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The development of projections and connections from transplanted locust sensory neurons

HILARY ANDERSON[1]

European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, Federal Republic of Germany

SUMMARY

Neurons innervating wind-sensitive hairs on the locust head form characteristic projections and connections within the CNS. These depend on intrinsic properties of the epidermis from which the hair and its neuron are formed (Anderson & Bacon, 1979; Bacon & Anderson, 1984). To investigate further these intrinsic properties and also extrinsic factors involved in guiding axon growth and determining synaptic connectivity, pieces of epidermis from the head were transplanted to the posterior head, prothorax, or mesothorax. Thus wind-sensitive neurons developing from the grafts were caused to grow into foreign parts of the CNS.

The neuronal projections from the graft hairs were examined by filling the axons with cobalt, and their connectivity with an identified interneuron, the Tritocerebral Commissure Giant, was examined by recording electrophysiologically the activity of the interneuron during stimulation of the graft hairs.

The results show that 1) the neuronal projections are confined to one tract, the median ventral tract, and to one arborization area, the ventral association centre, in all ganglia; 2) in all ganglia, neurons from different epidermal regions preserve their location-specific properties of forming ipsilateral or additional contralateral projections; 3) the extent of their projection in the CNS is not interpretable in terms of intrinsic instructions only; 4) in foreign ganglia, they fail to form connections with their normal target interneuron.

INTRODUCTION

In many insects the central nervous system (CNS) develops in the embryo, and at the time of hatching it has an almost full complement of neurons arranged in a pattern much like that of the adult. Sensory neurons on the other hand continue to be differentiated from epidermal cells throughout postembryonic life. What factors guide the growth of the sensory axons from the periphery through the CNS to their appropriate targets?

One approach to investigate this question is to transplant neurons to other regions of the nervous system and to examine their patterns of projection and connection. Sensory neurons associated with the wind-sensitive hairs on the head of the locust are a convenient system for this type of approach. These neurons

[1] *Author's present address*: Department of Zoology, University of California, Davis, California 95616, U.S.A.

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arise from epidermis which is readily transplantable to other regions of the body. New hairs and neurons are formed throughout larval development, so that after transplantation, neurons differentiated from the epidermal graft will be developing in an abnormal location and entering an abnormal area of the CNS.

The normal projections and connections of the wind-sensitive head hairs have been described in detail. There are five fields of hairs (F1–F5) on each side of the head. Neurons associated with hairs in F1 and F2, in F4 and F5, and in F3, form three distinct projection patterns (Tyrer, Bacon & Davies, 1979). They also form different functional connections with an identified interneuron, the Tritocerebral Commissure Giant (TCG); stimulation of hairs in F2, 4, 5 and the posterior part of F1 excites the TCG, while stimulation of hairs in F3 strongly inhibits it (Bacon & Mohl, 1983).

Transplantation of pieces of epidermis between F1 and F3 or between F2 and F3 on the head has shown that graft neurons form projections and connections not according to their actual location on the head but according to the original location of the graft epidermis (Anderson & Bacon, 1979; Bacon & Anderson, 1984). This suggests that a developmental programme is assigned to the epidermis at early developmental stages and that this programme governs aspects of the development of sensory neurons arising from the epidermis.

To investigate further the nature of this intrinsic developmental programme and the extrinsic cues from the CNS needed for sensory neurons to carry it out, the projections and connections of wind-sensitive neurons developing from grafts transplanted to other segments of the body were examined. The main findings have been briefly reported in a review (Anderson, 1981).

MATERIALS AND METHODS

Grafting operations

The grafting procedure was as reported previously (Anderson & Bacon, 1979). Six types of operation were performed on third instar locusts. Pieces of epidermis from either the F1–2 or the F3 head region were transplanted to either the posterior head, prothorax, or mesothorax (Fig. 1A). The F1–2 and F3 regions were chosen for grafting because they are technically easier to graft than other regions, because they form distinctly different projections and connections, and because they form many additional wind-sensitive hairs during late larval instars. When the operated animals became adults, the sensory projections from hairs which had subsequently developed on the grafts were examined morphologically and electrophysiologically.

Morphology of projections

Patches of hairs were isolated with a ring of wax. Sensory projections from the hairs were filled with cobalt chloride by breaking off the hairs and covering the patch with a drop of 5 % cobalt chloride in distilled water. Projections from individual hairs were filled either by inserting a glass micropipette filled with the same solution into a hole made in the cuticle at the base of the hair, or by carefully isolating single hairs with wax.

All preparations were left to fill for 24 h at 8 °C, and were then processed: the cobalt chloride was precipitated as cobalt sulphide, the nervous system was dissected out, fixed, hydrated,

intensified with silver, dehydrated, cleared, and mounted in Canada Balsam (Anderson & Bacon, 1979).

The wholemounts were drawn using a compound microscope with a drawing-tube attachment or photographed on Kodak Panatomic X film with a Zeiss Axiomat.

Specimens were then re-embedded in soft Epon or soft Araldite and sectioned at 25–40 μm in either the transverse or longitudinal plane. The sections were drawn or photographed as for wholemounts. Nerve roots, tracts and commissures were identified according to the scheme of Tyrer & Gregory (1982).

Electrophysiology

Extracellular recordings of the TCG were made from the tritocerebral commissure, as described in Bacon & Tyrer (1978), but using a suction electrode. Intracellular recordings were also made from the cell body of the TCG using the method described in Bacon & Mohl (1983). Hairs were stimulated singly or in groups using an eyelash mounted on a rod held in a micromanipulator.

RESULTS

Normal F1–2 and F3 projections

The projections from normal wind-sensitive hairs in F1–2 and in F3 are shown in Fig. 1B,C for comparison with projections from graft hairs. These projections have been described in detail previously (Tyrer *et al.* 1979). F3 neurons enter the brain via the ventral tegumentary nerve, arborize in the tritocerebrum, and descend to the suboesophageal ganglion, where they form short branches in ventral neuropil near the Median Ventral Tract (MVT). Most neurons terminate here but about 20 % descend in the MVT to the prothoracic ganglion where they form branches in the Ventral Association Centre (VAC). F1–2 neurons enter the brain via the dorsal tegumentary nerve, arborize in the tritocerebrum, and descend to the suboesophageal ganglion where they form short branches around the MVT, as do F3 neurons. In addition, the F1–2 neurons form branches in the homologous region on the contralateral side of the ganglion. Most F1–2 neurons terminate at this level, but about 20 % descend further via the MVT to the prothoracic ganglion where they also arborize in the VAC but form additional branches on the contralateral side of the ganglion.

