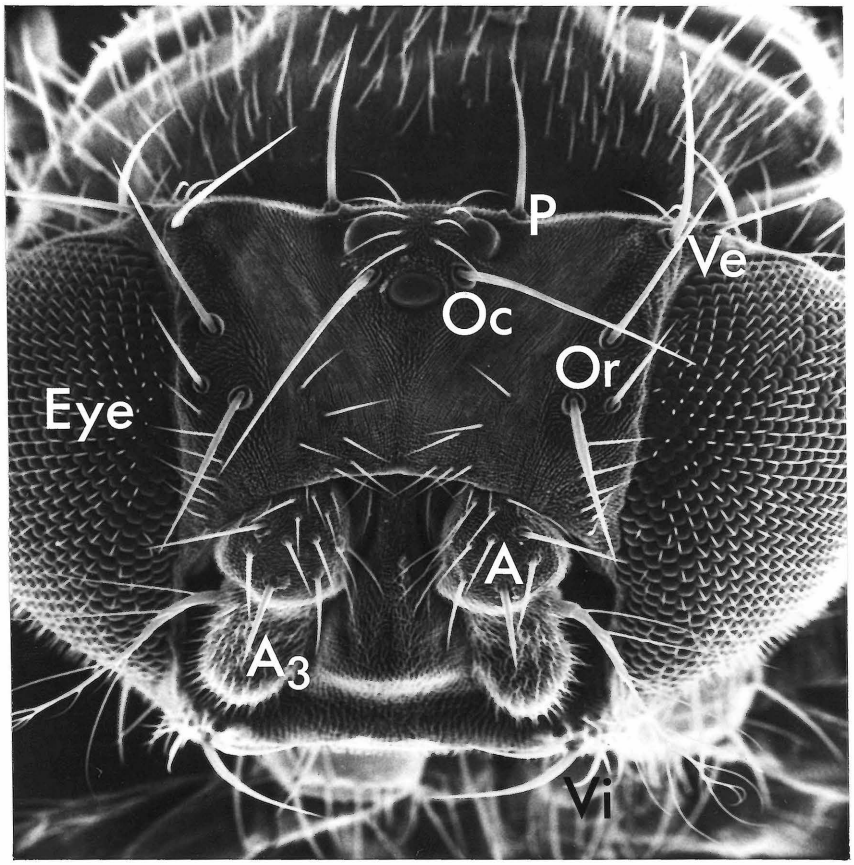
Or: orbital bristles (3)

P: postvertical bristle

Ve: vertical bristles (2)

Vi: vibrissae

Frontal view, dorsal at top. Scale bar = 100μ



2.

Figure 3. Camera lucida tracings of single head bristle cobalt fills illustrating all of the routes observed to be used by the sensory axons:

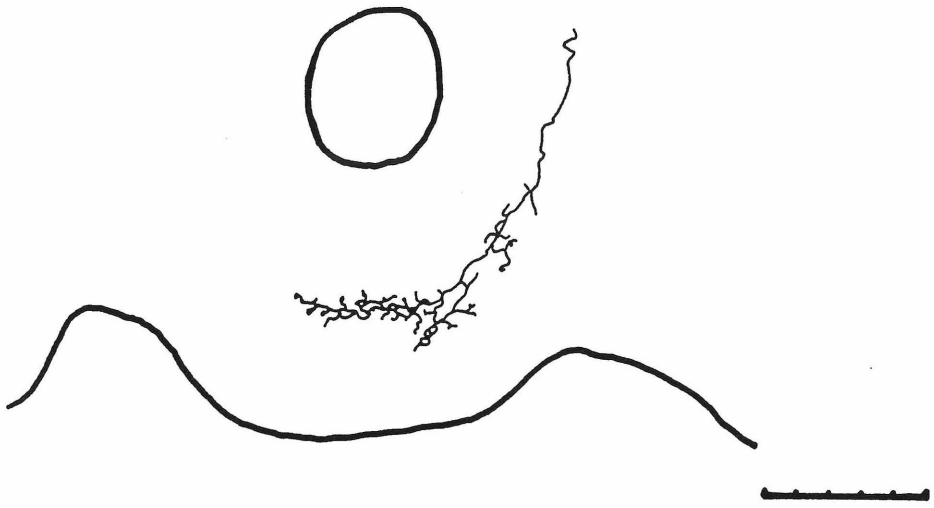
(a) bristle on the second antennal segment

(b) posterior orbital bristle

(c) vibrissa

(d) anterior vertical bristle

Frontal view, dorsal at top. Scale bar = 50μ



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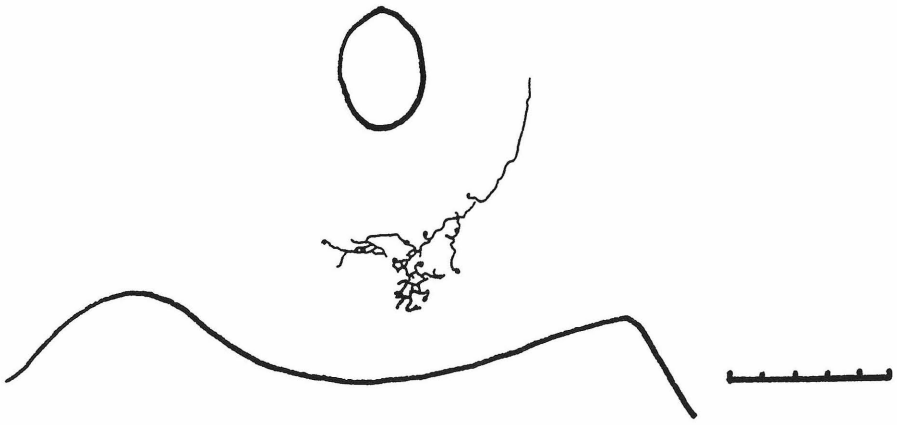

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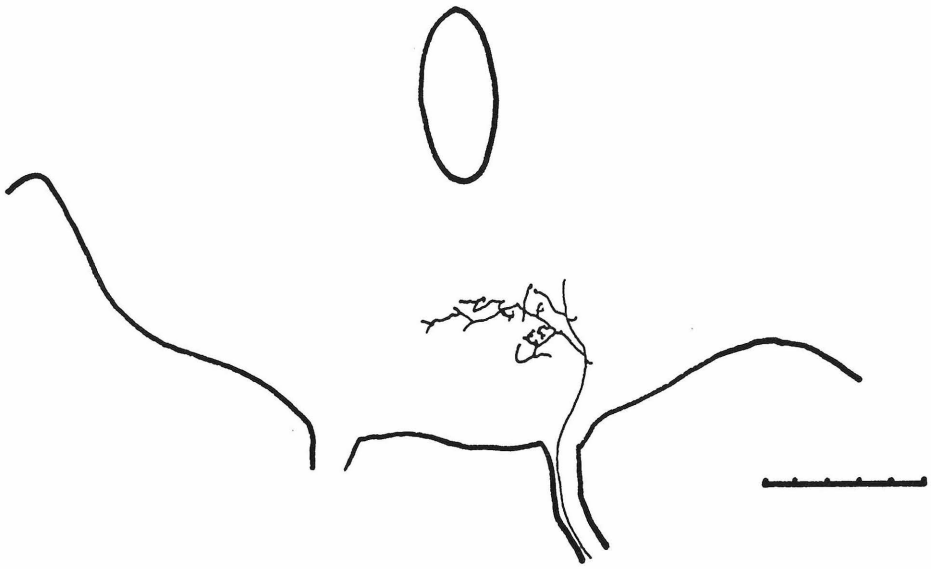

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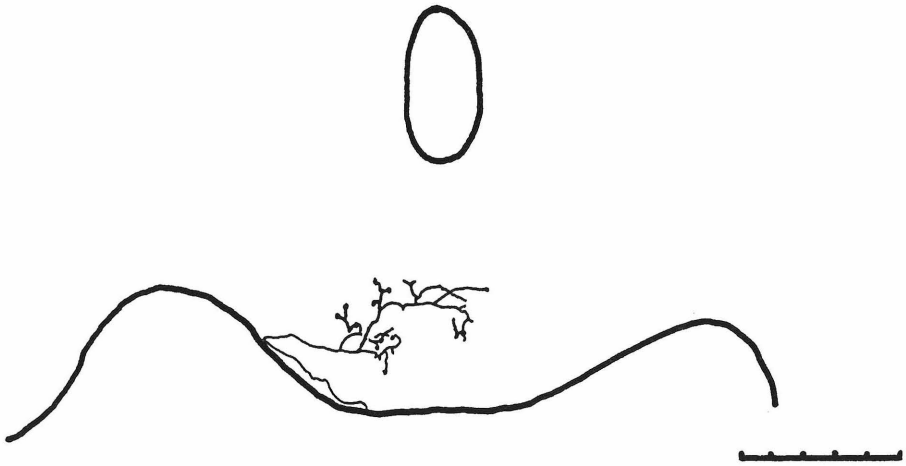


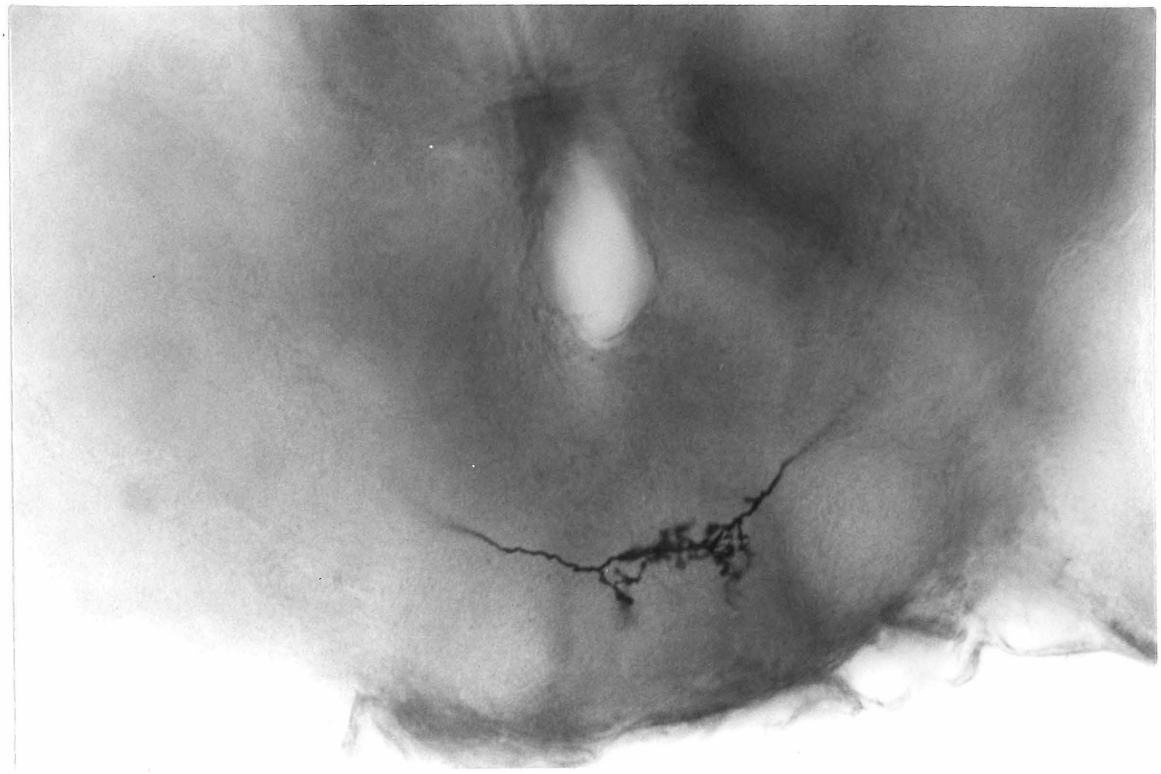
3d.

Figure 4. Photographs of two double bristle fills.

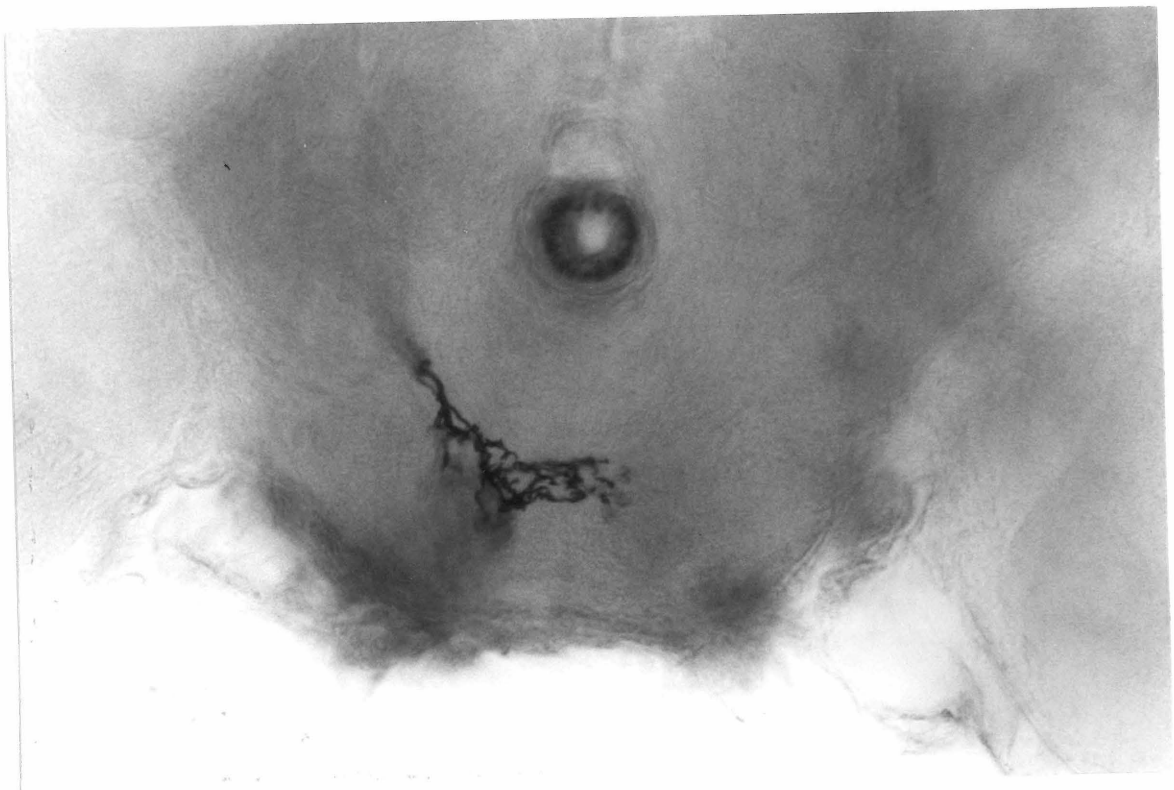
(a) Axons of ocellar (right) and postvertical (left) bristles showing their entry via the antennal nerve.

(b) Axons of posterior orbital bristle (thick) and adjacent fronto-orbital microchaete (thin) demonstrating close overlap of the two axons' routes and central arborizations.

Frontal view, dorsal at top. ×580



4a.



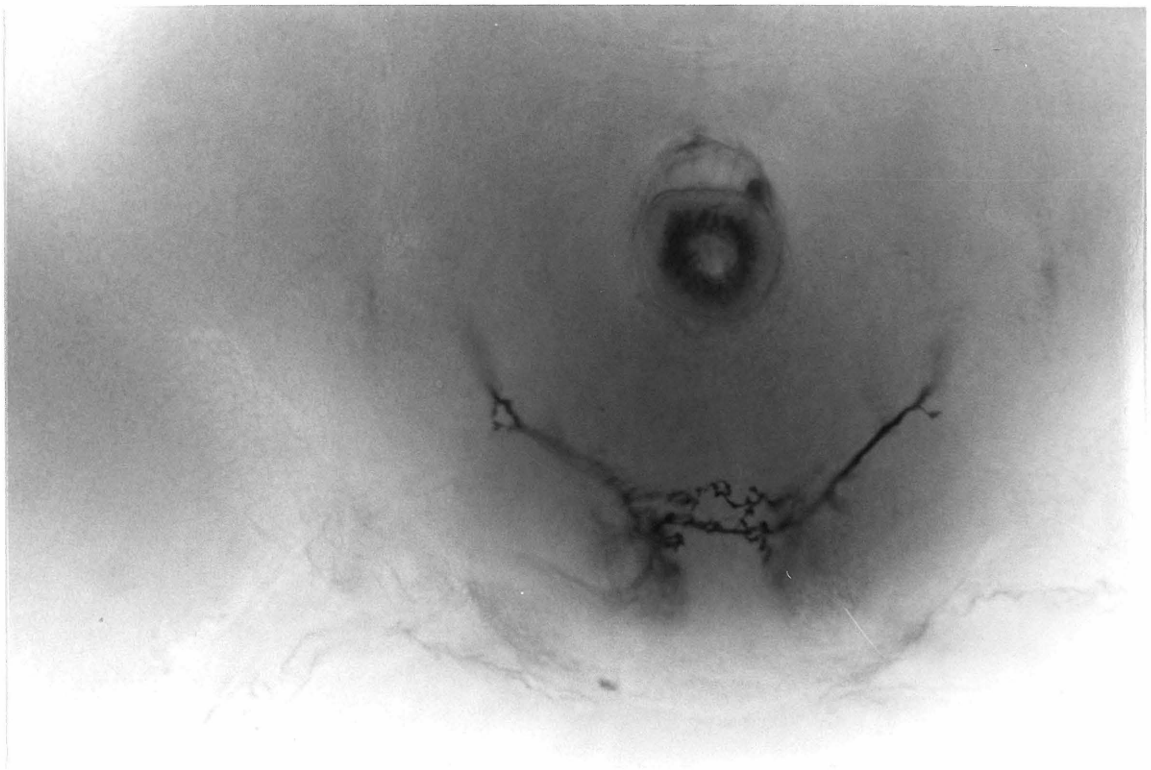
4b.

Figure 5. Photographs of a whole-mount preparation in which three bristles were cobalt filled showing the identity of their central arborizations and the different routes taken. The bristles are anterior orbital (right), anterior vertical (left), and posterior orbital (left).

(a) Anterior-medial part of the arborizations shown as well as the routes of the orbital bristle axons.

(b) The same preparation is shown with the focus farther back showing the ventral-posterior arborizations and the latter half of the route of the vertical bristle axon.

