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NEUROSPECIFICITY IN THE CRICKET CERCAL SYSTEM

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SUMMARY

Studies of neurospecificity in the cricket cercal sensory system are reviewed and a decade of experimentation is examined in the light of recently obtained anatomical data. The nearly complete description of the anatomy indicates that the excitatory receptive fields of directionally-selective interneurones are a joint function of an orderly afferent projection and the dendritic structure of the first order interneurones. The detailed understanding of the anatomy is shown to be a powerful tool in the interpretation of previously published physiological experiments and the design of new ones.

The mechanisms which shape the orderly afferent projection are then described and compared with the work on vertebrate sensory systems. It is concluded that both positional interactions of the type conceived by Sperry (1963) and competitive interactions of the type conceived by Hubel, Wiesel & LeVay (1977) are involved in producing the cercal afferent projection. Thus the two main components of the neurospecificity concept are shown to exist in the cricket nervous system. The limits of a purely anatomical approach to the study of neurospecificity are considered in light of the work on this cricket sensory system.

INTRODUCTION

Neurospecificity means different things to different people. However, most neuroscientists agree that the question of how selective synaptic connections are formed is basic to their science. One of the classical systems for studying this question has been the vertebrate visual system. In fact, many of the concepts involved in the idea of neurospecificity were first defined by investigators interested in vision. It is interesting that invertebrates, the *sine qua non* of selective connectivity, have been employed only sparingly in examining the question of specificity. One of the reasons for this is that the paradigm adopted by Sperry (1963) and his intellectual descendants has not been readily adapted to invertebrates. In particular, the existence of topographic maps between periphery and centre have previously been detected only in the visual system of various invertebrates where the afferents are small and numerous, defeating the advantages of invertebrates for the single cell approach. In the more accessible mechanosensory systems of invertebrates, topographic mapping had not been demonstrated. Recently however, in the cercal sensory system of crickets, a happy intermediate has

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been found. The cercal sensory cells are numerous enough to provide an array of afferents, yet many afferents are uniquely identifiable. More important, the afferent projection is highly ordered and can be called a topographic map. Thus, it is possible in crickets to study neurospecificity in the context of the classical paradigm, yet take advantage of the single cell approach to assess the mechanisms underlying specific neuronal connections.

The present review will summarize the state of our understanding of the mechanisms underlying synaptic connectivity in the cercal sensory system of crickets. It will try to give coherence to a wide range of experiments carried out during the last decade. The focal point will be the question: why does a given sensory neurone synapse with some interneurones and not others? The answer as it is now understood will be phrased in primarily anatomical terms. The review will also attempt to place findings on the cercal system in the context of previous attempts to understand neurospecificity.

DIFFERENTIAL CONNECTIVITY OF SENSORY NEURONES INNERVATING DIFFERENT TYPES OF RECEPTOR

Crickets possess a pair of abdominal appendages called cerci. The surface of a cercus is covered with receptors of four different types: three are hair-like receptors, filiform hairs, clavate hairs and bristle (appressed) hairs, and the fourth are the dome-shaped campaniform sensilla (Edwards & Palka, 1974). The central projections of the three types of sensory neurone associated with the hair-like receptors have been revealed using very simple methods (Tyrer, Bacon & Davies, 1979). The sensory neurones innervating the filiform and clavate hairs all arborize within a region of the terminal ganglion known as the cercal glomerulus (Fig. 1B and Murphey, 1981; Bacon & Murphey, 1984). The bristles arborize in a different region, anterior and ventral to the cercal glomerulus (Fig. 1A and R. K. Murphey, unpublished). Thus, based on the location of their central projections, the cercal receptors which have been mapped can be divided into only two classes – those projecting to the 'cercal glomerulus' and those projecting to the 'bristle neuropile'. This was the first clue to what now appears to be an important dichotomy of insect receptors. A series of transplantation experiments (Murphey, Bacon, Sakaguchi & Johnson, 1983*b*) have demonstrated that this dichotomy is present in other ganglia as well and is likely to be true for all the insects.

The important issue for this paper is how is this related to synaptic connectivity? Every interneurone in the cercal system which has been shown physiologically to receive its primary input from filiform hairs has its dendrites in the cercal glomerulus (Murphey & Levine, 1980; Levine & Murphey, 1980*b*; Bacon & Murphey, 1984). This class of 'filiform' interneurones is exemplified by the Medial Giant Interneurone (MGI) (Fig. 1D). A separate group of interneurones has now been identified which responds vigorously to touching the cerci, or the body surface where there are no filiform hairs (R. K. Murphey, unpublished). This group of interneurones, represented by Interneurone 7-1a (Fig. 1C), has its dendrites in the bristle neuropile and has no dendrites in the cercal glomerulus. There are a wide variety of these touch-sensitive interneurones, but each has its dendrites restricted to the bristle neuropile of the ganglion being examined. So far the correlation is perfect – interneurones with dendrites