Projections from segmental hairs

The projections from hairs on the posterior head, prothorax, and mesothorax, in the regions used as grafting sites, are shown in Fig. 1C for comparison with the projections of graft neurons developing at these sites. These projections have been described in detail elsewhere (Anderson, 1984). The neurons enter the ganglion of their host segment, and pass directly to the ventral neuropil where they arborize in the anterior part of the VAC or its homologue in the suboesophageal ganglion. Projections from the prothoracic hairs are confined to the prothoracic ganglion, but projections from the other hairs are multisegmental. Posterior head hairs *descend* to the prothoracic ganglion, whilst mesothoracic hairs *ascend* to the

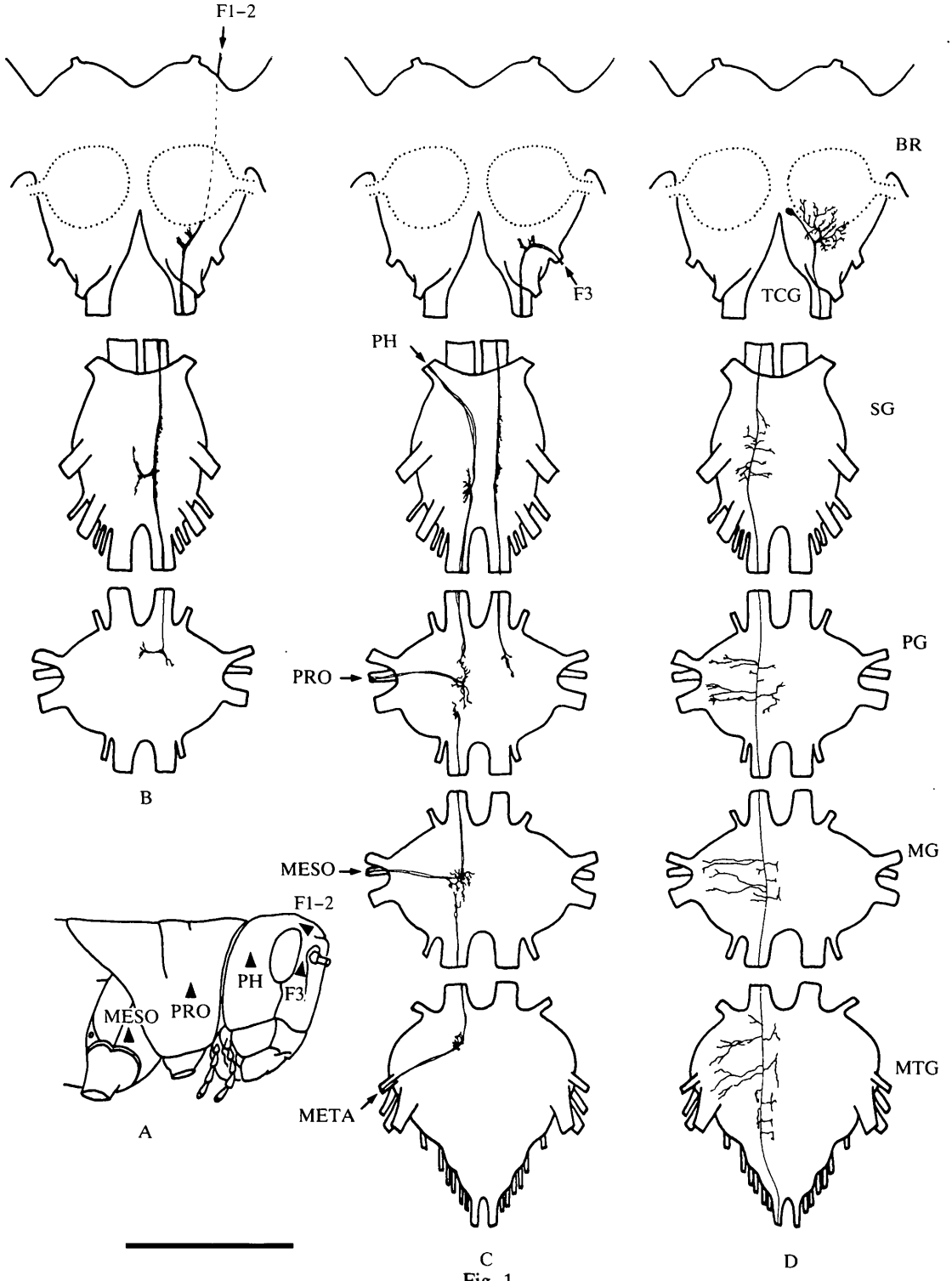

F1-2
F3
BR
TCG
PH
SG
PRO
PG
B
MESO
MG
F1-2
PH
F3
MESO
PRO
META
MTG
A
C
D

Fig. 1.

prothoracic ganglion, and metathoracic hairs *ascend* to the mesothoracic ganglion. In all cases the ascending or descending components are also restricted to the MVT longitudinal tract, and the secondary arborizations are restricted to the posterior or anterior part of the VAC respectively. There are no contralateral projections from these sites.

Projection of the TCG

Sensory inputs from the wind-sensitive hairs are used for the initiation, maintenance, and control of flight (Weis-Fogh, 1949). These functions are probably mediated by several different interneurons in the brain and thoracic ganglia. One interneuron has been identified and studied in detail – the TCG interneuron – and its anatomy is shown in Fig. 1D. Its cell body lies in the brain and it forms an extensive arborization in the tritocerebrum where the wind-sensitive sensory neurons also arborize. It descends through the circumoesophageal connective as far as the tritocerebral commissure through which it crosses to the contralateral side. It remains on the contralateral side throughout the rest of its course through the suboesophageal and thoracic ganglia. In these ganglia, the main axon passes through the Dorsal Intermediate Tract, and the branches are confined to dorsal neuropil (Bacon & Tyrer, 1978).

Projections from graft neurons

The extent of representative projections from groups of hairs on each of the grafts is shown in Figs 2 and 3, and the tracts and arborization areas used within the CNS are illustrated in Figs 4 and 5. Examples of projections of single neurons are shown in Fig. 6. The major observations will be discussed one by one.