Frontal view, dorsal at top. ×580



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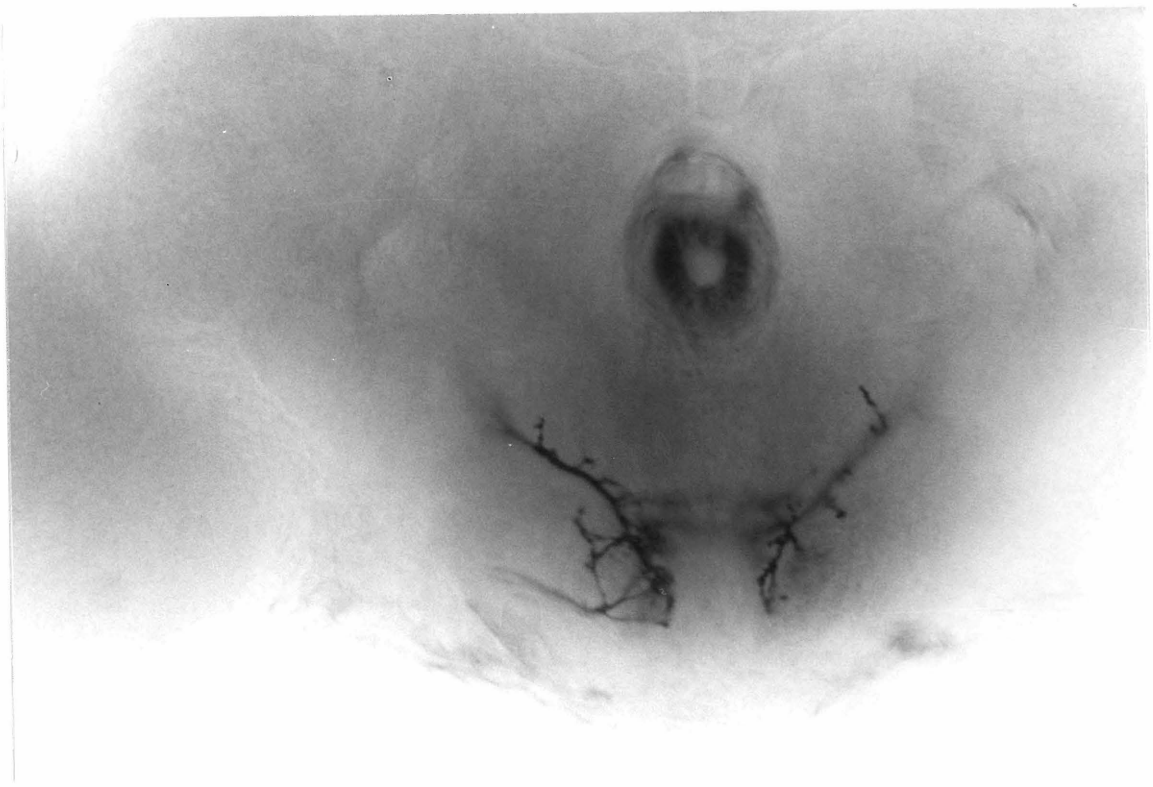


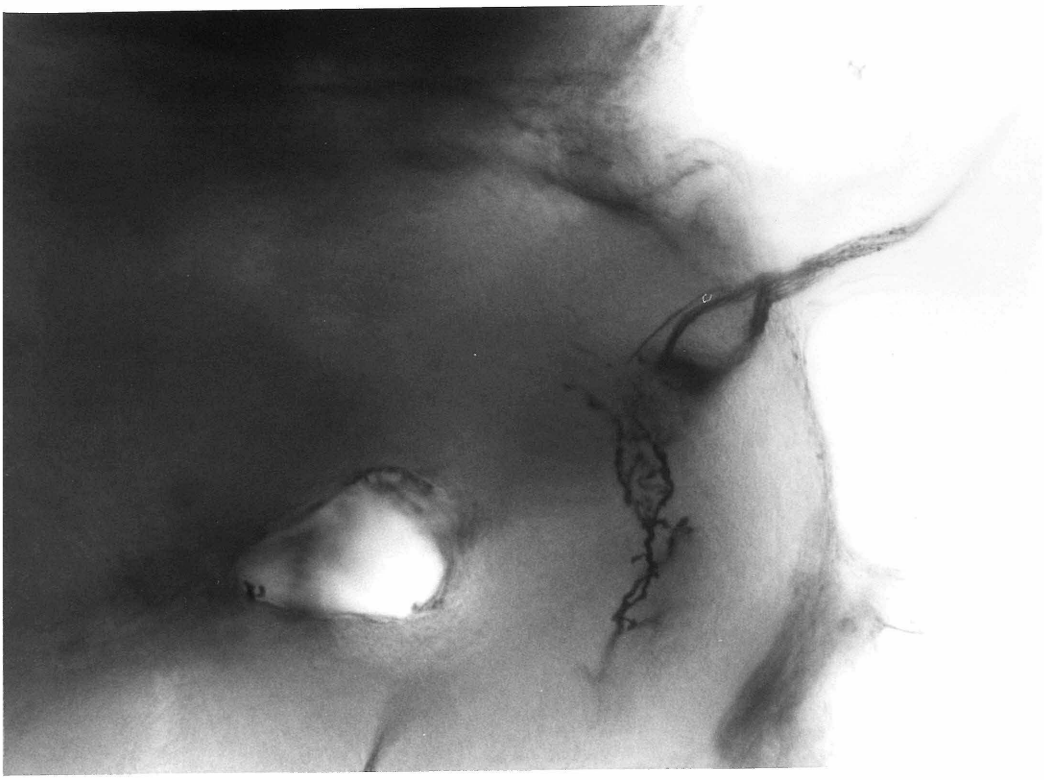
5b.

Figure 6. Photographs showing projection of eye interommatidial bristles and its relationship to that of the macrochaetes. The right eye and left ocellar bristle were cobalt filled.

(a) View of the anterior-medial arborization of the ocellar bristle and the route of the interommatidial bristle axons.

(b) The same preparation is shown with the focus farther back showing the ventral-posterior arborization of the ocellar bristle and its relation to the more lateral arborization of the interommatidial bristle axons.

Frontal view, dorsal at top. ×580



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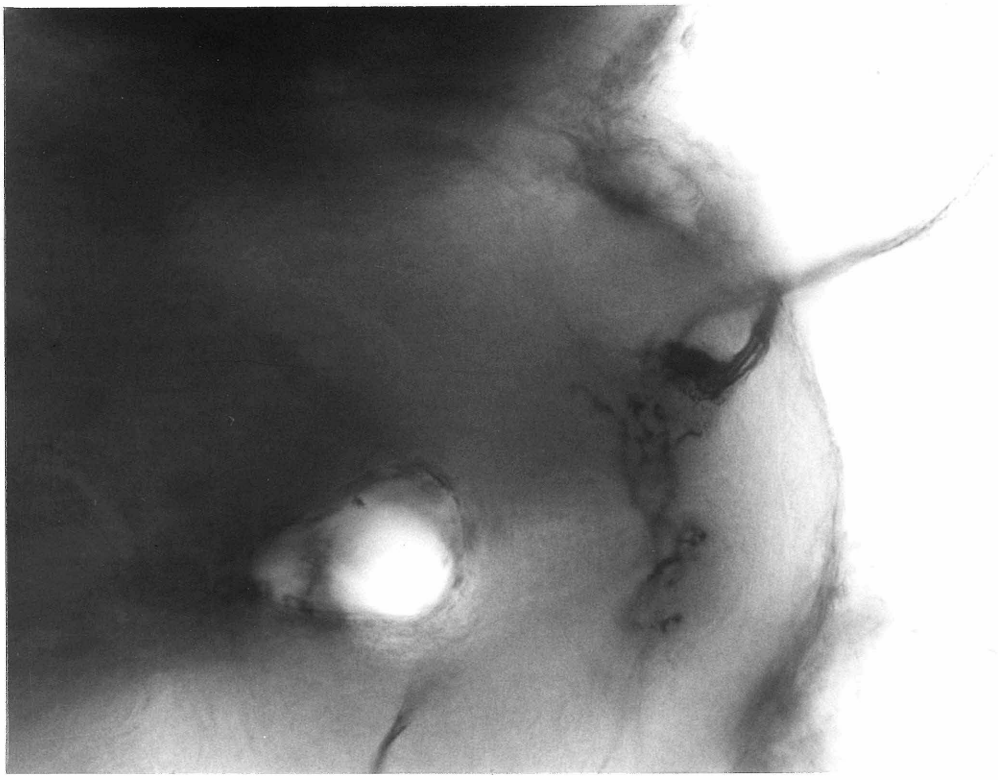


6b.

Figure 7. Projections of antennal sensilla into the olfactory lobe.

(a) Photograph of a whole mount preparation showing general features of the olfactory projection and its organization into bilaterally symmetric sets of glomeruli. The right side shows the cobalt filled antennal nerve and ipsilateral olfactory lobe with more densely filled glomeruli.

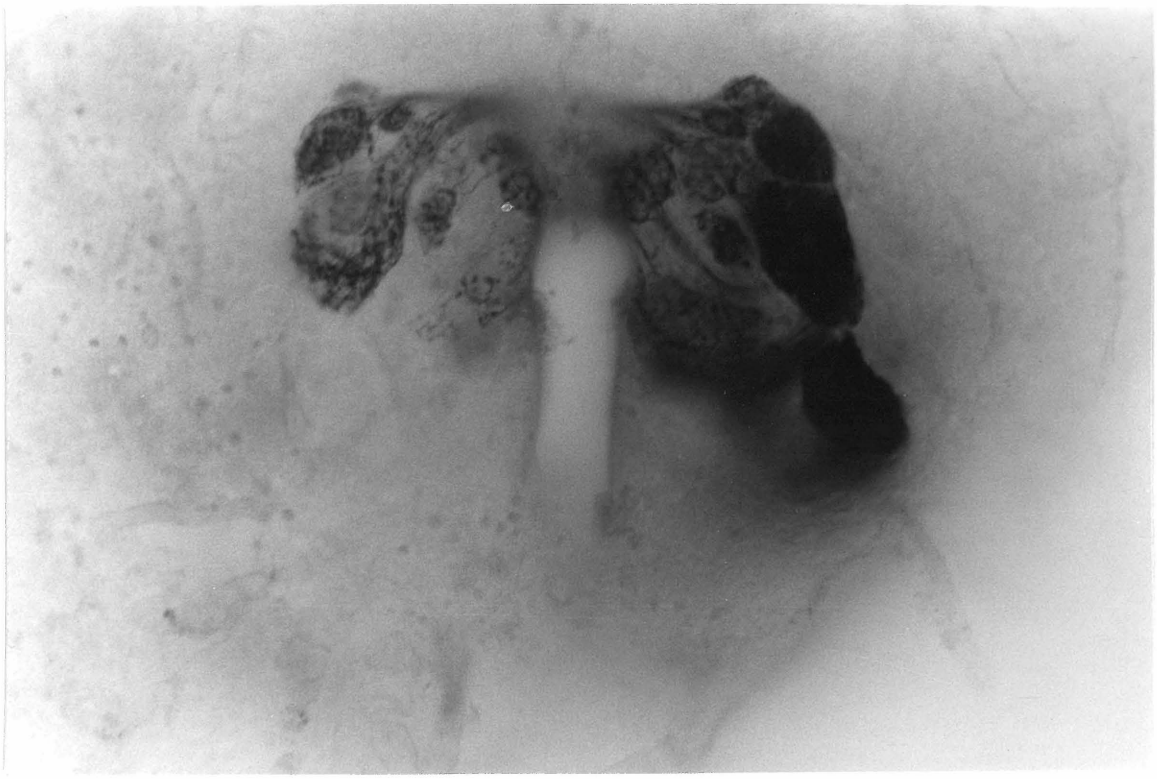
(b) Photograph of a frontal section cut through the brain 12μ from the anterior surface showing the olfactory fibers crossing from the antennal nerve into the ventrolateral corner of the olfactory lobe. Note particularly the entry of the axons into the superficial fiber layer.

(c) Photograph of a frontal section cut through the brain 27μ from the anterior surface near the middle of the olfactory lobe. Shown are the peripherally located glomeruli with the interior nearly devoid of sensory axons.

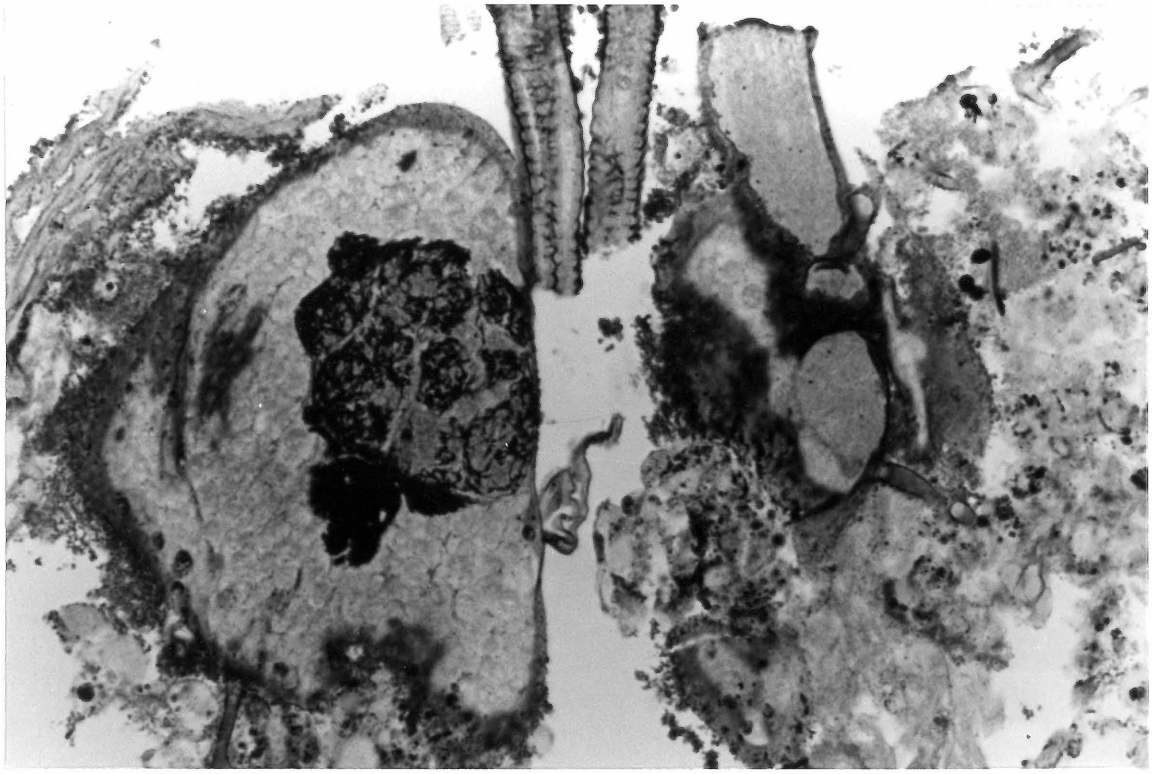
(d) Photograph of a frontal section cut through the brain 48μ from the anterior surface in the posterior olfactory lobe. Shown are the bundles of axons making up the antennal commissure and their entry into the superficial fiber layer and some individual glomeruli.

(e) Tracing of a single olfactory axon. The initial portion of the route is in the superficial fiber layer on the anterior surface of the olfactory lobe. Scale bar = 50μ

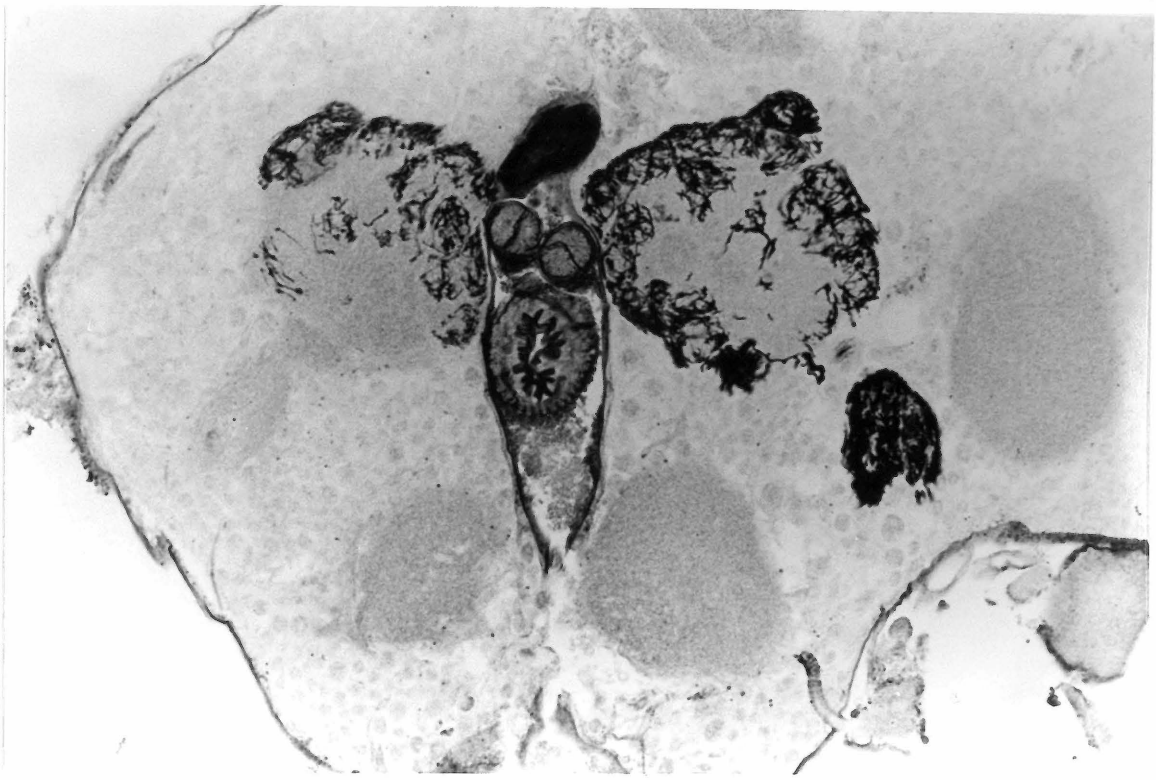
Dorsal at top in all cases. a-d:×580



7a.



7b.



