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SHORT COMMUNICATION

DEVELOPMENTAL DETERMINATION OF CENTRAL CONNECTIONS OF SENSORY NEURONES FROM WIND-SENSITIVE HAIRS IN THE LOCUST, *SCHISTOCERCA GREGARIA*

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The nervous system of hemimetabolous insects is a favourable preparation in which to study how sensory neurones form particular projections and connections within the CNS. One reason is that the development of the sensory neurones is physically separate from the development of the CNS; sensory neurones are derived from epidermal cells under the cuticle, in contrast to interneurones and motor neurones which are derived from central neuroblasts. In addition, whereas the CNS has an almost full complement of neurones at the end of embryogenesis, neuroblast division having ceased in all but a few brain centres, sensory neurones continue to be generated from their epidermal precursors throughout the postembryonic life of the animal. Therefore transplanting pieces of epidermis during postembryonic development places sensory neurones in altered environments without surgery to the CNS. Furthermore, since sensory neurone development continues within the transplanted epidermis, the influence of these perturbations can be tested on developing as well as regenerating sensory neurones.

We have examined the determination of central connections of sensory neurones of the locust's wind hairs. There are five fields of wind-sensitive hairs on each side of the locust head (Fig. 1A). Sensory neurones associated with these hairs have different anatomical projections and functional connections within the CNS according to their location on the head. There are three classes of central projection, one for neurones associated with hairs in Field 3, one for Fields 1 and 2, and another for Fields 4 and 5 (Tyrer, Bacon & Davies, 1979). In addition to their different branching patterns in the CNS, axons from Field 3 enter the brain *via* the ventral tegumentary nerve (VTN) while axons from the other fields enter *via* the dorsal tegumentary nerve (DTN). These sensory neurones provide input to an

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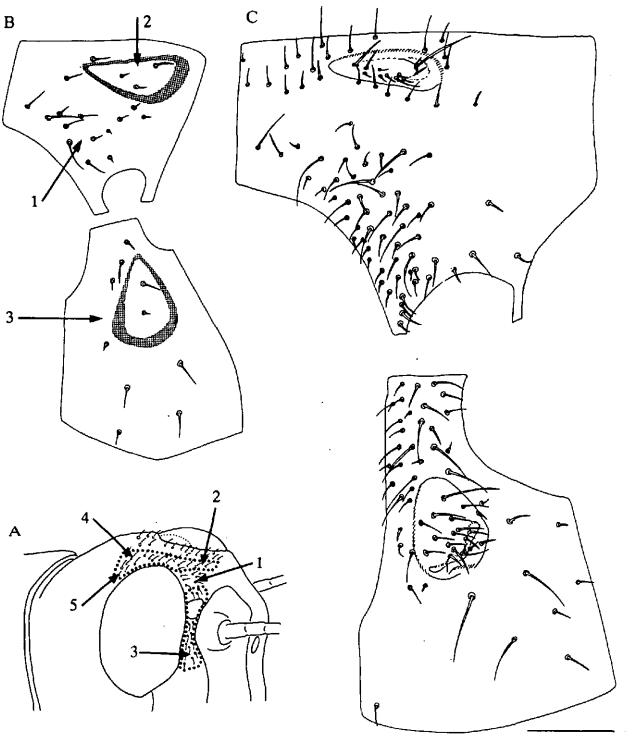

Fig. 1. (A) The locust's wind-sensitive head hairs are arranged in five fields (1–5) on each side of the head. The dotted lines delineate the region of hairs on the top of the head which excite the TCG and those on the side of the head which inhibit it. Hairs in the intermediate region seem to provide no input to the TCG. (B),(C) Drawings of pieces of the second instar cuticle (B) and adult cuticle (C) from the right side of the animal's head; wind hair Fields 1, 2 and 3 are labelled. In this animal, a piece of Field 2 epidermis and a piece of Field 3 epidermis were exchanged in the second instar. Shading in (B) indicates the region of clotted haemolymph around the transplants where they were held in place next to the host epidermis. Individual hairs present at the time of transplantation are recognized by their location and size in the adult stage. Scale: A, 3 mm; B and C, 1 mm.

identified wind-sensitive interneurone, the tritocerebral commissure giant (TCG): the TCG is excited by stimulation of hairs in Fields 2, 4, 5 and the posterior part of Field 1, and inhibited by stimulation of hairs in Field 3 and the anterior part of Field 1 (Fig. 1A; Bacon & Möhl, 1983).

In a previous study, we transplanted epidermis and cuticle between Fields 1 and 3 on the head at the third instar stage (Anderson & Bacon, 1979). When we stained the adult projections from the grafts, we found that regenerating axons from hairs

present at the time of operation, and newly-formed axons from hairs developing after transplantation, enter the CNS by the same nerve as those from the surrounding undisturbed cuticle: that is, the VTN in the case of transplants into the Field 3 region and the DTN in the case of transplants into the Field 1 region. Despite the fact that the graft axons take an altered trajectory to the CNS, once they enter the CNS they diverge from their neighbouring undisturbed axons and form a pattern of projections which is appropriate to the original location of the graft epidermis and not its current location. The arborizations formed in the CNS are therefore not determined by interactions with the altered environment in which the graft neurones grow, but by an intrinsic property of the epidermis from which they differentiate (Anderson & Bacon, 1979).

To test whether connectivity patterns of these sensory neurones are also determined by properties of their epidermis of origin, we exchanged pieces of epidermis between Fields 2 and 3 on the right side of the head in 30 animals at the second instar stage of development, using methods described previously (Anderson & Bacon, 1979). In 10 control animals, the triangular pieces of cuticle and epidermis were cut out, lifted up, and then replaced in their original locations. The operated animals developed as normal throughout the four remaining instars to become adults. The cuticles shed at each of the moults were collected and drawn to make maps of the hairs formed at each stage of development.