1) *Graft axons enter the ganglion of the host segment*

In each case axons from the neurons on the graft joined with surrounding axons from adjacent segmental hairs and entered the host ganglion by the nearest segmental nerve (Figs 2 & 3). Neurons developing from grafts to the posterior head therefore grew into the suboesophageal ganglion (a ganglion to which all F1–2 and F3 head hairs normally project), from the prothorax into the prothoracic ganglion (a ganglion to which about 20 % of the hairs normally project), and from

Fig. 1. Neuronal projections from graft and host hair regions and the TCG interneuron in normal locusts. (A) Diagram of the locust head and thorax showing the location of the two graft regions, F1–2 and F3, and the three host regions, PH, PRO, and MESO. (B) Sensory projections from hairs in F1–2. (C) Sensory projections from hairs in F3, and the three host regions PH, PRO, MESO and from the META for comparison with graft projections. (D) Projection of the TCG interneuron. PH, posterior head; PRO prothorax; MESO, mesothorax; META, metathorax; BR, brain; SG, suboesophageal ganglion; PG, prothoracic ganglion; MG, mesothoracic ganglion; MTG, metathoracic ganglion. Scale for B,C,D equals 1000 μm.

the mesothorax to the mesothoracic ganglion (a ganglion to which none of these hairs would normally project).

2) *Graft projections are not random but regular*

Projections from graft neurons were not random or chaotic even when they entered ganglia to which they would not normally project. In all ganglia the projections were confined to the MVT, and the arborizations to the VAC (Figs 4 &

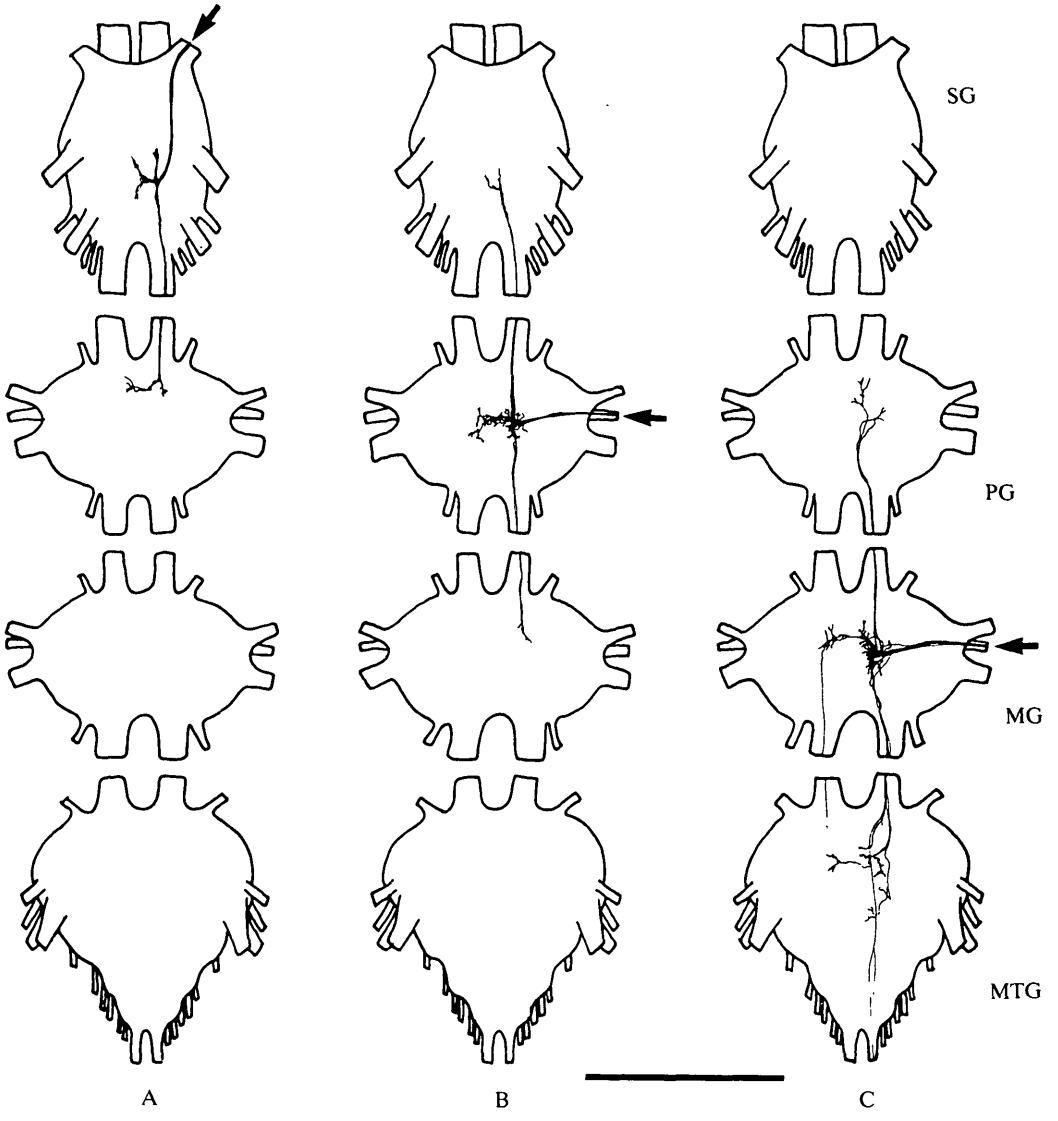


Fig. 2. Sensory projections from F1–2 neurons developing on the posterior head (A), prothorax (B), and mesothorax (C). Arrowheads indicate the nerve of entry into the CNS. Abbreviations as for Fig. 1. Scale equals 1000 μm.

5). This tract and this arborization area is the one used by normal head hairs (Tyrer, Bacon & Davies, 1979) and host segmental hairs (Anderson, 1984).

Projections from grafts to the posterior head entered the suboesophageal ganglion via nerve 1, passed ventrally through the mandibular and maxillary neuromeres (Fig. 4A), then curved dorsally to the region of the MVT where they extended short branches into surrounding neuropil (Fig. 4B,C), before descending in the MVT to the prothoracic ganglion, where they formed branches in the VAC (Fig. 4D).