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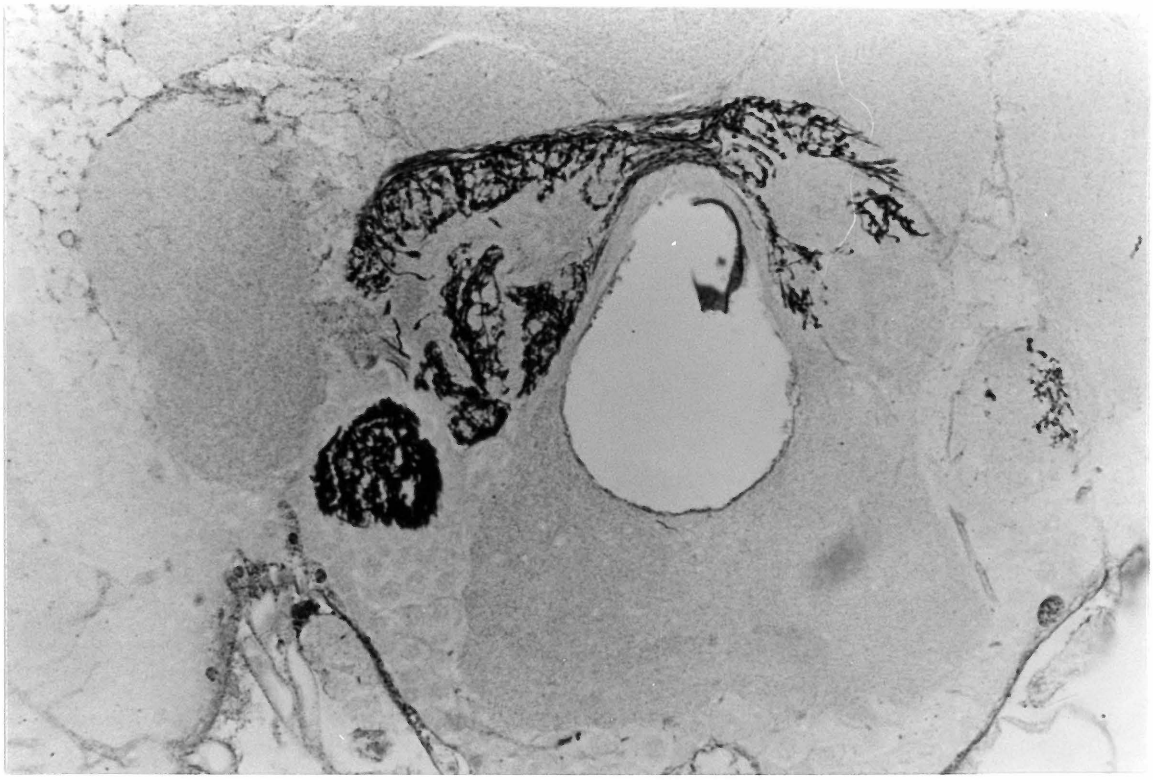
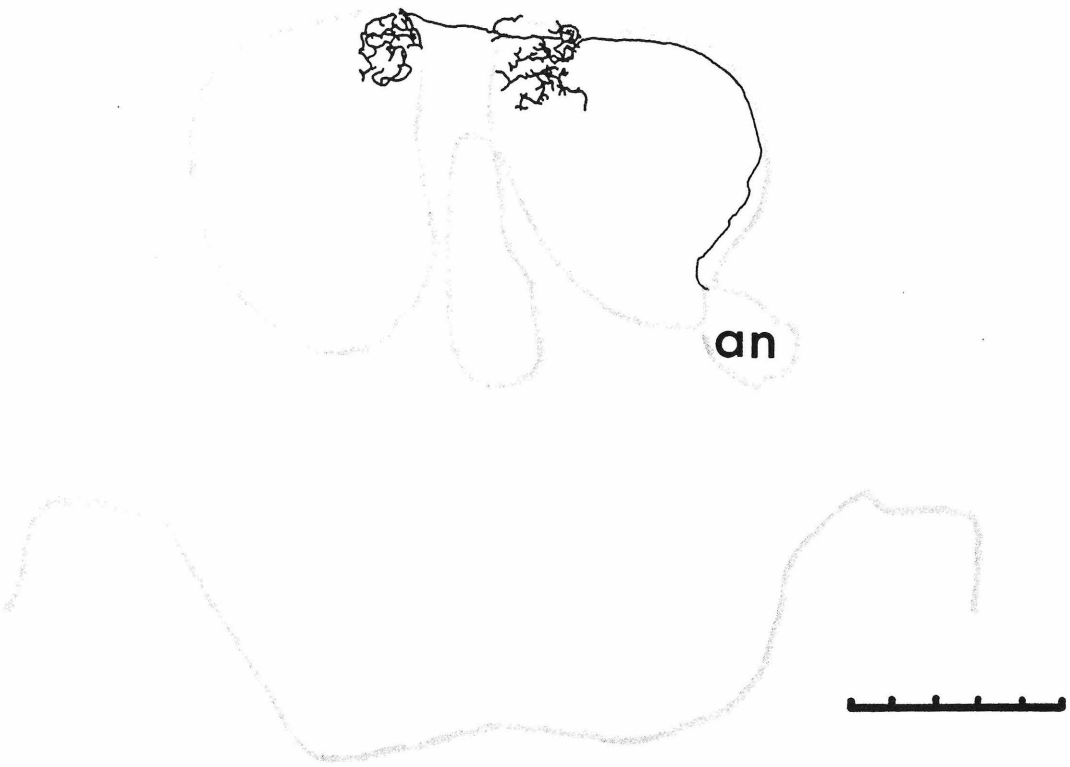


7d.



7e.

Figure 8. Non-olfactory lobe projections of antennal sensilla.

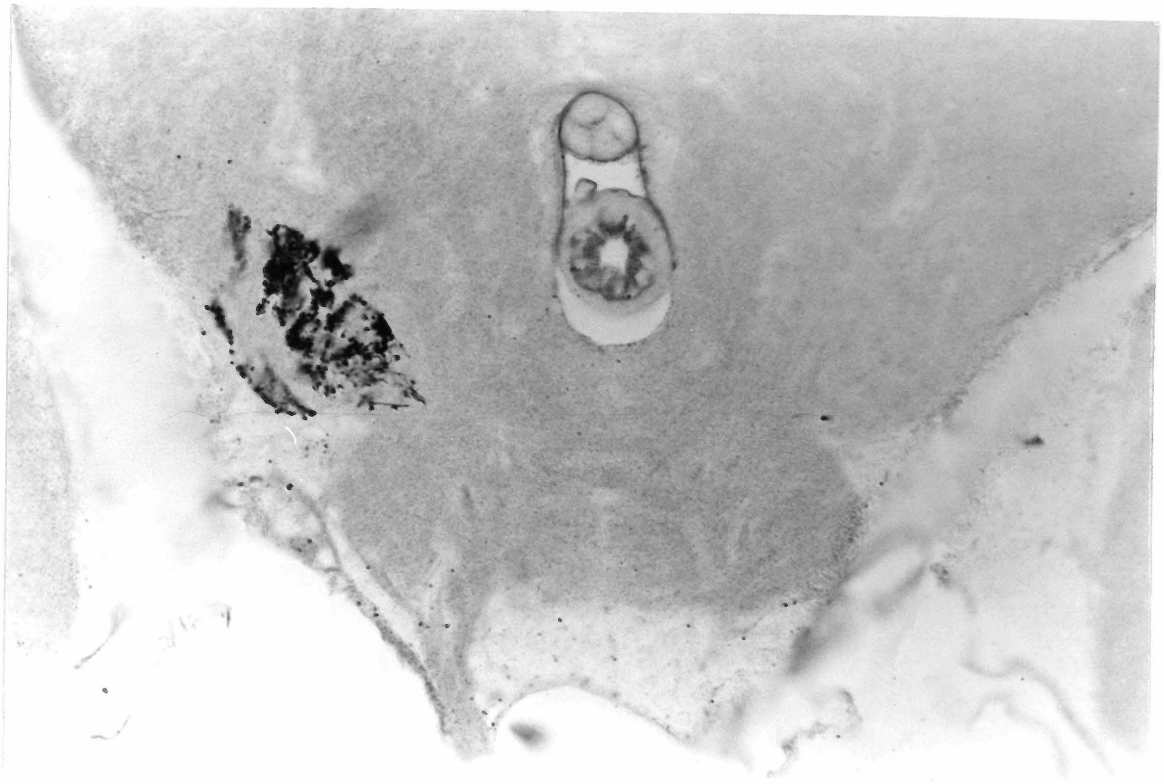
(a) Photograph of a frontal section cut through the brain 65μ from the anterior surface (just posterior to the olfactory lobe) showing the separation of the lateral antennal nerve fibers into dorsal and ventral bundles.

(b) Photograph of a frontal section cut through the brain 76μ from the anterior surface showing the anterior edge of the HB-like or L-fiber projection extending ventromedially from the antennal nerve into the subesophageal ganglion. Dorsal to the antennal nerve the the posterior-dorsal termination of the dorsal bundle of lateral fibers can be seen. Just ventral to the antennal nerve the ventral bundle of lateral fibers can be seen as they loop around the nerve and enter the subesophageal ganglion.

(c) Photograph of a frontal section cut through the brain 104μ from the anterior surface showing parts of all three antennal projections into the subesophageal ganglion. Most ventrally the posterior-ventral arborization of the HB-like projection can be seen. Just dorsal to it can be seen the projection into the subesophageal ganglion of the ventral bundle of lateral fibers. Dorsalmost are anterior axons of the medial fiber projection.

(d) Photograph of a frontal section cut through the brain 110μ from the anterior surface showing the major part of the medial fiber projection.

Dorsal at top. ×580



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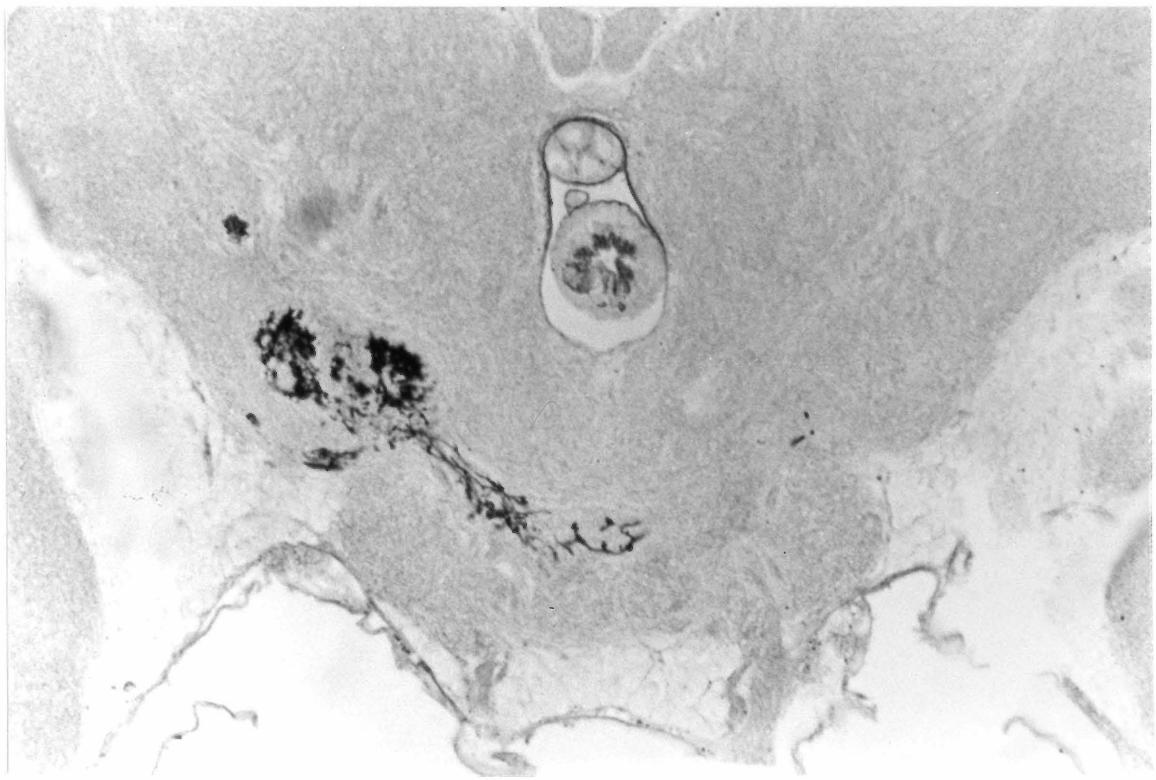

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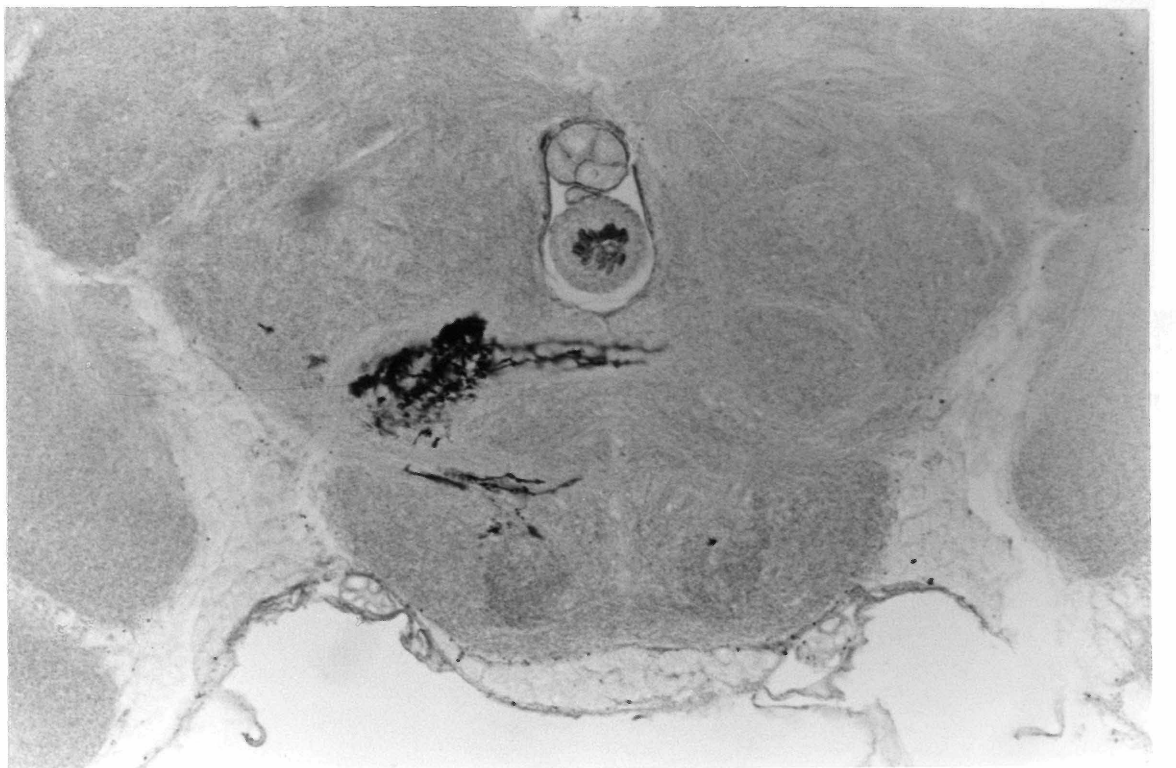

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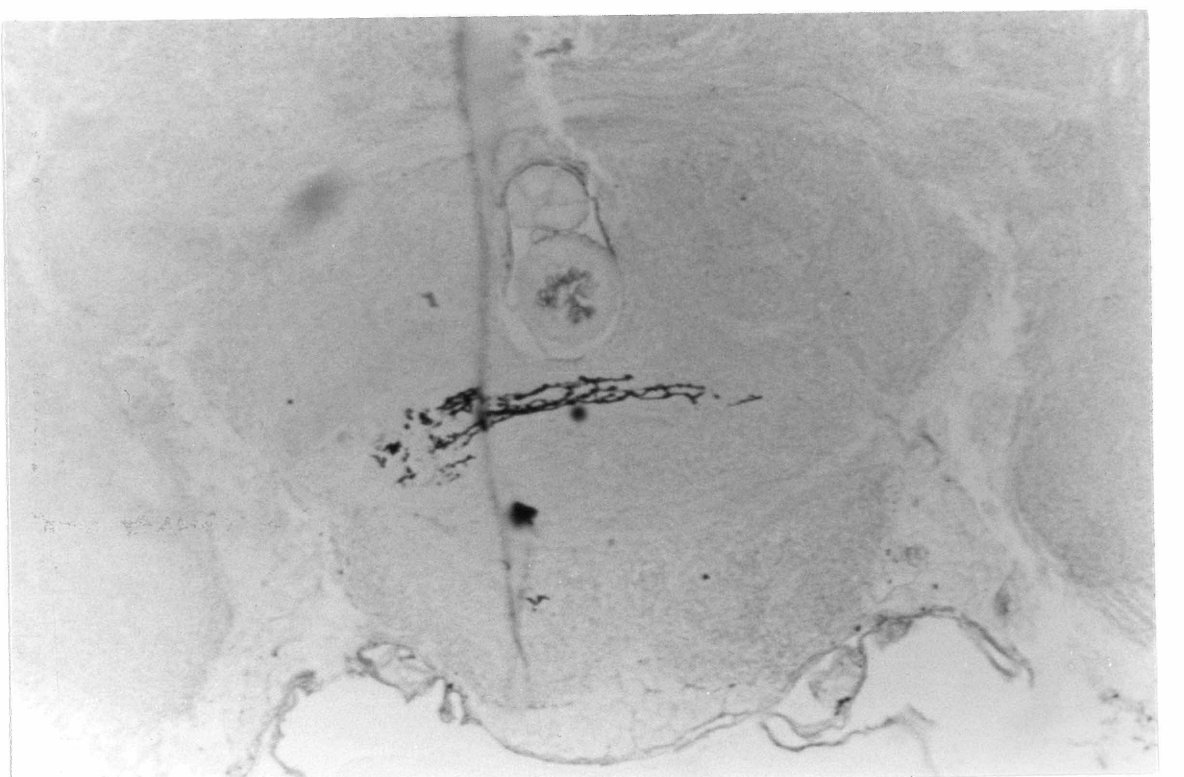


8d.

Figure 9. Diagram of the tarsal sensory projection. This was drawn from three superimposed camera lucida tracings of cobalt fills of each of the legs.