The exchanged pieces of epidermis typically formed rounded bumps or depressions in their new locations (Fig. 1B,C). This is a common response when grafting together different regions of epidermis, and is thought to represent local differences in cellular adhesion properties (Nübler-Jung, 1977). Many new wind-sensitive hairs developed after the operation. A typical Field 2 graft bearing 2–3 hairs at the time of transplantation would bear some 15–20 hairs at the adult stage. Typical Field 3 grafts would bear 2–3 hairs at transplantation, and about 9–14 hairs as adults (Fig. 1B,C). Of these hairs, the longest (350–450 μm) were those present in the second instar, and the shortest (140–200 μm) were those formed in the later instars.

When the animals became adult, the nature of the central connections made by afferents from transplanted hairs was examined by intracellular recording from the TCG while individual hairs or groups of hairs were mechanically stimulated, as described in detail elsewhere (Bacon & Möhl, 1983). Mechanical stimulation of groups of Field 2 hairs which had not been transplanted excited the TCG (Fig. 2A) as in normal animals (Bacon & Möhl, 1983). Mechanical stimulation of hairs on Field 2 cuticle transplanted to a Field 3 position also excited the TCG (Fig. 2B). No hairs on these grafts ever inhibited the TCG, even though adjacent undisturbed Field 3 hairs would invariably do so (Fig. 2D). Hairs on the control Field 2 replacement grafts excited the TCG (Fig. 2C) and therefore the important observation is that Field 2 neurones developing on grafts in an abnormal Field 3 location on the head showed the same functional connections as those developing on grafts in their normal location.

Stimulation of Field 3 hairs which had not been grafted strongly inhibited the TCG (Fig. 2D); displacement of a single hair would often completely terminate the tonic activity of the TCG when it was firing at about 10 Hz. Simultaneous

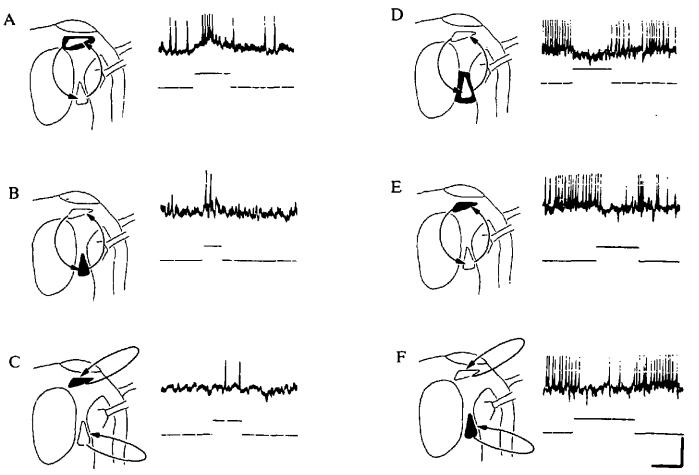

Fig. 2. Intracellular recordings from the TCG of operated animals (top trace) during mechanical stimulation of Field 2 or Field 3 hairs (bottom trace, the stimulus is on when the trace is in the upper position) show that epidermis of origin determines neuronal connectivity. The dark patch on the diagram of the head next to each intracellular record shows the location of the hairs stimulated in each experiment. (A) Stimulation of undisturbed Field 2 hairs; (B) exchange graft Field 2 patch in Field 3 position; (C) control graft Field 2 patch; (D) undisturbed Field 3 hairs; (E) exchange Field 3 patch in Field 2 position; (F) control graft Field 3 patch. Scales: vertical, 10 mV; horizontal, 400 ms A–C, 1 s D–F.

stimulation of several hairs on Field 3 grafts developing in a Field 2 location inhibited the TCG activity (Fig. 2E), but stimulation of individual hairs did not produce a clear inhibitory response. Similarly, the inhibition from hairs on control Field 3 replacement grafts was also weaker than normal, requiring stimulation of several hairs together to produce a response (Fig. 2F). This reduced synaptic efficacy, observed when testing individual hairs, therefore reflects some effect of the transplantation rather than where the hair develops on the head. Despite this, it is clear that neurones developing in Field 3 grafts in an abnormal Field 2 location develop the same functional connections as Field 3 neurones in their normal location.

In summary, after exchanging pieces of epidermis between Fields 2 and 3, both regenerating and newly-developing sensory neurones take abnormal routes to the CNS (Anderson & Bacon, 1979), but are able to form functional connections with an identified interneurone in the CNS which are of the type (excitatory or inhibitory) appropriate to the original location of the graft epidermis, and not to its new foreign location. It is clear that the epidermis contains some intrinsic property which determines these features of the neuronal projection, and the present study demonstrates the stability of this commitment in transplanted pieces of epidermis.

This kind of study is possible because in the unperturbed locust wind-hair system, there exists such a good correlation between a sensory neurone's position in the periphery and its central connections. Such correlations are also found in other insect sensory systems, notably in the moth pupal gin trap (Bate, 1973) and the cricket cercus (Bacon & Murphey, 1984). We conclude that these results on neurones developing from epidermis in its normal position and those of the present study on neurones developing from transplanted epidermis are consistent with the hypothesis, proposed by Bate & Lawrence (1973), that developing sensory neurones acquire location-specific properties in response to their level in a segmental gradient of positional information, and that these properties define the synaptic connections they form in the CNS.

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