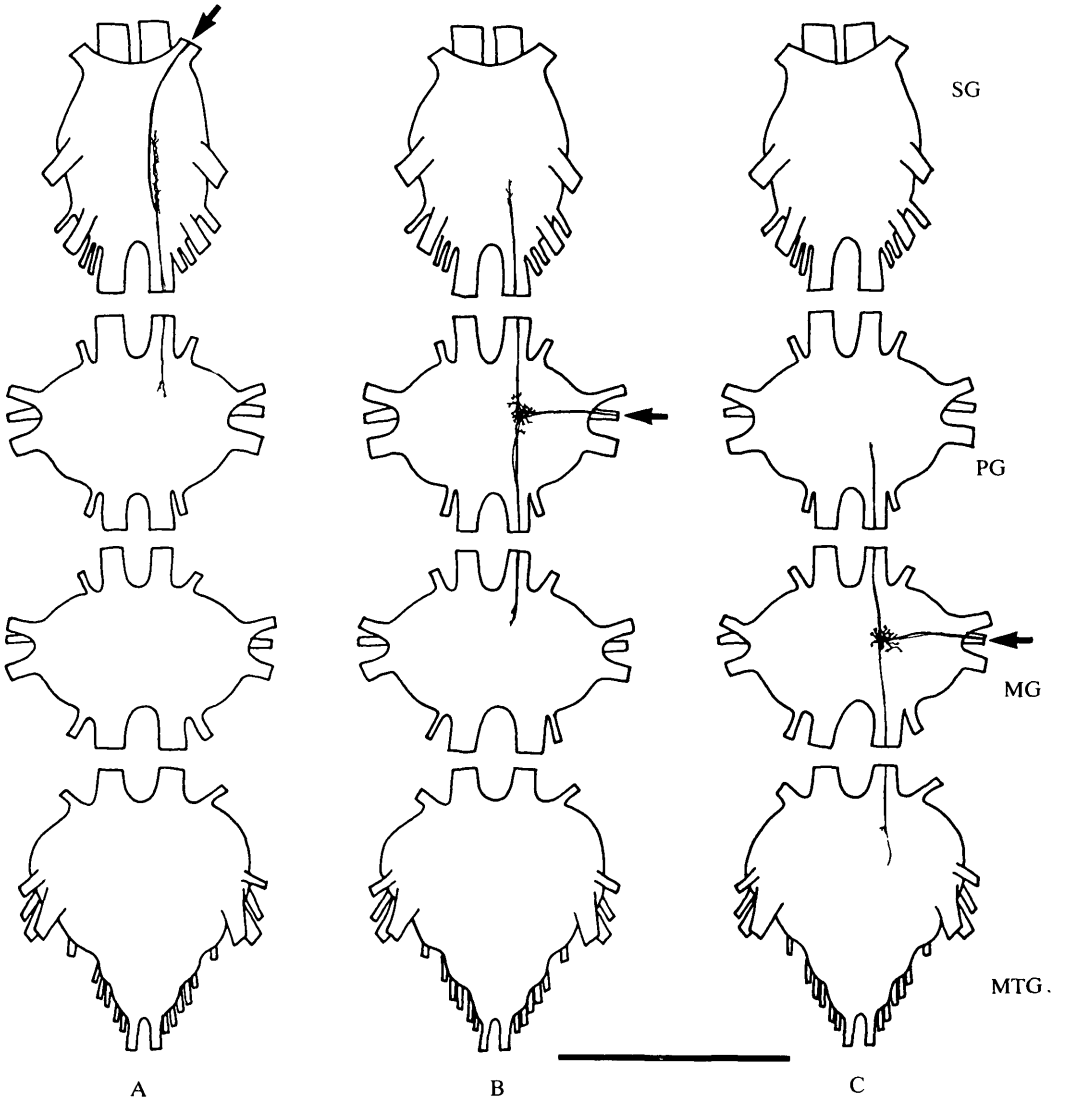
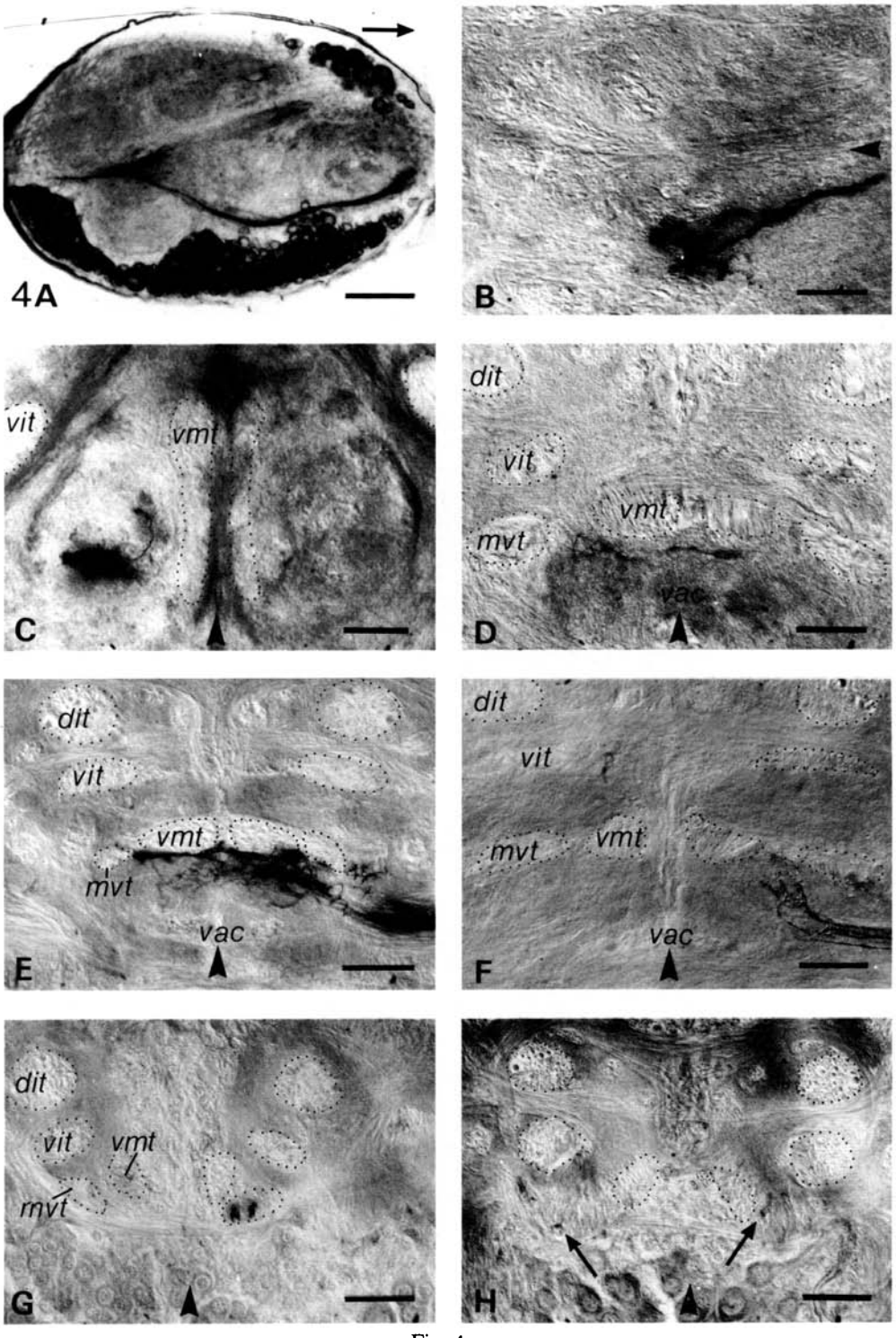


Fig. 3. Sensory projections from F3 neurons developing on the posterior head (A), prothorax (B), and mesothorax (C). Arrowheads indicate nerve of entry into the CNS. Abbreviations as for Fig. 1. Scale equals 1000 μm.