Pr: Prothoracic neuromere

Ms: Mesothoracic neuromere

Mt: Metathoracic neuromere

Horizontal view with anterior at left. Scale bar = 50μ

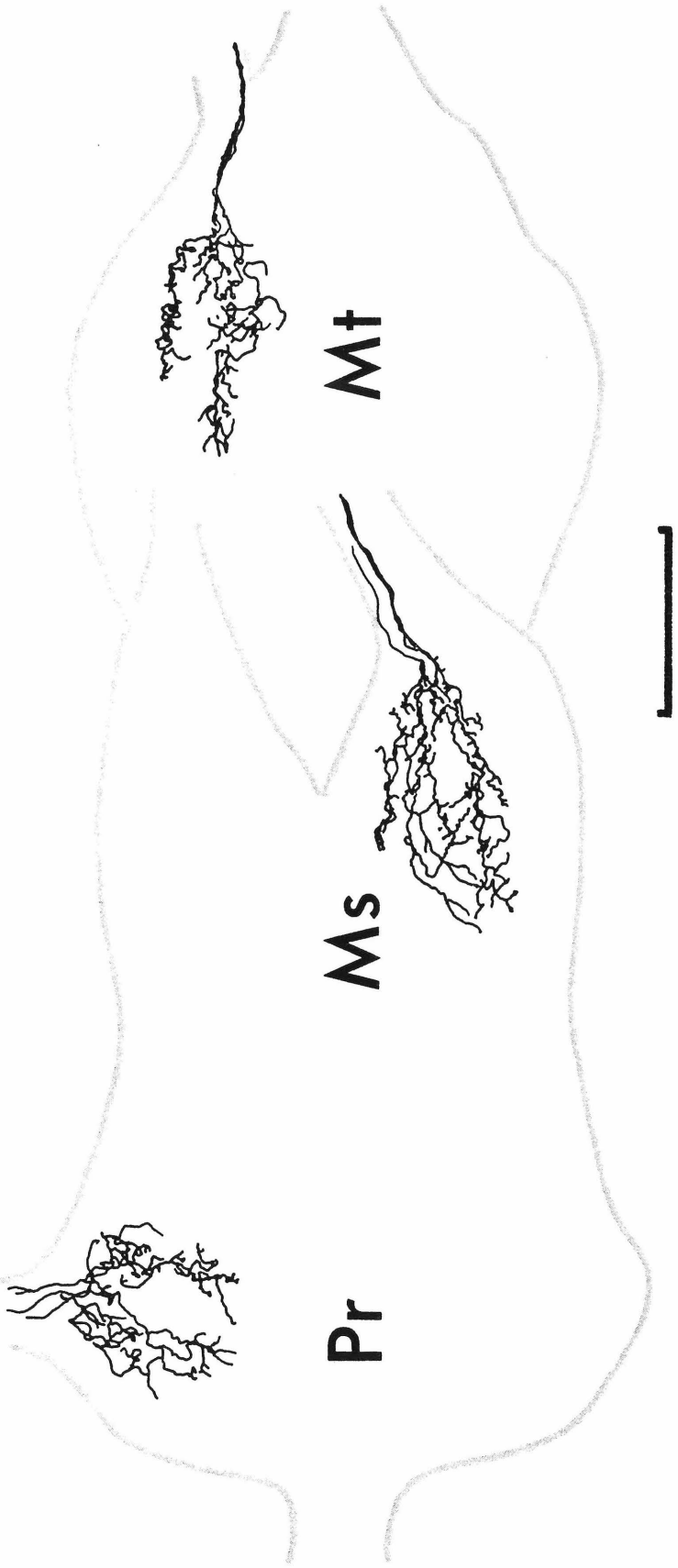
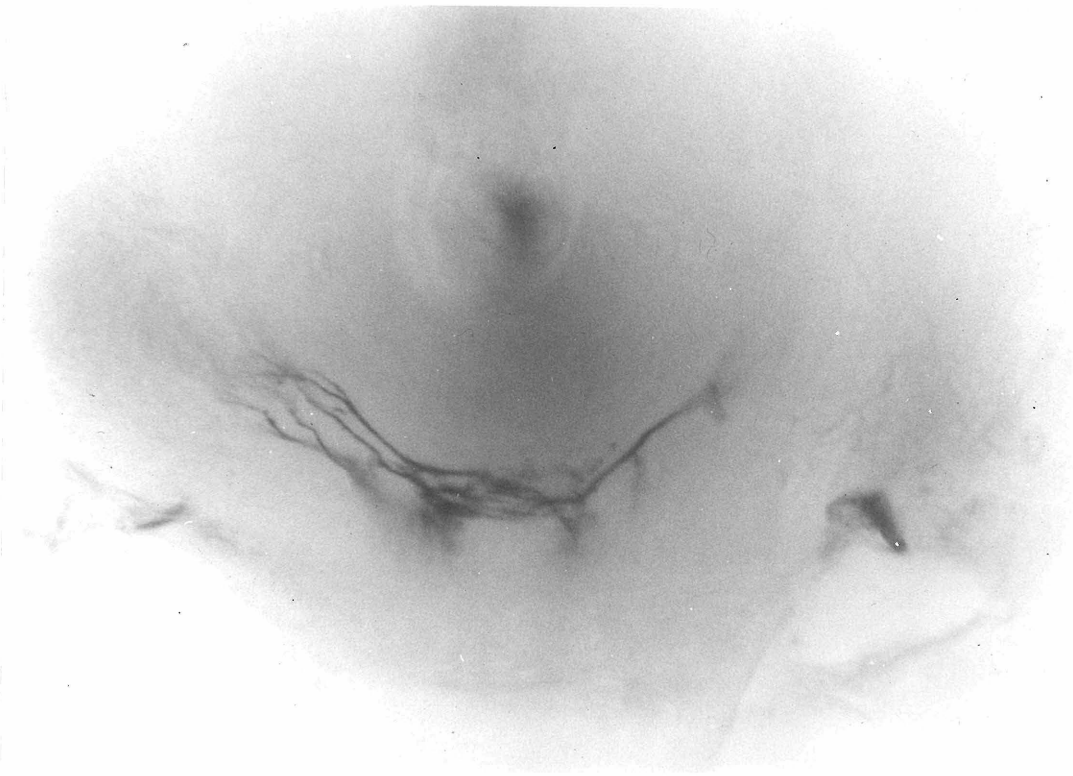


Fig.9

Figure 10. Photograph of a double bristle fill in an *ecd* fly. Right is a posterior orbital bristle, left is a bundle of axons from supernumerary bristles. Frontal view, dorsal at top. ×580



10.

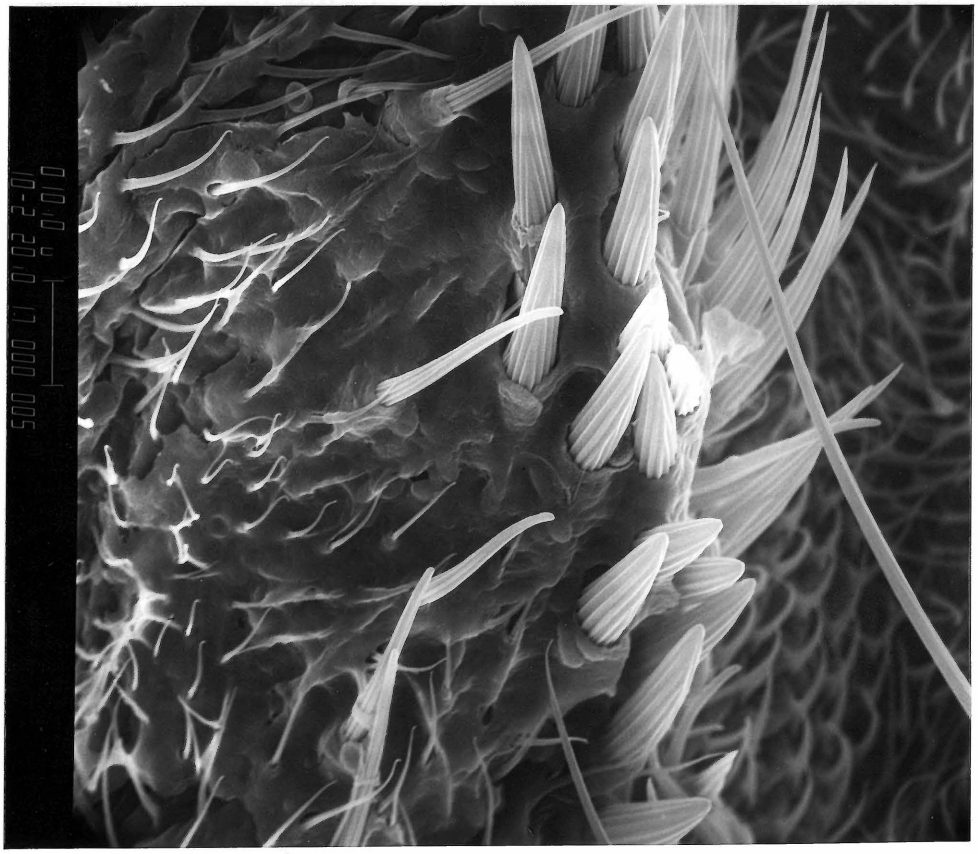
Figure 11. Photographs showing the nature of the ey^{Opt} phenotype.

(a) The eye and surrounding cuticle of an ey^{Opt} homozygote showing the winglike knob occupying much of the surface of the eye and large ectopic bristles dorsal to it. All flies used for cobalt fills had one or both of these features.

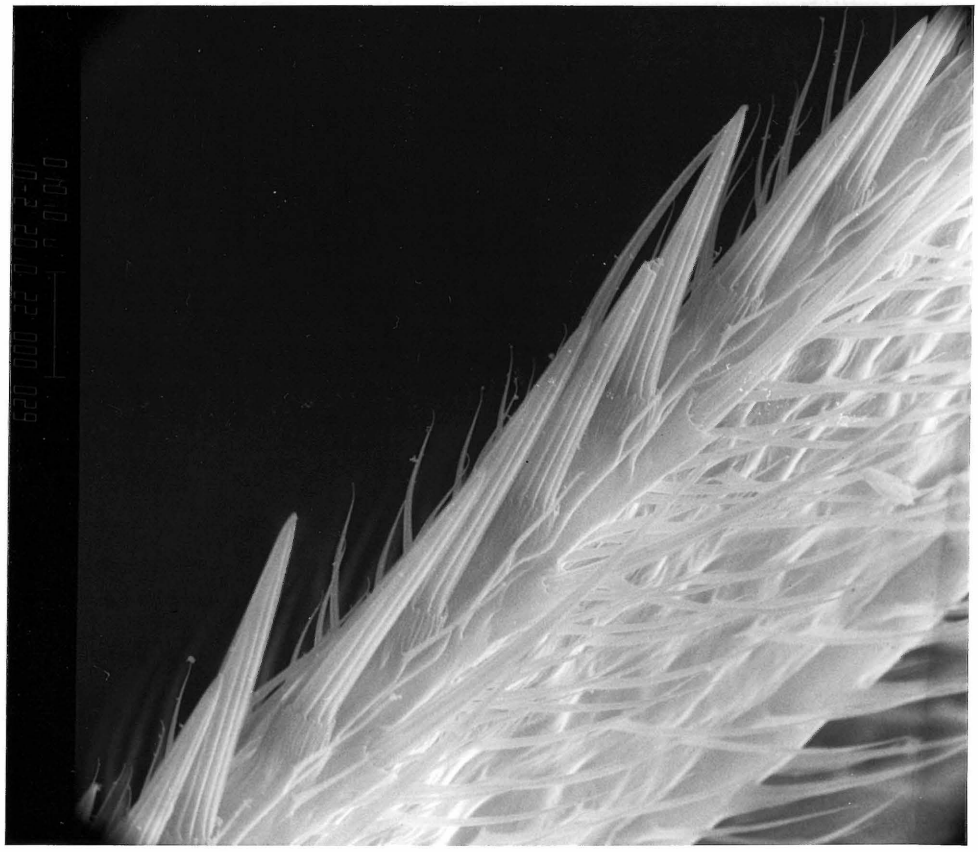
(b) Higher magnification view of the anterior edge of the winglike knob. The triple row of bristles characteristic of the anterior wing margin can be seen in a distorted, though recognizable, form: a dorsal row of double-curved bristles, a middle row of stout blunt-tipped bristles and a ventral row of sharp-tipped bristles punctuated with dorsal row-type double-curved bristles. Compare with (c)

(c) Anterior wing margin of a wild type (Canton-S) fly at the same magnification as (b).

11a.



11b.



11c.

Figure 12. Camera lucida tracings of projections into the brain from ectopic bristles in ey^{Opt} flies.

(a) An axon bundle entering through the antennal nerve and forming a projection roughly like that of wild-type head bristles but with a greatly reduced anterior-medial arborization. In addition, two axons can be observed to project laterally into the ventrolateral protocerebrum.

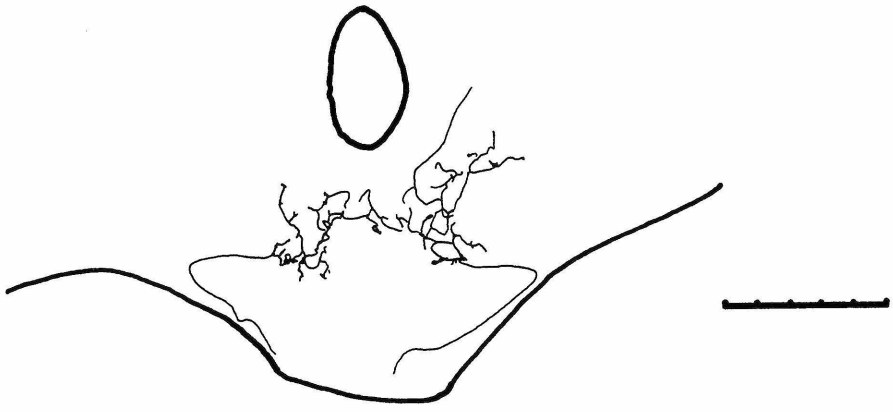
(b) Two bristles cobalt filled in the same fly: a posterior vertical bristle (left) and an ectopic bristle (right). Both enter in the posterior tegumental nerve and their arborizations can therefore be directly compared. The ectopic bristle axon lacks an anterior-medial arborization and projects into the dorsal subesophageal ganglion.

(c) Two axons entering through the antennal nerve but branching in the dorsal subesophageal ganglion.

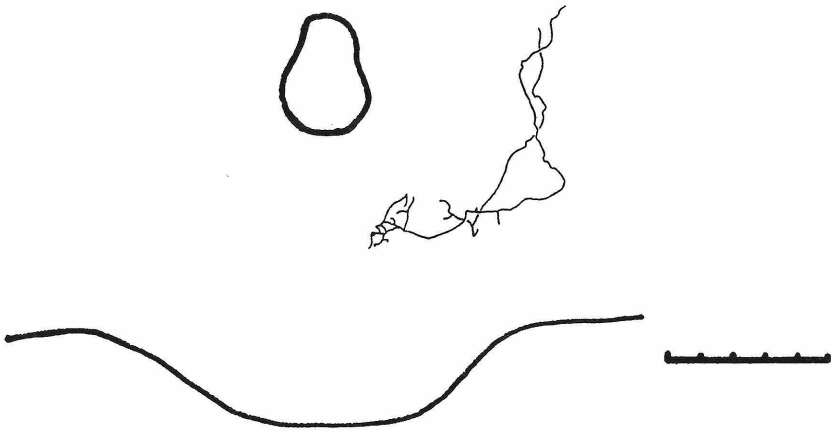
(d) Axon bundles entering through both the antennal and posterior tegumental nerves, making overlapping irregular projections in the dorsal subesophageal ganglion and deuterocerebrum.

Frontal view, dorsal at top. Scale bar = 50μ

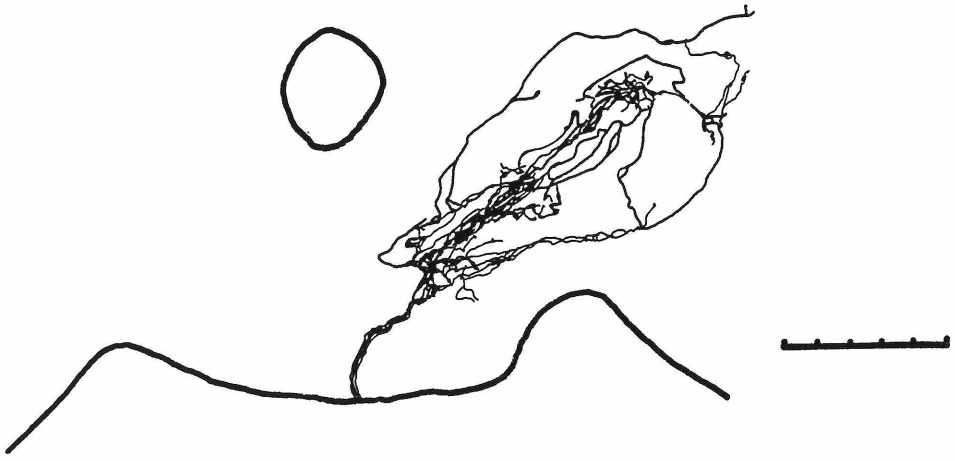
12a.



12b.



12c.



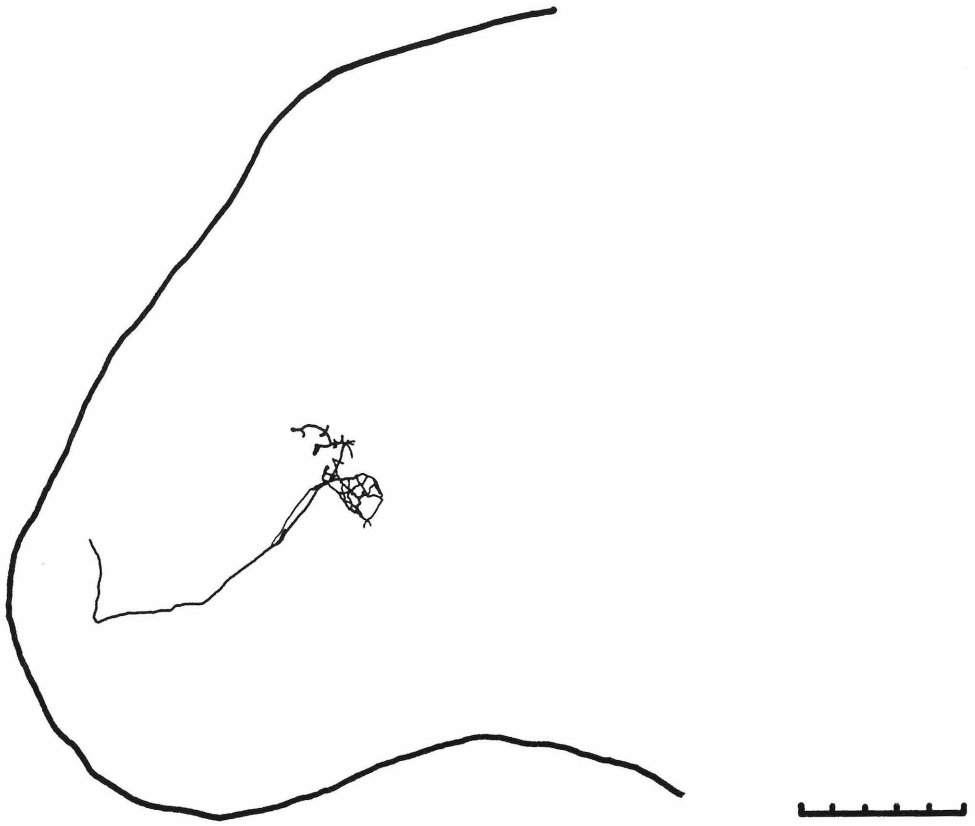
12d.

Figure 13. Camera lucida tracings of two projections into the optic lobe from ectopic bristles in ey^{Opt} flies.

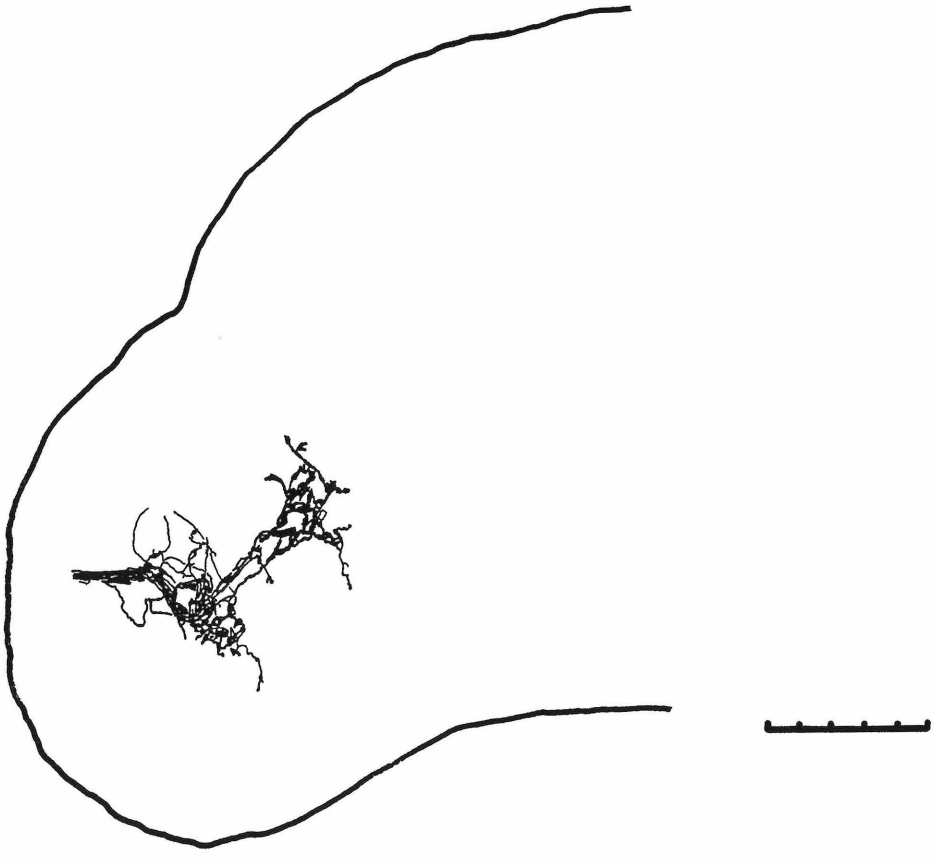
(a) Two axons entering the optic lobe and branching in the posterior lobula.

(b) A bundle of axons entering the optic lobe and branching in the medulla's serpentine layer as well as in the posterior lobula.

Frontal view, dorsal at top. Scale bars = 50μ



13a.



13b.

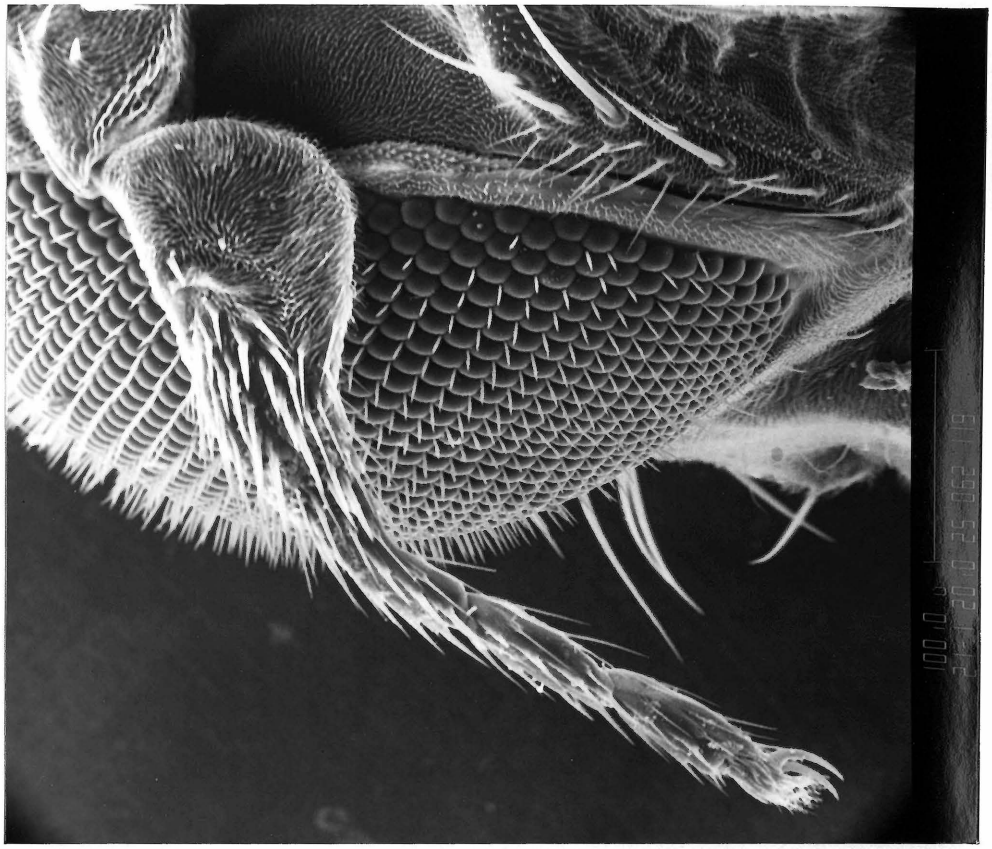
Figure 14. Photographs showing the nature of the antennal leg phenotype.

(a) An antennal leg from a fly of the genotype ss^{a}/sbd^{104}. The flagellum is reduced, the arista is missing and the distal antenna is instead a copy of the distal tarsus with all of the characteristic bristles included.

(b) An antennal leg from a fly of the genotype $Antp^{73b}/Pc^{3}$. Although there is nearly no antennal tissue and all leg segments are represented the appendage differs from a normal leg in at least two respects: There is a protuberance on the antennal leg coxa bearing a small number of bristles characteristic of the antennal flagellum. The distal tarsus is not completely transformed from antenna to leg but terminates in an arista-like structure instead of a claw.

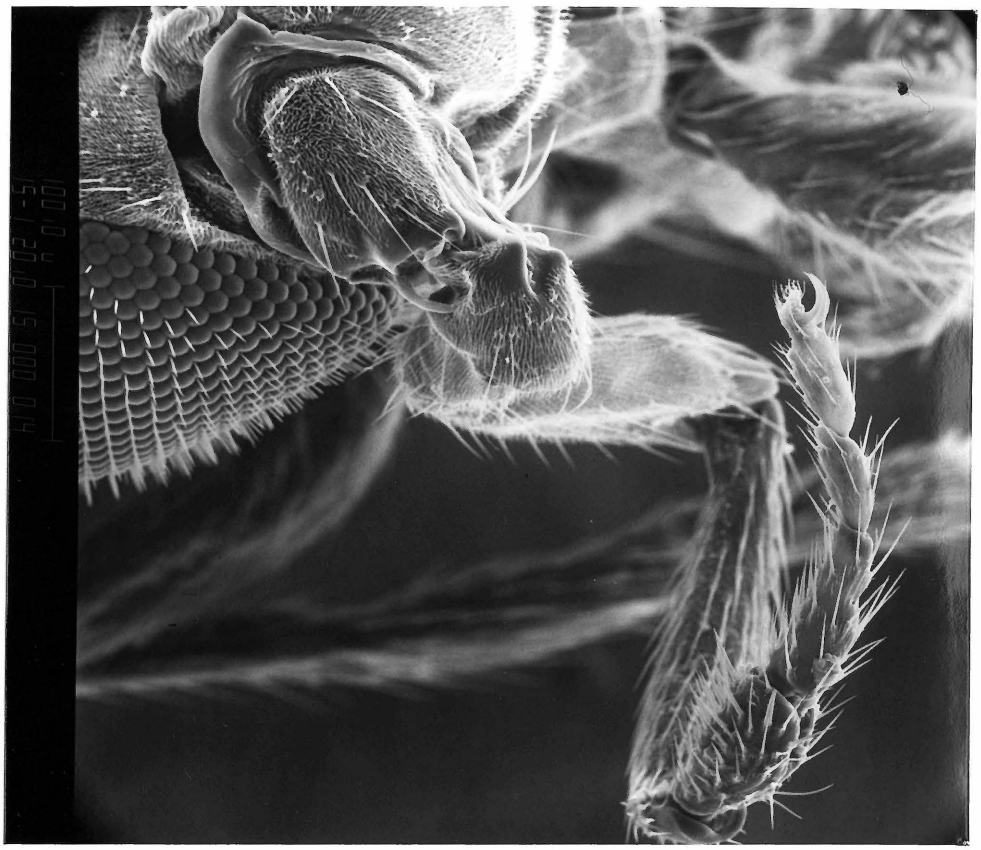
(c) An antennal leg from a fly of the genotype $Antp^{73b}\ Df(3R)sbd^{104}/\ Pc^{3}\ ss^{a}$. The transformation of antenna to leg appears to be complete externally.

(d) The distal tarsus of a leg from a wild-type (Canton-S) fly.



14a.

14b.



14c.

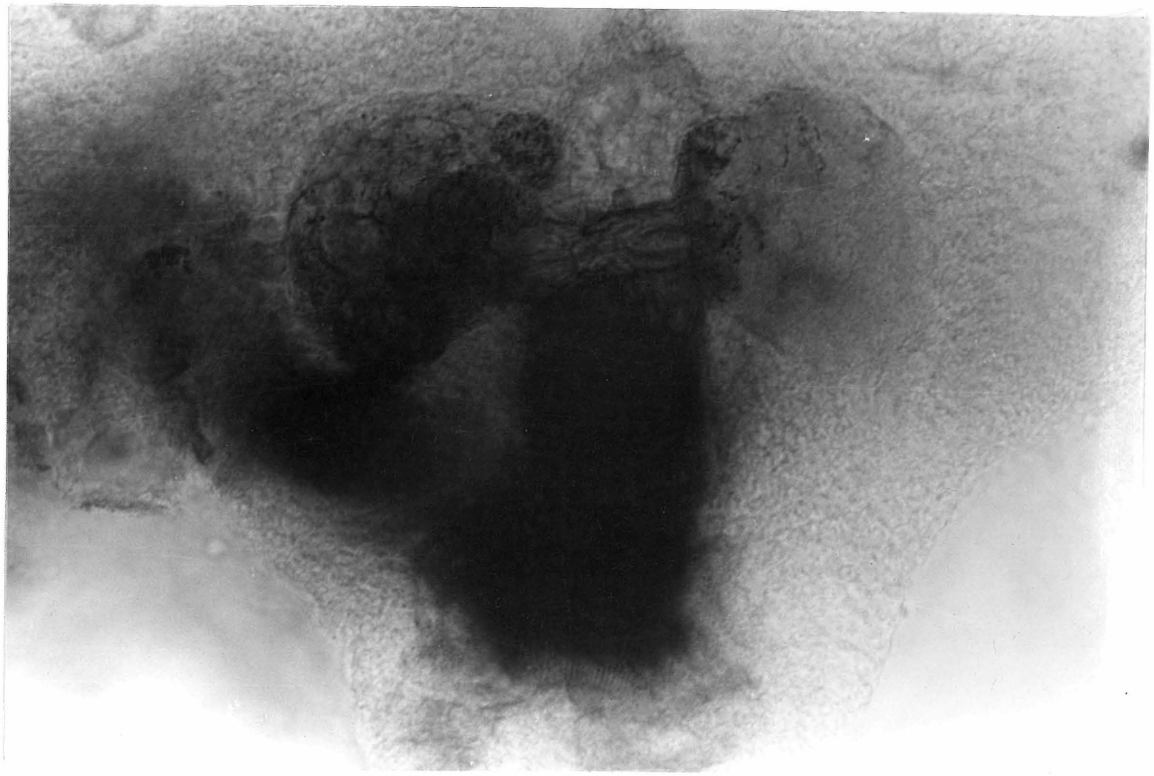
14d.

Figure 15. Projections of antennal leg sensilla in flies of the genotype ss^{a}/sbd^{104} into the olfactory lobe.

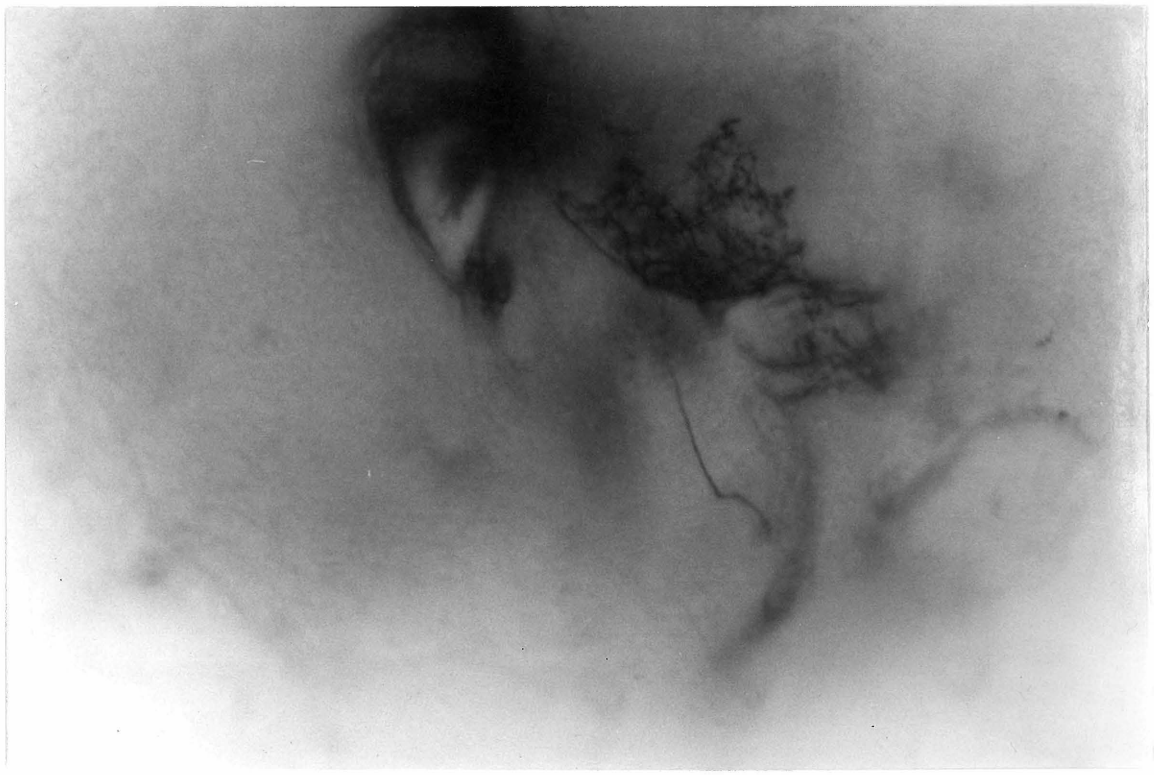
(a) Whole mount preparation in which the antennal leg was cut so as to expose distal tarsal and flagellar sensilla to the $CoCl_2$. The ventral olfactory lobe is densely filled with an irregular projection but axons forming heterolateral glomerular arborizations can be seen in the dorsal lobe.

(b) Whole mount preparation in which the antennal leg was cut in the distal distal tarsus. Only an irregular projection can be seen.

Frontal view, dorsal at top. ×580



15a.



15b.

Figure 16. Projections of antennal leg distal tarsal sensilla in *Antp*^{73b} *Df(3R)sbd*^{104} / *Pc*^{3} *ss*^{a} flies into the olfactory lobe, anterior subesophageal ganglion and tritocerebrum.

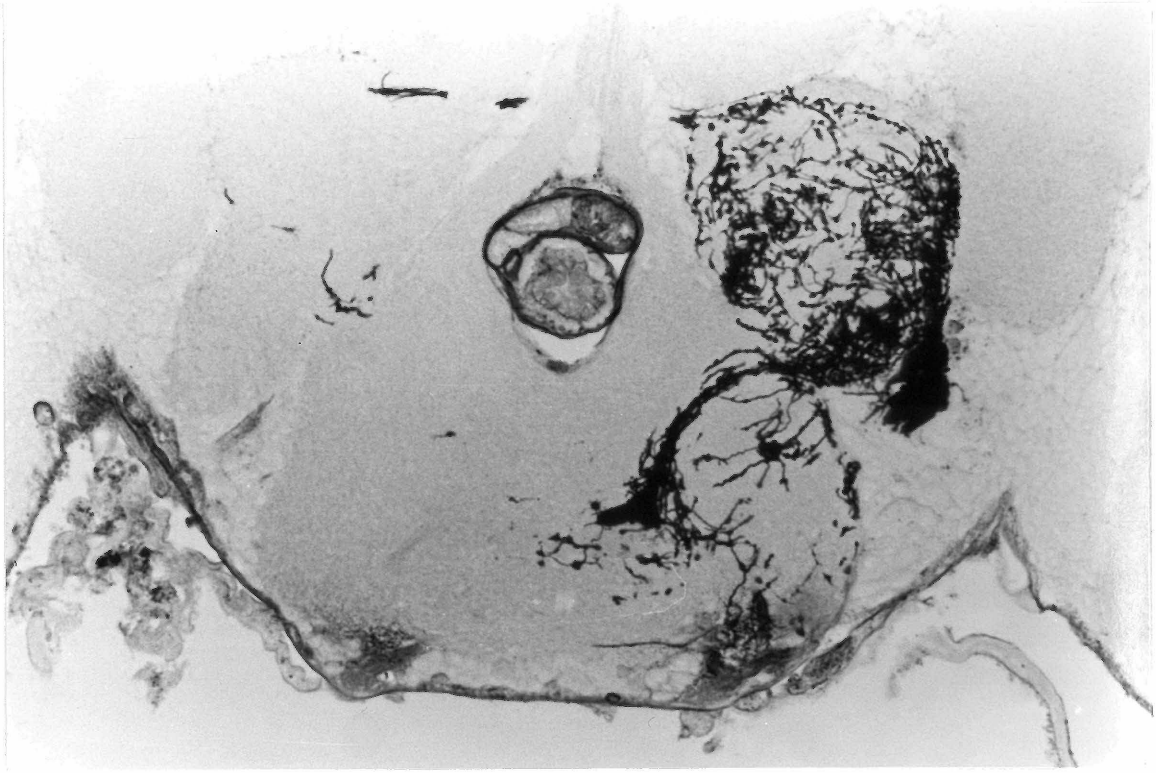
(a) Photograph of a frontal section cut through the brain 37μ from the anterior surface showing the irregular sensory arborization in the olfactory lobe and part of the anterior-ventral group of adventitious axons leaving the lobe to enter the subesophageal ganglion.

(b) Photograph of a frontal section cut through the brain 46μ from the anterior surface showing the olfactory lobe and part of the posterior-dorsal group of adventitious axons leaving the lobe to enter the subesophageal ganglion. This is from the same preparation as (a).

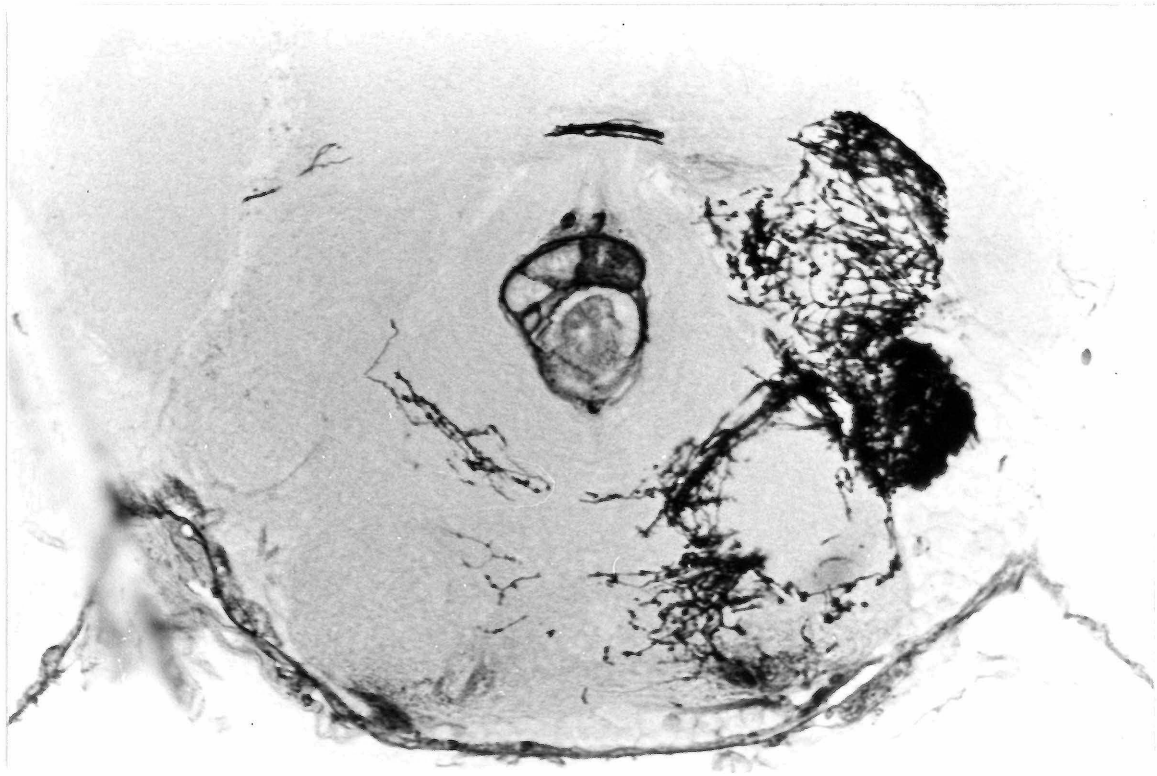
(c) Photograph of a frontal section cut through the brain 39μ from the anterior surface. Unlike the preparation shown in (a) and (b) this has an adventitious group of axons which enter the subesophageal ganglion directly from the antennal nerve in the region of the olfactory lobe (distinguishing it from the HB-like projection which is more posterior) in addition to those entering the subesophageal ganglion from the lobe. Axons can be seen entering both ventral and dorsal regions of the subesophageal ganglion.