4A
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D
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Fig. 4.

Projections from grafts to the prothorax entered the prothoracic ganglion via nerve 3, passed directly to the ventral neuropil where they formed branches within the VAC (Fig. 4E,F) and either terminated, or ascended to the suboesophageal ganglion (Fig. 4G), or descended to the mesothoracic ganglion (Fig. 4H), within the MVT.

Projections from grafts to the mesothorax entered the mesothoracic ganglion in nerve 3, formed arborizations within the VAC (Fig. 5A,B), and either terminated in this ganglion, or ascended within the MVT to the prothoracic ganglion (Fig. 5B,D) or descended to the metathoracic ganglion (Fig. 5C).

3) *Graft projections do not simply follow host projections or normal head hair projections*

Since graft neurons used the same tracts and arborizations areas as surrounding host neurons, it could be that they simply followed them. This appears not to be the case. Although many graft neurons do follow the same path as host segmental neurons (indicated in Table 1), in every case some of the neurons from each graft (Figs 2, 3, 6) behaved differently from surrounding host neurons (Fig. 1C), and from hair neurons elsewhere on the segment (Anderson, 1984). For example, graft neurons on the posterior head formed arborizations in the suboesophageal ganglion which extended much further anteriorly than did surrounding posterior head neurons. Many neurons from grafts to the prothorax arborized in the prothoracic VAC, as for prothoracic hairs, but then also formed additional ascending or descending branches to adjacent ganglia. Many neurons from grafts to the mesothorax formed branches descending to the metathoracic ganglion,

Fig. 4. Photomicrographs of sections through the suboesophageal and prothoracic ganglia showing the tracts and arborization areas of cobalt-filled graft neurons on the posterior head and prothorax. (A) F1–2 neurons from a graft to the posterior head within a longitudinal section through the suboesophageal ganglion. Anterior is to the right, dorsal uppermost. (B,C) Transverse sections of the suboesophageal ganglion showing F3 neurons from a graft to the posterior head branching in the region of the MVT, between the VIT and VMT tracts. (D) Transverse section of the prothoracic ganglion showing F1–2 neurons from a graft to the posterior head forming an arborization in the ipsilateral and contralateral parts of the prothoracic VAC. (E) Transverse section through the prothoracic ganglion showing F1–2 neurons from a graft to the prothorax forming arborizations in the ipsilateral and contralateral halves of the prothoracic VAC. (F) Transverse section through the prothoracic ganglion showing F3 neurons from a graft to the prothorax forming only an ipsilateral arborization in the prothoracic VAC. (G) Transverse section through the anterior part of the prothoracic ganglion showing F3 neurons from a graft to the prothorax, ascending to the suboesophageal ganglion within the MVT. (H). Transverse section through the posterior part of the prothoracic ganglion showing F1–2 neurons from a graft to the prothorax, descending to the mesothorax within the MVT (arrows). Only the major tracts are labelled. *dit*, dorsal intermediate tract; *vit*, ventral intermediate tract; *vmt*, ventral median tract; *mvt*, median ventral tract; *vac*, ventral association centre. The arrowhead in each figure indicates the midline and points dorsally. Scale equals 400 μm (A), 100 μm (B,C,D), 160 μm (F), and 200 μm (E,G,H).

which was never the case for mesothoracic hairs. Neither did the graft neurons always simply follow their normal counterparts. This could be the case for graft neurons on the posterior head since 20 % of normal F1–2 and F3 neurons descend from the suboesophageal ganglion to the prothoracic ganglion, as did the graft neurons, but in no case did the graft neurons also ascend to the brain alongside normal F1–2 and F3 neurons. Furthermore, no normal F1–2 or F3 graft neurons descend further than the prothoracic ganglion. Graft neurons on the prothorax which descended to the mesothoracic ganglion, and all graft neurons on the mesothorax therefore had no wind-sensitive neurons to follow. In addition, even neurons from the same graft did not always follow one another (see section 5 for