(d) Photograph of a frontal section cut through the brain 41μ from the anterior surface showing the olfactory lobe and axons projecting from its dorsal surface into the dorsal protocerebrum.

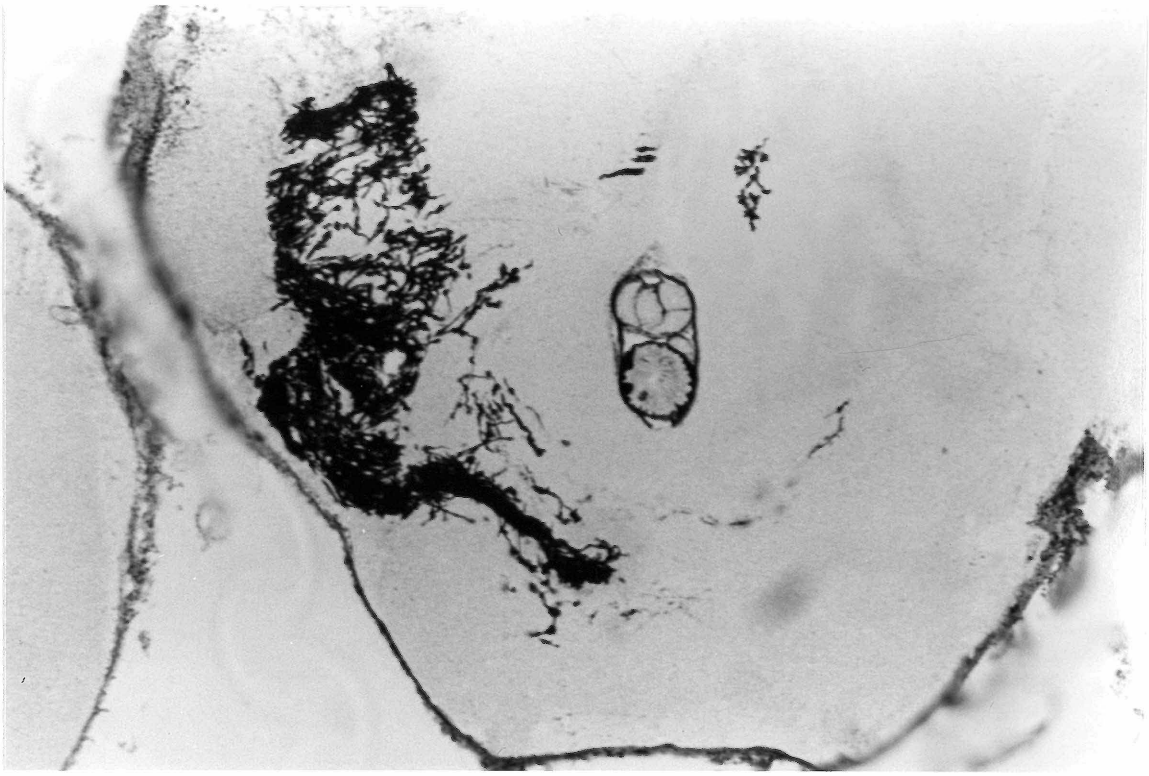
Frontal sections, dorsal at top. ×580



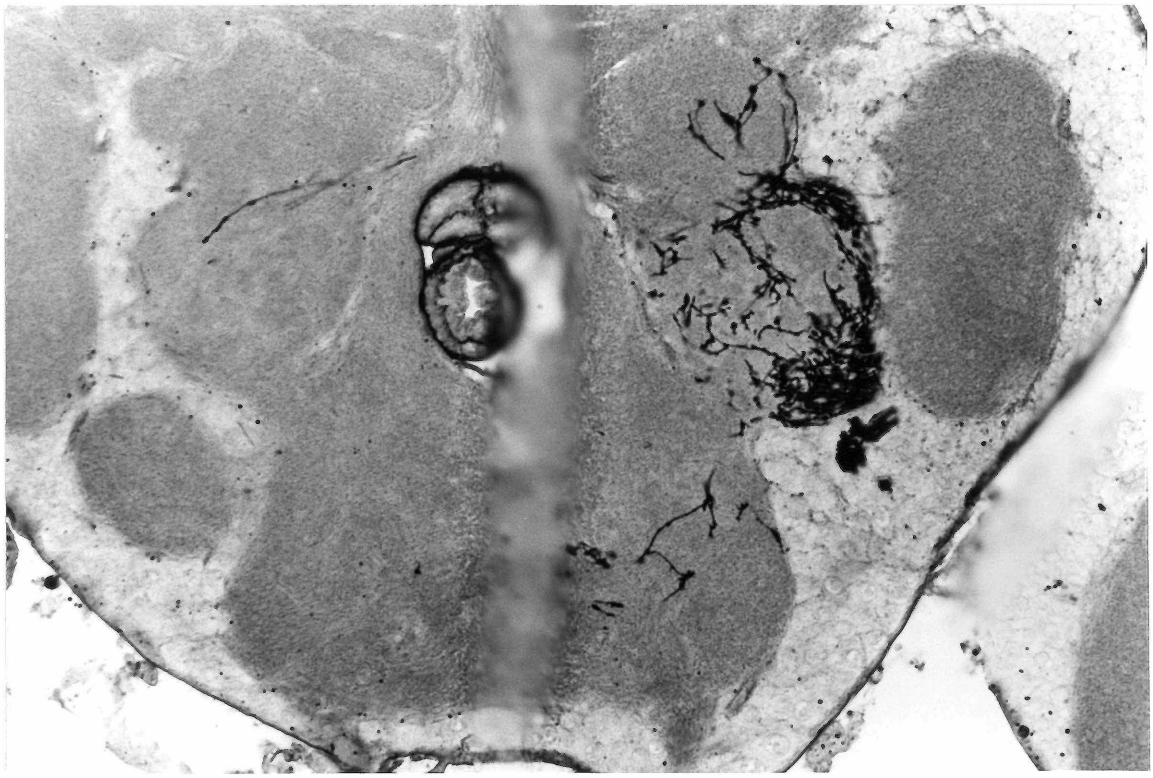
16a.



16b.



16c.



16d.

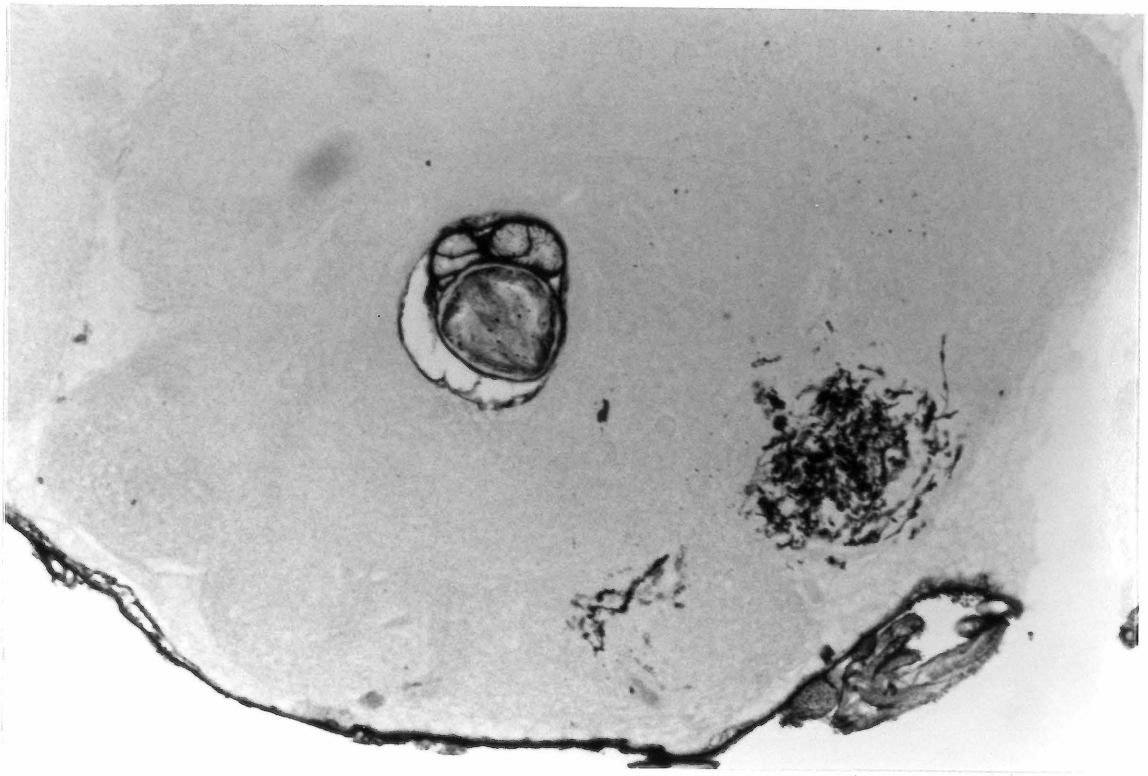
Figure 17. Non-olfactory lobe projections of antennal leg distal tarsal sensilla in flies of the genotype $Antp^{73b}$ $Df(3R)sbd^{104}$ / Pc^{3} ss^{a}.

(a) Photograph of a frontal section cut through the brain 54μ from the anterior surface showing part of the HB-like projection.

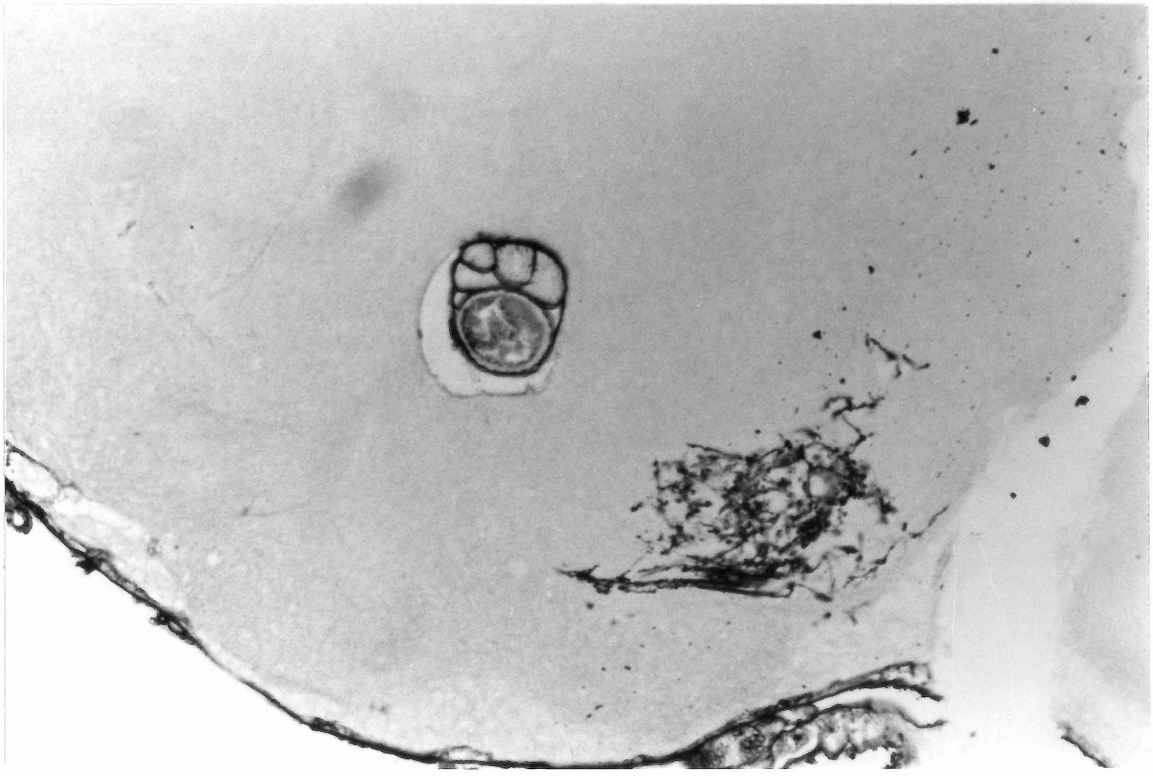
(b) Photograph of a frontal section cut through the brain 80μ from the anterior surface showing the projection into the subesophageal ganglion of the ventral bundle of lateral antennal nerve fibers.

(c) Photograph of a frontal section cut through the brain 96μ from the anterior surface showing the projection of the medially projecting antennal nerve fibers.

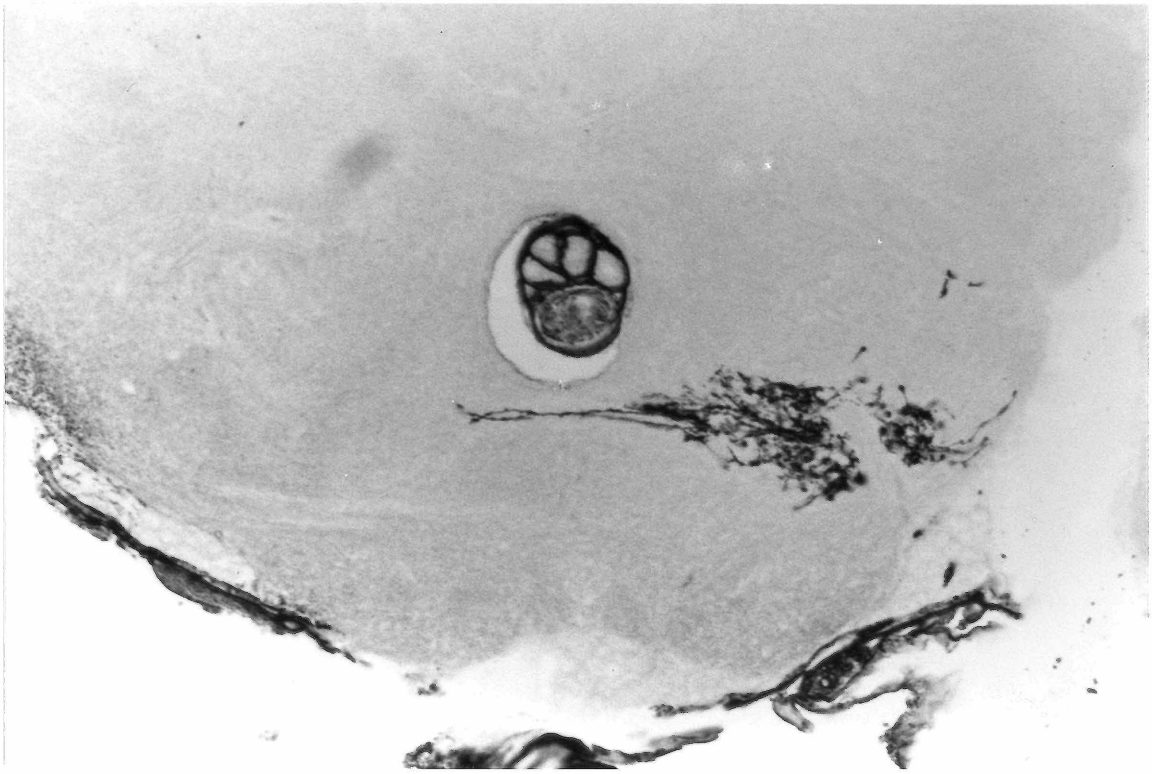
Frontal sections, dorsal at top. ×580



17a.



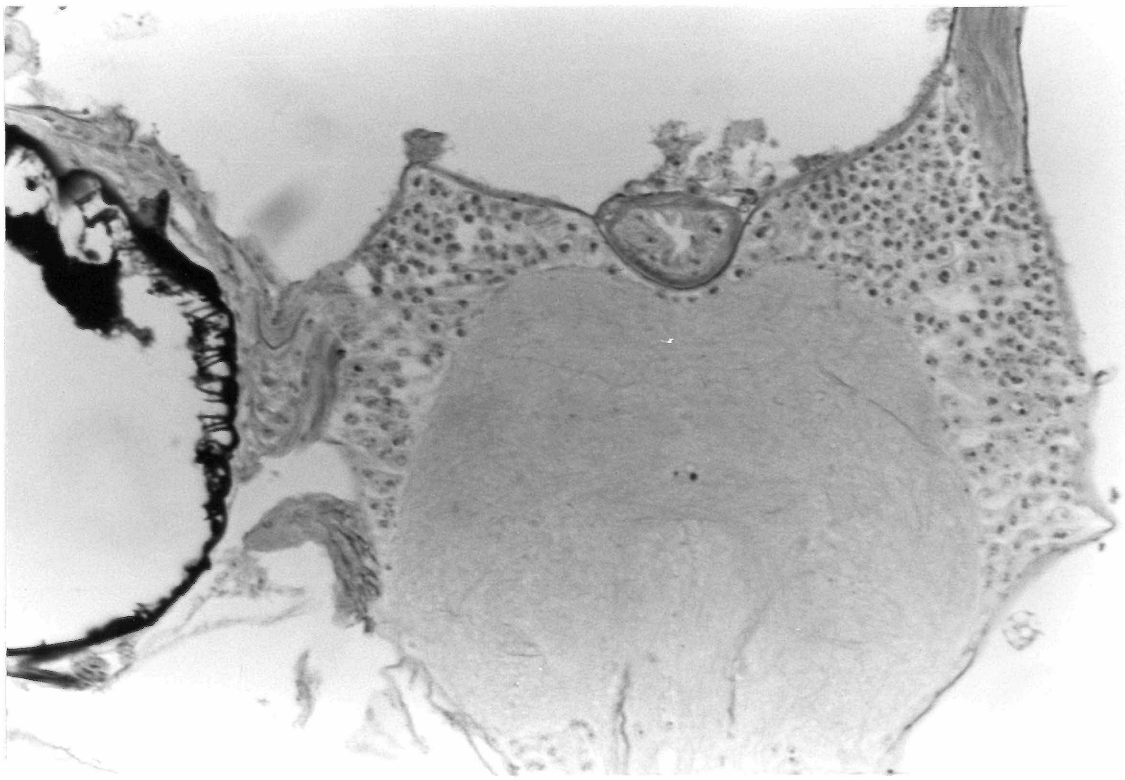
17b.



17c.

Figure 18. Silver stained preparation showing a noneverted antenna in the optic lobe of an *ant* fly. A large bundle of fibers can be seen leaving the antenna and running through the cellular cortex to enter the subesophageal ganglion and, presumably, other antennal targets. Part of the contralateral, unaffected antenna and antennal nerve can be seen.

Horizontal section, anterior at top. ×580



18.