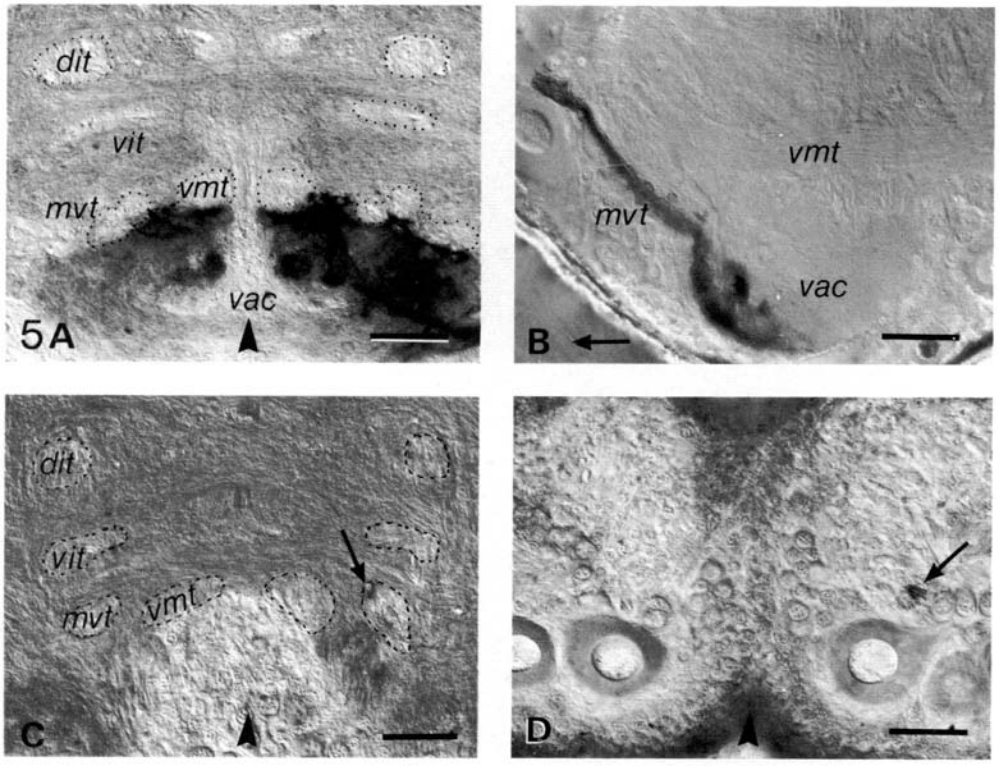

Fig. 5. Photomicrographs of sections through the mesothoracic ganglion showing the tracts and arborizations of cobalt-filled neurons from grafts to the mesothorax. (A) Transverse section showing F1–2 neurons arborizing in the ipsilateral and contralateral halves of the VAC. (B) Longitudinal section showing F3 neurons arborizing in the VAC and ascending to the prothoracic ganglion. Anterior is to the left and dorsal is uppermost. (C) Transverse section through the posterior part of the mesothoracic ganglion showing F1–2 neurons descending within the MVT (arrow) to the metathoracic ganglion. (D) Transverse section through the anterior part of the mesothoracic ganglion showing F3 neurons ascending (arrow) to the prothoracic ganglion. Only the major tracts are labelled. Abbreviations as in Fig. 4. Arrowheads indicate the midline and point dorsally. Scale equals 200 μm in all cases.

further discussion). Thus the wind-sensitive neurons do not passively follow other similar neurons which use the same tracts and neuropil areas but apply their own developmental programme.

4) *F1–2 and F3 hairs maintain their characteristic differences in all ganglia*

The normal projections from F1–2 and F3 neurons differ in that the former send additional branches to the contralateral side of the suboesophageal and prothoracic ganglia (compare Figs 1B, and 1C). Reciprocal grafts between F1–2 and F3 have shown that this is an intrinsic difference and is not related to their actual location on the head (Anderson & Bacon, 1979). Projections from F1–2 or F3 neurons grafted to other segments also maintained this feature (compare Figs 2, 3); F1–2 neurons formed additional contralateral branches in the foreign ganglia to which they first projected, even though the grafts were placed in a lateral position where all host hairs form only ipsilateral projections (Fig. 1C). However, in a few cases the F1–2 neurons extending through a second ganglion formed only an ipsilateral projection e.g. compare the prothoracic arborizations from normal F1–2 hairs (Fig. 1B) with those from F1–2 hairs grafted to the mesothorax (Fig. 2C).

5) *Graft projections are more variable in more posterior ganglia*

Normal projections from head hairs *in situ* and from segmental hairs show almost no variation from animal to animal. As Table 1 indicates, all F1–2 and F3 neurons project to the tritocerebrum and suboesophageal ganglion. Of these about 20 % descend further to the prothoracic ganglion. The segmental hairs show no variation in extent (Anderson, 1984 and Table 1).

Projections from graft hairs on the posterior head showed no variation i.e. 100 % of filled projections showed arborizations in the suboesophageal ganglion and in the prothoracic ganglion (Table 1). Fills of individual neurons or patch fills

Table 1. *Percentage of projections containing components*

BR	SG	PG	MG	MTG	Hair region filled
100	100	20	–	–	Normal F1–2 & F3
–	100	100	–	–	Grafts to PH (20)
–	100	100	–	–	Normal PH
–	40	100	75	–	Grafts to PRO (20)
–	–	100	–	–	Normal PRO
–	–	94	100	53	Grafts to MESO (17)
–	–	100	100	–	Normal MESO

% of projections containing components within the brain (BR), suboesophageal (SG), prothoracic (PG), mesothoracic (MG) or metathoracic (MTG) ganglia.

The number of graft projections examined is indicated in brackets.

The figures underlined are those for the ganglion of entry, those to the left indicate ascending components, those to the right descending components.

in which individual projections could be clearly distinguished confirmed that all neurons arborized in the suboesophageal ganglion, descended, and arborized in the prothoracic ganglion.

Graft neurons on the prothorax on the other hand showed variation both from graft to graft (Table 1) and from neuron to neuron within a graft (Fig. 6). The projections shown in Figs 2 and 3 are examples of patch fills which showed the

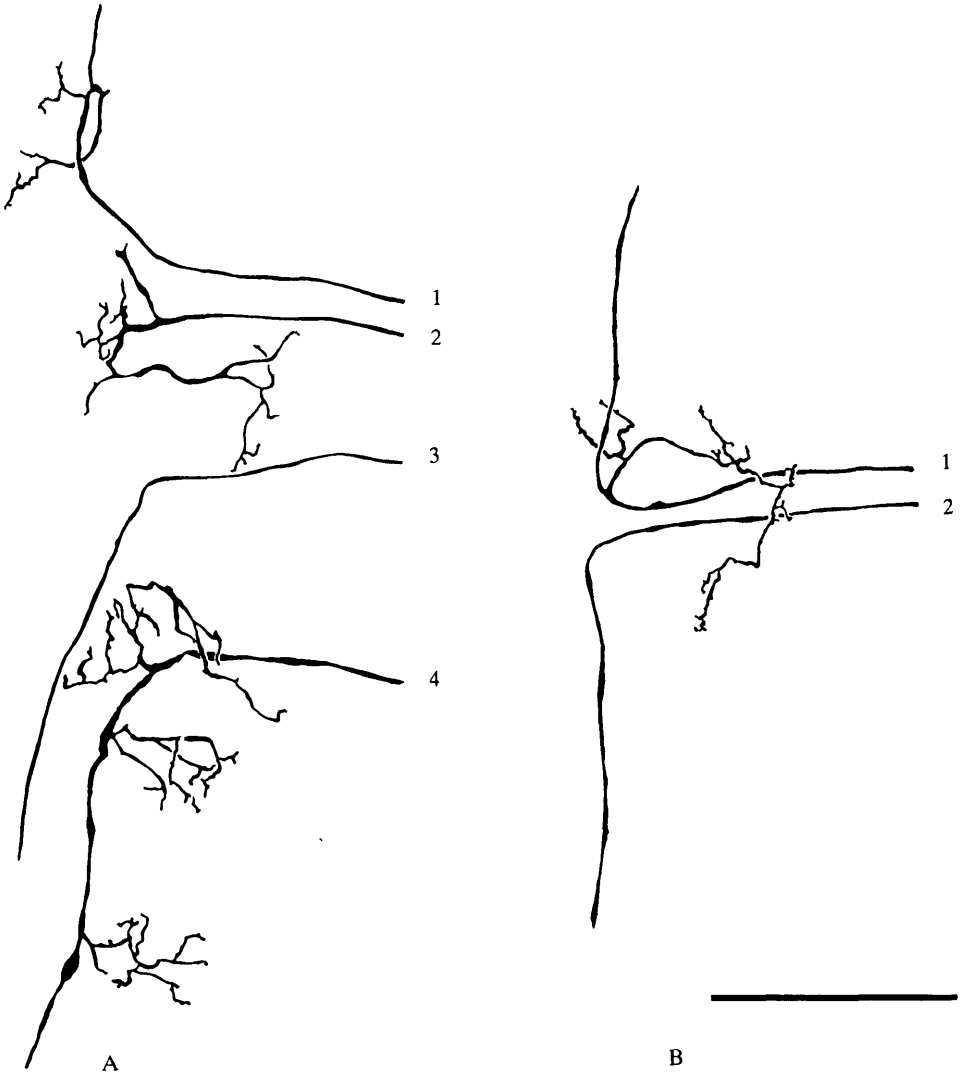

Fig. 6. Camera lucida drawings of individual neurons from an F3 graft to the prothorax (A) and an F3 graft to the mesothorax (B). Anterior is uppermost. Scale equals 50 μm.

maximum extension observed. Not all grafts showed such extensive projections. 100 % of grafts showed some arborization in the prothoracic ganglion. 42 % of graft projections also included branches extending anteriorly to the suboesophageal ganglion while 74 % included branches extending posteriorly to the mesothoracic ganglion. In addition to the variation between the projections from individual grafts, there was variation between individual neurons from the same graft. A particularly instructive example is shown in Fig. 6A. Neuron 4, which was the most common type observed, showed an extensive arborization in the prothoracic ganglion and then descended to the mesothoracic ganglion. Other neurons showed much more limited projections, e.g. neuron 1 exhibited only a small arborization in the prothoracic ganglion, and ascended to the suboesophageal ganglion, neuron 2 arborized only in the prothoracic ganglion, and neuron 3 descended to the mesothoracic ganglion without forming a prothoracic arborization. Neuron 3 was the only example of its kind observed.

Considerable variation was also found in the projections from grafts to the mesothorax. 100 % of the fills from grafts on the mesothorax formed arborizations in the mesothoracic ganglion, and 94 % of these cases also had branches to the prothoracic ganglion while 53 % of cases had branches descending to the metathoracic ganglion (Table 1). The projections illustrated in Figs 2 and 3 are examples of maximally extensive projections. However, as for grafts to the prothorax, not all neurons from the same graft showed the same projection. Individual neurons had a variety of configurations as illustrated in Fig. 6B. In two cases (e.g. neuron 2 in Fig. 6B) only a descending component was formed without a local mesothoracic arborization. All other projections had a local arborization and could in addition have either an ascending (e.g. neuron 1 in Fig. 6B), or descending component, or, more rarely, both.

6) *Graft neurons do not form functional connections with the TCG*

Stimulation of F1–2 neurons normally excites the TCG whilst stimulation of F3 neurons normally inhibits it (Bacon & Mohl, 1983). The connections between the hair neurons and the TCG are located in the tritocerebrum of the brain, since cutting the circumoesophageal connective close to the suboesophageal ganglion does not alter the response of the TCG to hair stimulation (Bacon & Tyrer, 1978).

Stimulation of hairs on the transplants failed to produce any response in the TCG (Fig. 7). This failure was not a result of abnormality in the TCG in experimental animals since in all cases recorded the TCG functioned quite normally when normal hairs on the head were stimulated (Fig. 7). Neither was it a result of alteration to the sensory neurons themselves as a consequence of the grafting operation since similar grafting operations between F1–2 and F3 head regions does result in innervation of the TCG (Bacon & Anderson, 1984).

DISCUSSION

Determination of sensory neuron developmental programmes

Earlier experiments in which pieces of epidermis were transplanted between F1 and F3 or between F2 and F3 on the head, showed that graft neurons form projections and connections not according to their actual location on the head but according to the original location of the graft epidermis (Anderson & Bacon, 1979; Bacon & Anderson, 1984). This suggested that a developmental programme is assigned to the epidermis at early developmental stages and that this programme governs aspects of the development of sensory neurons arising from the epidermis.

Subsequent work comparing the projections from head hairs and from other hairs of the same type on different segments of the body (Anderson, 1984), has shown that all these neurons are restricted to the MVT and VAC, but that within these restricted areas those arising from different segments may consistently differ in the distance and direction that they travel, and those arising from different parts of the segment may consistently differ in forming additional contralateral projections or not.

These results fit well with the hypothesis, based on the examination of projection patterns from homeotic mutants in *Drosophila*, that the choice of a pathway is strictly encoded in the developmental programme of a neuron, which partly depends upon its developmental history, such that sensory neurons of the same modality throughout the body grow along the same general pathways which are further restricted depending upon the segmental and compartmental determination of the neuron, and partly depends upon the position of the neuron,

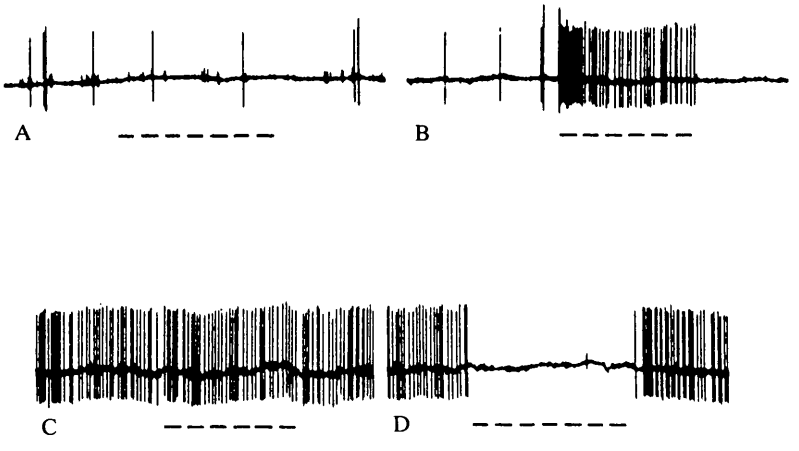

Fig. 7. Extracellular recordings from the TCG of operated animals during stimulation (dashed line) of F1–2 hairs on grafts to the posterior head (A), ungrafted F1–2 hairs (B), F3 hairs on grafts to the posterior head (C), and ungrafted F3 hairs (D).

which defines the fine details of its projection (Ghysen, 1980; Ghysen, Jansen & Santamaria, 1983).