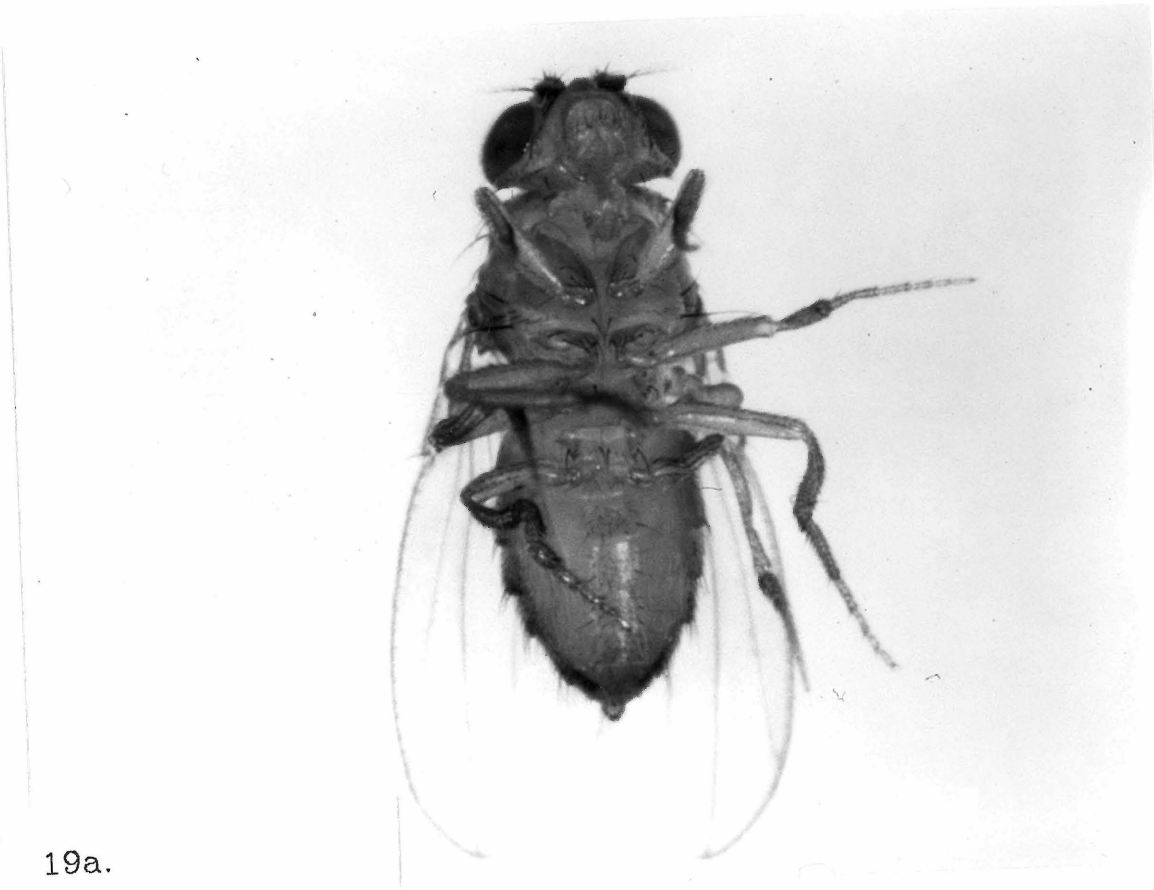
Figure 19. Comparison of the sensory projections from the distal tarsus of a wild-type metathoracic leg and a leg on the first abdominal segment of a *bxd*^{51j} / *Df(3)P2* fly.

(a) Photograph of the ventral side of an eight-legged fly of the genotype *bxd*^{51j} / *Df(3)P2* showing the external phenotype.

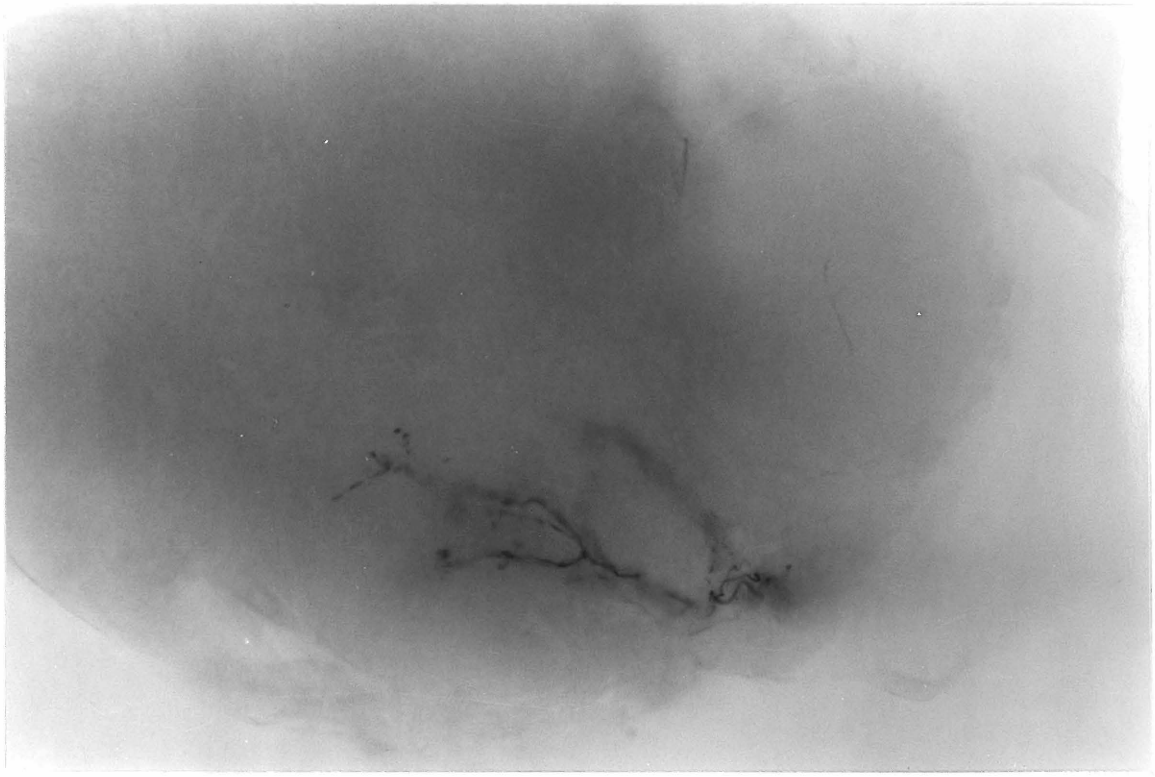
(b) Horizontal view of the metathoracic neuromeres of a *bxd*^{51j} / *Df(3)P2* fly, HRP filled from the first abdominal leg tarsus showing a projection like that of the wild-type metathoracic leg shown in (d). Anterior at left. ×580

(c) Horizontal view of the metathoracic neuromeres of a *bxd*^{51j} / *Df(3)P2* fly, HRP filled from the first abdominal leg tarsus showing a projection that comprises only the medial branch of the normal U-shaped arborization. Anterior at left. ×580

(d) Horizontal view of the metathoracic neuromeres of a wild-type fly, anterior at left. The metathoracic leg tarsus was cut and exposed to horseradish peroxidase (HRP). The sensory projection is medial and the motorneurons that took up HRP can be seen on the lateral edge of the neuromere. ×580



19a.



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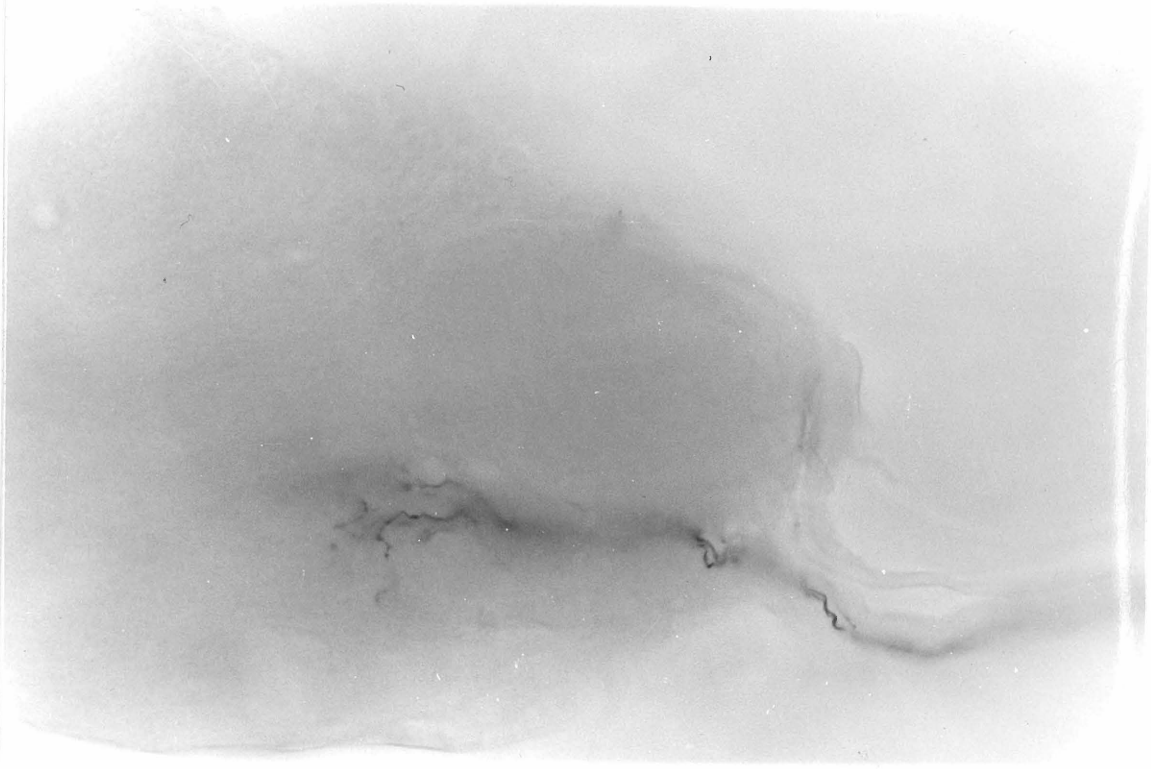

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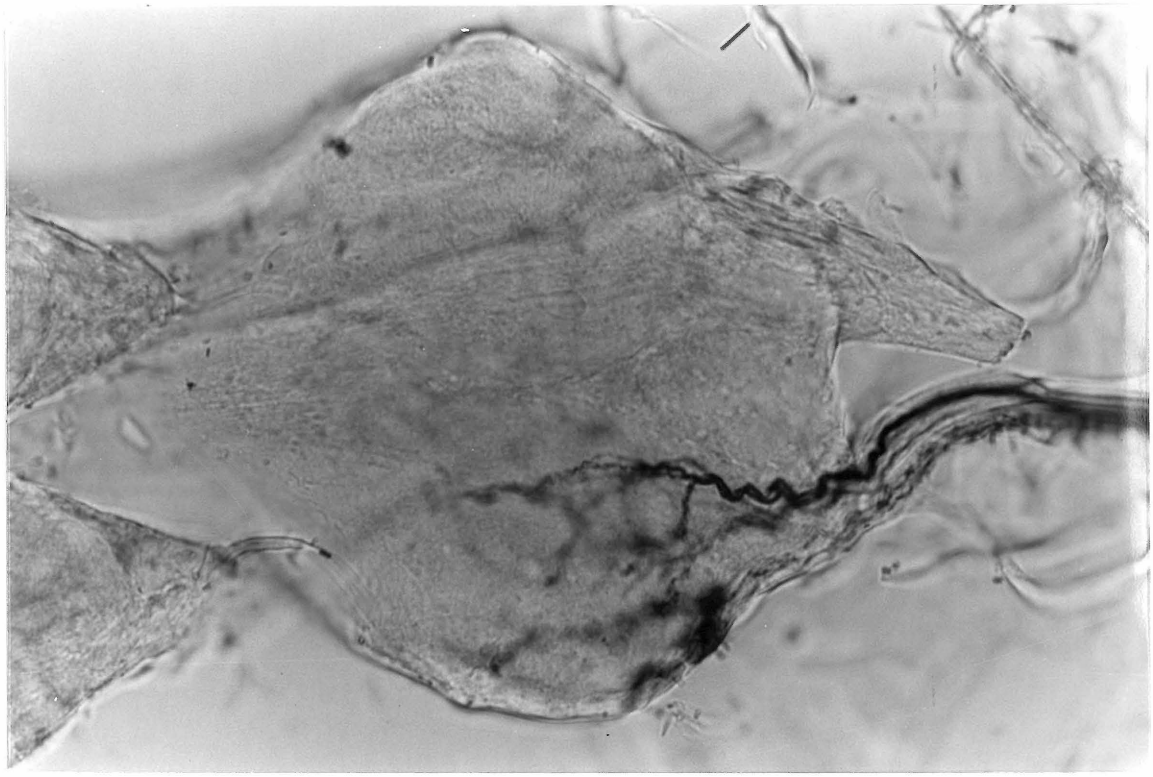

19d.

Figure 20. Diagram of those CNS neurons transneuronally cobalt filled from the antenna (or antennal leg) that ramify primarily ipsilaterally. Transneuronally filled cells are in black and primary sensory projections in gray. The sensory axons shown on the left side are those within the antennal nerve and the complete projection of the lateral fibers. In black is shown the group of small central neurons with fine axons that appear to associate the dorsal bundle of lateral antennal nerve fibers with the ventrolateral protocerebrum.

On the right side the rest of the primary antennal sensory projection is shown: the heterolateral olfactory lobe projection (wild-type), the HB-like projection and the medial fiber projection. From the olfactory lobe a thick bundle of axons ascends the antennal-glomerular tract to the ipsilateral mushroom body calyx and the dorsolateral protocerebrum. The cell bodies of these axons are those just lateral to the olfactory lobe. A small number of thin axons runs directly to the dorsolateral protocerebrum from the antennal mechanosensory area.

This was drawn from three superimposed camera lucida tracings of wild-type antennal cobalt fills. Frontal view, dorsal at top. Scale bar = 50μ

20

Figure 21. Diagram of those CNS neurons transneuronally cobalt filled from the antenna (or antennal leg) that ramify heterolaterally. Transneuronally filled axons and cells are in black or dark gray and primary sensory projections are in gray. Shown are all of the non-olfactory lobe primary sensory projections as they all seem to be associated with some of the transneuronally filled neurons drawn here. The commissure dorsal to the esophagus (central commissure) contains the large axons that run dorsally from the antennal mechanosensory area. These axons, including the giant fibers, have cell bodies located in the cellular cortex at the posterior surface of the brain and not shown here.

Just ventral to the esophagus are several large axons with cell bodies in the cortex lateral and anterior to the antennal mechanosensory area. Ventral to these are bundles of axons with cell bodies in the cortex ventral and posterior to the axons.

This was drawn from five superimposed camera lucida tracings of wild-type antennal cobalt fills. Frontal view, dorsal at top. Scale bar = 50μ

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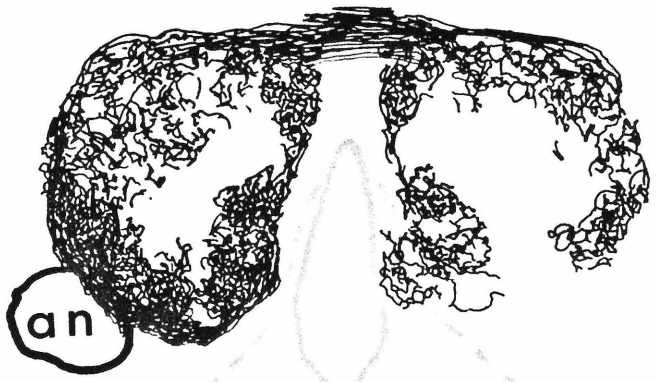
Figure 22. Summary diagram of the wild-type antennal sensory projection into the olfactory lobe. To show most of the characteristic features this was drawn so that successively more dorsal portions of the diagram represent successively more dorsoposterior portions of the projection. *a n*: antennal nerve.

Frontal view, dorsal at top. Scale bar = 50μ



22

Figure 23. Summary diagram of the *Antp*^{73b} *Df(3R)sbd*^{104} / *Pc*^{3} *ss*^{a} antennal leg tarsal sensory projection into the olfactory lobe. Also shown are the adventitious projections from the olfactory lobe and antennal nerve into the subesophageal ganglion, tritocerebrum and protocerebrum. This was drawn from five superimposed camera lucida tracings so as to show all possible adventitious projections. An individual preparation will generally display only a subset of them. Specifically, what is shown outside of the olfactory lobe (roughly ventral to dorsal) are: the axons of the anteroventral adventitious projection with an irregular arborization in the subesophageal ganglion and tritocerebrum and many axons coursing in a layer just under the cellular cortex (a common feature), axons running from the antennal nerve directly into the subesophageal ganglion (an uncommon feature), the dorsoposterior adventitious projection with axons entering the dorsal subesophageal ganglion especially a discrete region of neuropil between the ventromedial borders of the two olfactory lobes, axons leaving the dorsal border of the olfactory lobe and entering the protocerebrum. *a n*: antennal nerve.

Frontal view, dorsal at top. Scale bar = 50μ

23

Figure 24. Summary diagram of the antennal or antennal leg HB-like projection into the subesophageal ganglion. *a n*: antennal nerve.

Frontal view, dorsal at top. Scale bar = 50μ

24

Figure 25. Summary diagram of the antennal or antennal leg medial fiber and lateral fiber projections into the subesophageal ganglion. *a n*: antennal nerve.

Frontal view, dorsal at top. Scale bar = 50μ

25

Part II

Segment-specific organization of leg motoneurones is transformed in bithorax mutants of *Drosophila*.*

* Green, S. H. (1981) Segment-specific organization of leg motoneurones is transformed in bithorax mutants of *Drosophila*. *Nature* **219**, 652-4.

In Drosophila, genes controlling segmentation in the thorax and the abdomen are clustered in one region of the genome known as the bithorax complex. Studies of the genetics of this complex suggest that loss of activity of a gene causes transformation of a particular segment to a more anterior one, mesothorax representing the ultimate transformation[1]. This transformation is well described for the epidermis, but it is not clear whether other segmentally arranged tissues are also transformed. The segmental ganglia are fused, in Drosophila, into a single compact mass termed the thoracic ganglion but the segmental organization of the nervous system is still apparent. There are discrete regions of neuropil, termed neuromeres, corresponding to the three thoracic segments: prothorax, mesothorax and metathorax. A small terminal neuromere corresponds to the abdominal segments. Evidence is presented here that the leg motorneurons of each of the three thoracic segments are arranged in a segment specific pattern in the thoracic ganglion. In mutant flies which have the metathoracic cuticle transformed to mesothoracic, the arrangement of the metathoracic leg motorneurons can be altered to resemble that of the mesothoracic leg motorneurons.

The present study makes use of horseradish peroxidase (HRP) filling of cells to study the innervation of the leg. This enzyme is applied to a cut made in the tarsus and is transported through those sensory axons that have been cut. It is also taken up by motorneuron terminals and transported in a retrograde fashion so as to fill the entire cell. The HRP filled cells can be visualized by any one of several methods[2, 3]. Fig. 1 is a composite drawing showing leg motorneurons in each neuromere. The arrangement of the leg motorneurons is different in the three neuromeres. In the prothoracic (anterior) neuromere, the cell bodies are in a region of the cortex anterior to the neuropil. The axons project back into the neuropil, into which they branch, collect into a loose bundle and course laterally

and ventrally to leave the ganglion in the prothoracic leg nerve. In the mesothoracic (middle) neuromere, the cell bodies are in the cortex anterior and ventral to the neuropil. The axons project posteriorly to exit through the mesothoracic leg nerve. Branches are directed dorsally into the lateral neuropil, segregated from sensory neuron arborizations which are in the medial region. In the metathoracic (posterior) neuromere, the motorneuron cell bodies lie lateral and somewhat posterior to the neuropil. Their axons project anteriorly from the cell bodies, then loop back and then run posteriorly to the nerve. All the axons loop back at the same point although the cell bodies are spread through a region 30-60 μ posterior to the loop. As in the mesothoracic neuromere, the motorneurons' arborizations are laterally placed in the neuromere, not overlapping the medially placed sensory neurons' arborizations. In each neuromere a single motorneuron sends a branch contralaterally. These cross in commissures located posteriorly in the prothoracic and mesothoracic neuromeres but anteriorly in the metathoracic neuromere. In the mesothoracic neuromere this motorneuron has its cell body located anterior to the other motorneuron cell bodies. Thus, in terms of the positions of the leg motorneuron cell bodies and of the commissure, the metathoracic neuromere is the reverse of the anterior two neuromeres along the anterior-posterior axis.