The transplantation experiments in the present work were undertaken to examine further the proposed aspects of the intrinsic developmental programme assigned to the head hairs, and the extrinsic cues from the CNS needed for the neurons to carry it out.

The results show that the wind-sensitive head hair neurons are found in the same tracts and arborization areas as other neurons from segmental mechanoreceptive hairs (Anderson, 1984) even when transplanted to other segments. The behaviour of the grafted neurons therefore conforms to the modality rule proposed by Ghysen (1980) and discussed above. This tract and arborization area is not used by sensory neurons of other modalities such as proprioreceptors (Bräunig, Hustert & Pflüger, 1981), hair plates (Pflüger, Bräunig & Hustert, 1981), or campaniform sensilla (Hustert, Pflüger & Bräunig, 1981).

Transplanted wind-sensitive neurons also continue to display location-specific features in their projections, even in completely foreign ganglia; graft F1–2 projections consistently differ from those of graft F3 hairs and of segmental hairs at the host site, in forming additional contralateral branches. Similar transplantation experiments on crickets show a similar result (Murphey, Bacon, Sakaguchi & Johnson, 1983). Sensory neurons on the cricket cercus normally project to the terminal ganglion where they form a somatotopic map i.e. the location of each neuron's projection in the ganglion is related to the location of its cell body on the cercus. Neurons from cerci transplanted to the mesothorax project to the corresponding region of the mesothoracic ganglion where they show location-specific features by again forming a somatotopic map.

It was suggested above that the segmental determination of hair neurons might influence the distance and direction they grow within the CNS. Both F1–2 and F3 neurons normally enter and arborize in the tritocerebrum, descend and arborize in the suboesophageal ganglion, and then about 20% pass to the prothoracic ganglion (Fig. 1). When these neurons are transplanted to other segments, they do not always form projections which resemble host segmental projections in either the distance or the direction grown. This autonomous behaviour supports the view that head hair neurons are intrinsically different from hairs on other segments. However, neither do graft projections consistently resemble normal head hair projections. Rather, the most striking points about the graft projections are that individual neurons within a graft, which should have the *same* segmental determination, may show *different* projections, and that even those graft projections encountering the posterior parts of their normal pathways do not then continue and complete the pathway.

It is therefore difficult to interpret these aspects of the behaviour of graft neurons solely in terms of intrinsic instructions, such as to grow posteriorly through a fixed number of ganglia or form branches of a constant length or grow along specified sections of the MVT in the brain, suboesophageal and prothoracic

ganglia, since all these parameters can differ between normal and graft projections. It therefore seems that to act out the proposed segment-specific features of the programme in fact requires access to extrinsic information which differs in different segmental ganglia. The increasing variability of projection patterns in more posterior ganglia could reflect the absence of specific cues in these ganglia, such as, for example, the presence of particular interneurons with which to synapse.

How might the different features of this programme be assigned to the epidermal precursors of the wind-sensitive hairs? It seems likely that the underlying processes are very similar to those that determine the development of the cuticle-secreting components of the epidermis. Segment-specific features could be assigned at the time when segments are first determined in early embryogenesis (Lawrence, 1981) and location-specific features could be assigned according to the position of a precursor cell within an epidermal field of positional information (Ghysen, 1980; Murphey, Johnson & Sakaguchi, 1983), or according to its compartment (Ghysen, 1980).

CNS pathways

Several authors have suggested that neurons navigate through the CNS by recognizing defined pathways (Ghysen, 1978; Katz & Lasek, 1980; Raper, Bastiani & Goodman, 1983). This view is supported by the present observations on transplanted wind-sensitive sensory neurons. The graft neurons only arborize in one region of the neuropil, the VAC, and only travel in one set of longitudinal tracts, the MVT. They recognize these regions in all ganglia, even those to which they would not normally project.

What might be the nature of these pathways? Several possibilities have been proposed (Katz & Lasek, 1980), and include extracellular matrix, glial cells, chemotactic gradients, preformed spaces, and pioneer neurons. An attractive hypothesis is that the MVT and VAC bear a particular molecular label which is recognized by the growth cones of all those sensory neurons which normally use this pathway. This molecular label could be present on a persisting pioneer neuron (Bate, 1976; Bate & Grunewald, 1981), or could be present on later developing neurons which follow the pioneer and which the hair neurons can also recognize (Raper *et al.* 1983). This requires further investigation.

Recognition of interneurons

Neurons developing from epidermis exchanged between F1–2 and F3 on the head innervate the TCG (Bacon & Anderson, 1984), but those developing from F1–2 or F3 epidermis transplanted to other body regions (posterior head, prothorax, or mesothorax) fail to grow towards and connect with the TCG even when they encounter and grow over part of their normal pathway.

Neurons transplanted between hair fields on the head grow into the brain where the TCG is in close proximity to the path of the head hair neurons (Anderson & Bacon, 1979; Bacon & Anderson, 1984). Neurons transplanted to other regions of the body grow into the suboesophageal, prothoracic or mesothoracic ganglia of the CNS. In these ganglia, the arborization of the TCG is restricted to the dorsal part of the ganglion and the DIT (Bacon & Tyrer, 1978) precluding the possibility of direct synapses from the ventrally restricted head hair transplant neurons.

It seems likely that the primary strategy for head hair neuron development is to first grow along defined pathways, to form or not form contralateral branches at homologous sites in each ganglion, to arborize in certain homologous regions of neuropil in each ganglion, and only then to recognize and form synapses with particular target interneurons within this region of neuropil. Only in the brain is the TCG in close proximity to the arborization area of the head hair neurons.

It is not known whether graft neurons form connections with other normal target interneurons in the suboesophageal and prothoracic ganglia. Nor is it known whether they also form connections with interneurons which are not their normal targets but which are homologous to them, or which simply have arborizations in the VAC. Answers to these questions would be useful for understanding further how sensory neurons recognize appropriate interneurons, and for investigating the possible role of interneurons as cues influencing the extent of the projection pathway of neurons.

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