Similar results were obtained by cobalt filling of axons at the cut end of the coxa using the method of ref. 4. Furthermore, observations of silver stained ganglia by Power[5], repeated in this lab, reveal large cells with an arrangement like that of the backfilled motor-neurons and with a similar nuclear morphology. Therefore, the differences in the motorneuron pattern in the three neuromeres do not appear to result from uptake of HRP by different subpopulations of an identically arranged motorneuron population in each neuromere. The arrangement of the leg motorneurons as described above

is the same in males and females of the D. melanogaster wild type strains Canton-S and Oregon-R, the mutant spineless-aristapedia, and Drosophila virilis.

The bithorax complex mutations abx (ref. 6) and bx transform anterior metathorax to anterior mesothorax, pbx does the same to the posterior compartment. Flies of the genotypes abx bx^3 pbx/abx bx^3 pbx and abx bx^3 pbx/Df(3R)P2 (ref. 7) (derived from stocks provided by Dr. E. Lewis) can have the cuticle of the metathoracic segment nearly identical to that of the mesothoracic, although expression (degree of change) is variable and incomplete transformations are seen. In all cases studied, the metathoracic leg itself was completely transformed into a mesothoracic leg, as determined by the pattern of leg bristles. Metathoracic legs of these flies were cut and HRP applied as for the wild-type flies. Of 27 successful backfills, 4 showed altered patterns of motorneuron arrangement that resembled mesothoracic motorneurons and 9 showed intermediate phenotypes; i.e. unusually positioned motorneurons, suggestive of a mesothoracic pattern but resembling neither pattern entirely. The remaining 14 backfilled legs showed the wild type arrangement of motor neurons. Of 41 successful metathoracic leg backfills in flies wild-type for bithorax, no deviations from the metathoracic pattern were ever observed. This is consistent with the hypothesis that the central nervous system (CNS) can be segmentally transformed by the mutations. As with the epidermis, there is variability in expression although the mutations' expression and penetrance (fraction of flies affected) are far less in the CNS than in the cuticle.

Fig. 2 shows an example of a metathoracic neuromere with a mesothoracic pattern of motorneuron arrangement. The motorneuron cell bodies lie anterior and ventral in the cortex, the axons project posteriorly through the neuropil. Fig. 2 also shows that the cell which projects contralaterally is anterior to the other cells, just as in the mesothoracic neuromere. Thus, a unique metathoracic neuron appears to be converted into its mesothoracic homologue.

Nevertheless, the transformation to the mesothoracic pattern is not complete. The contralaterally projecting fiber takes an anterior route as in the wild-type metathoracic neuromere.

Two examples of intermediate phenotypes are shown in fig. 3: fig. 3a shows a metathoracic neuromere containing motorneurons in intermediate positions. Fig. 3b shows a ganglion with metathoracic leg motorneurons that are positioned properly but have axons that project posteriorly into the nerve instead of looping anteriorly. Possibly, cell body position and direction of axon growth are under separate control by anterior-posterior positional information.

Previous neuroanatomical studies[8, 9] of bithorax used these mutants as a system for the study of sensory projections from the ectopic cuticle. These studies did raise the question of whether the mutations affect the CNS itself. Palka et al. found sufficient differences between bithorax and wild-type ganglia and sensory pathways to suggest that the CNS was altered by the mutations. They found additional evidence for this by considering the pattern of projection of sensory neurons from clones of mutant cuticle into presumably wild-type ganglia. Ghysen, stressing the overall similarity in the anatomy of wild-type and bithorax ganglia and sensory pathways concluded that the CNS was not altered in the specific pathways studied.

This study approaches the question directly by studying neurons with cell bodies in the CNS. It has also profited from the use of the recently constructed combination abx bx^3 pbx to obtain more extreme transformations than were previously available. The observations reported here do not imply that the genes of the bithorax complex directly controls the CNS segmental pattern. They may result from a direct effect of the genes in another tissue which in turn affects the CNS by induction or by mechanical constraints on CNS growth. Experiments using mosaic flies should determine which tissue must be mutant for the pattern of organization

of leg motorneurons to be transformed. This should be relevant to the general problem of mechanisms underlying segmental differences in the CNS.

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FIGURE 1: A wild-type thoracic ganglion is shown in horizontal plane. This drawing is a composite of three separate backfills, traced from photomicrographs onto one thoracic ganglion outline. Only one side is shown for each neuromere. As many as 9 motorneurons have been filled from a given leg but generally fewer are seen as in the pro- and mesothoracic examples shown here. In anterior to posterior order, the prothoracic (Pr), mesothoracic (Ms), metathoracic (Mt) and abdominal (Ab) neuromeres are labeled. The leg nerves are also labeled: PrLN for prothoracic leg nerve, MsLN for mesothoracic leg nerve and MtLN for metathoracic leg nerve. Sensory axons filled in these preparations were not included in the drawings.

The motorneurons were filled by applying 20% HRP + 3% α-lysolecithin to a cut at the distal end of a leg. Backfill time was 10-14 hours. The ventral thorax was removed and fixed with 1.25 glutaraldehyde + 1% formaldehyde in 0.1M phosphate buffer (pH 7.3) for 30 minutes. The ganglion was removed from the cuticle, fixed an additional 45 minutes, and washed 3 x 30 minutes in phosphate buffer + 10% sucrose.

The HRP was visualized by one of the following two methods: (1) A method modified from one described in reference 2: the ganglion was washed 2 x 5 minutes in Hanker-Yates reagent (1.5 mg/ml in 0.05M Tris buffer, pH 7.6). This was followed with a 3-6 minute incubation in fresh reagent + 0.006% hydrogen peroxide. The

reaction, which colors the HRP filled neurons reddish-brown, was observed with a dissecting microscope to determine the appropriate endpoint. The ganglion was then washed 3 x 5 minutes in Tris buffer, dehydrated in ethanol (one minute each 70% and 95%, 3 x one minute 100%), cleared in methyl salicylate and mounted in immersion oil. (2) A method modified from one described in reference 3: the ganglion was kept for 15 minutes in a medium made up as follows: 0.25 ml of 3,3 , 5,5 -tetramethyl benzidine solution (2 mg/ml in 100% ethanol) was added to 10 ml of sodium ferricyanide solution (1 mg/ml in 0.01M acetate buffer, pH3.3), both solutions freshly made up. Hydrogen peroxide was then added to a final concentration of 0.006% and the ganglion was kept in this for 4-6 minutes. This reaction colors the HRP filled neurons blue. As above, the actual reaction time was determined by observing the reaction with a dissecting microscope. The ganglion was washed 3 x 5 minutes with acetate buffer and further processing was as described above.

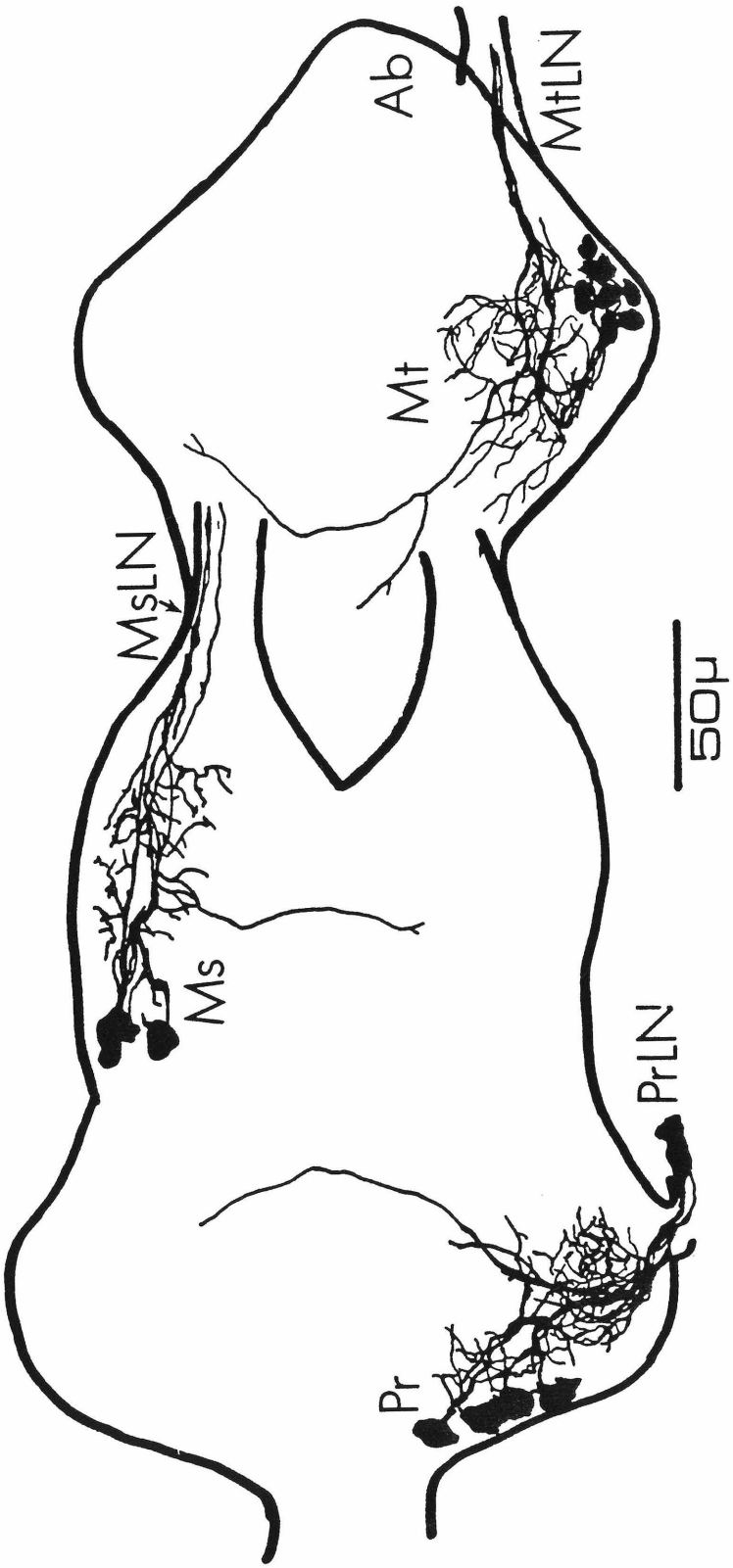

Ab
MtLN
Mt
MsLN
Ms
PrLN
Pr
50µ

FIGURE 2: A thoracic ganglion from an animal of the genotype abx bx^3 pbx/abx bx^3 pbx is shown in horizontal plane. A metathoracic leg was backfilled and the HRP visualized as in method (1) in figure 1.

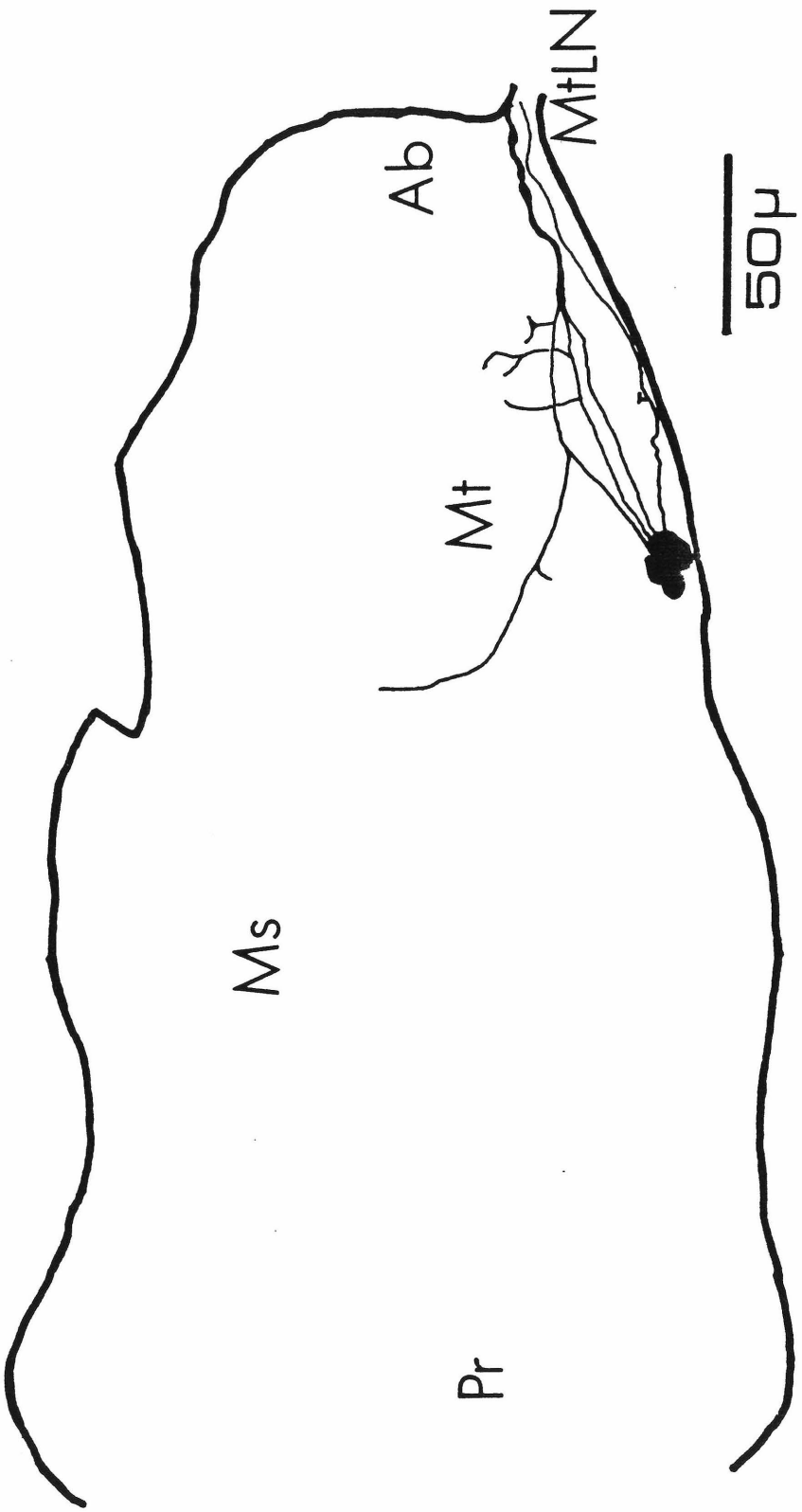
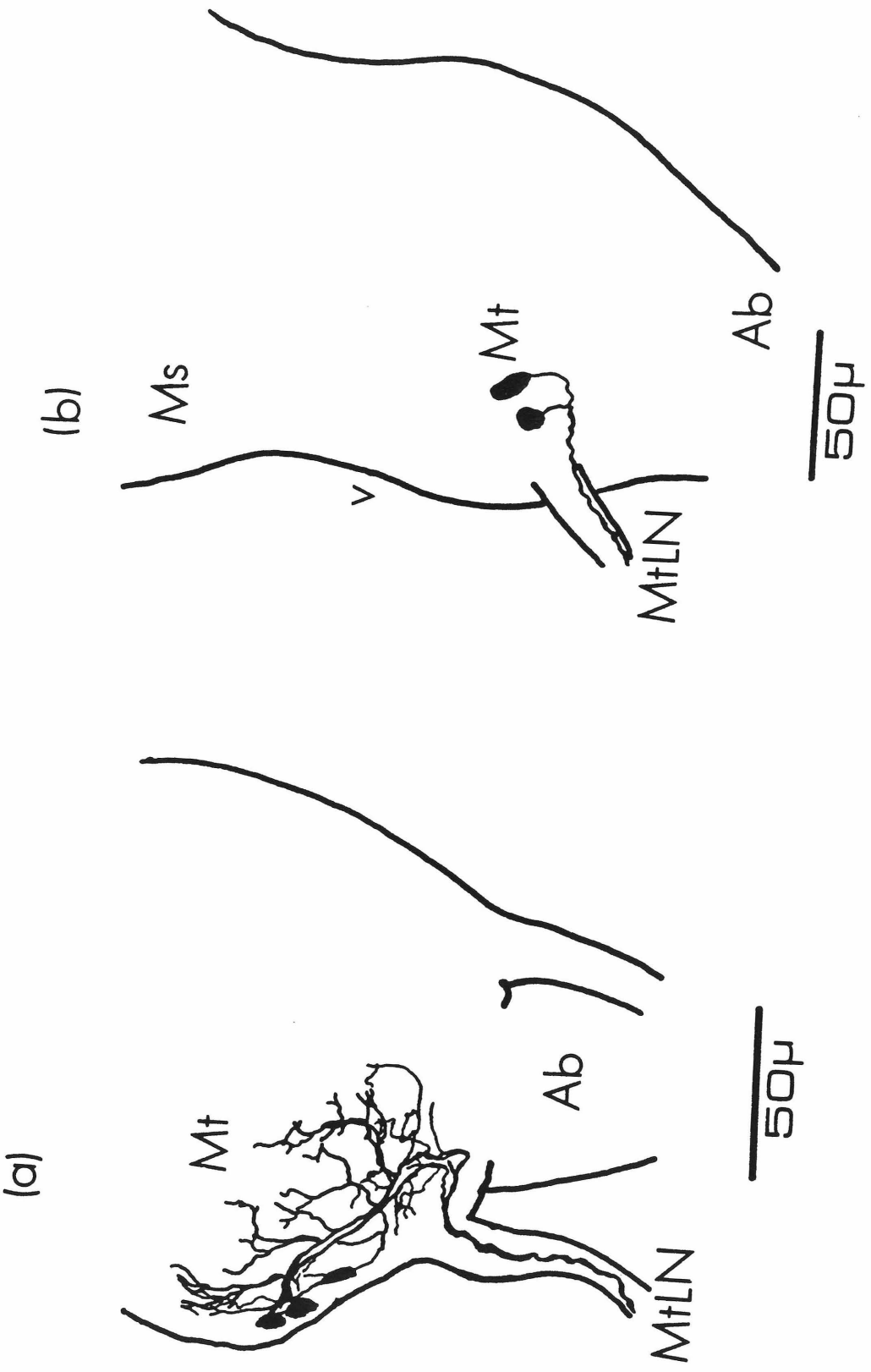

Ab
M+LN
Mt
Ms
Pr
50μ

FIGURE 3: This figure shows two "intermediate" transformations. (a) A thoracic ganglion from an animal of the genotype abx bx^3 pbx/abx bx^3 pbx is shown in horizontal plane. A metathoracic leg was backfilled and the HRP visualized using method (1). (b) A thoracic ganglion from an animal of the genotype abx bx^3 pbx/Df(3)P2 is shown in sagittal plane, V marking the ventral surface. A metathoracic leg was backfilled and the HRP visualized using method (2).


(a)
Mt
Ab
MtLN
50μ
(b)
Ms
v
Mt
MtLN
Ab
50μ