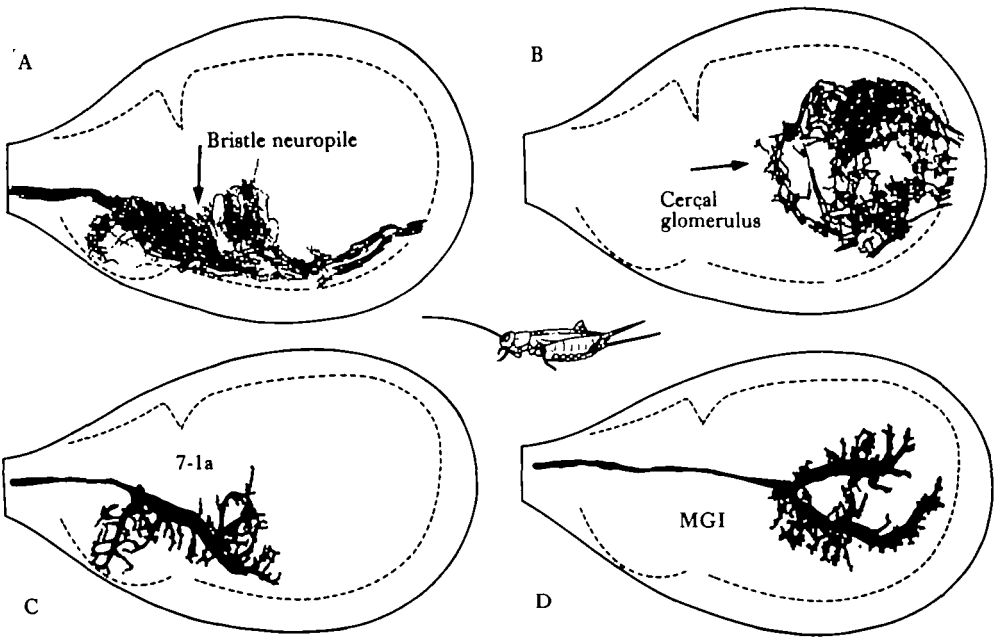


Fig. 1. A dichotomy of afferents and interneurones. (A), (B) The afferent projection from cercus to terminal abdominal ganglion shown in sagittal view. (A) The projection from bristles to the terminal ganglion. Note the afferent axons coursing under the cercal glomerulus to end in the 'bristle neuropile'. (B) The projection from filiform and clavate neurones terminating in the 'cercal glomerulus'. (A) and (B) were prepared from sagittal sections of a single specimen. The two projections which have virtually no overlap were separated during reconstruction. (C), (D) Two types of interneurone. (C) Interneurone 7-1a, which is sensitive to touch and has its dendrites located exclusively in bristle neuropile. (D) The medial giant interneurone (MGI) which is sensitive to wind and has its dendrites exclusively in the cercal glomerulus. (C) and (D) were obtained from the same preparation and were separated during the reconstruction. All four reconstructions represent a 80–100 μm slab of ganglion lateral to the midline. Dashed lines represent the boundary between soma and neuropile. The inset shows the location of the ganglion (darkened) in a sagittal view of a cricket.

in the bristle neuropile respond vigorously to touching the bristles and those with dendrites in the cercal glomerulus respond to filiform or clavate input. It appears that the anatomical separation of the afferent terminals, combined with the restriction of dendrites to one or the other area, is one important step in creating specific synaptic connections.

The mechanism for the separation of afferent terminals remains to be resolved but some clues about the process exist. Bristles are innervated by two to five sensory cells, while filiform and clavate hairs are innervated by one sensory cell (Gnatzy & Schmidt, 1971). Receptors are produced when a single epidermal cell enlarges to become a mother cell which gives rise to the four cells of a singly-innervated hair like the filiform hair. One cell secretes the hair, one secretes the socket, one forms a glia-like accessory cell and one produces the sensory neurone. In multiply-innervated receptors, the limb of the lineage containing neurones is extended to produce additional sensory cells (reviewed in Bate, 1978). Our working hypothesis is that these extra rounds of cell division are in some way coupled to the target area in the central nervous system (CNS). How this linkage is accomplished remains obscure. How it is brought about can be addressed in some respects. In the appendages, all types of sense cells are

bundled together in common nerves (Shankland & Bentley, 1983), so there is no obvious separation which might lead to the observed central separation. In crickets, the bristle afferents diverge from the other afferents immediately upon reaching the CNS, growing on a separate pathway under the cercal glomerulus to their target region. Thus one might suppose they are never allowed to come in contact with the dendrites of the 'inappropriate' targets. However, in locusts, some of the first bristle afferents grow through the middle of the filiform target area and then branch in the appropriate bristle neuropile (Shankland, 1981). Thus a very selective mechanism exists for separating these two populations of afferents and is one step in determining which afferents will synapse with which interneurones.

In summary, the differentiation of cercal sensory neurones commits them to arborize in a particular area of neuropile where they encounter and synapse with non-overlapping subsets of interneurones. Whatever the forces are that divide the terminals into these two main classes they are crucial to the formation of selective synaptic connections.

DIFFERENTIAL CONNECTIVITY OF ONE CLASS OF RECEPTOR

The original studies of the cricket cercal system made it clear that all receptors of the filiform type do not connect with all interneurones having dendrites in the cercal glomerulus (Edwards & Palka, 1974). This is a second form of specificity where cells of similar type synapse with different target neurones. When the anatomy of the afferent projection was determined and it became possible to consider the physiology in an anatomical context, the role of structure in this process was revealed.

Each filiform hair is constrained by the mechanics of its hinge joint to vibrate in a single plane (Palka, Levine & Schubiger, 1977). Each hair is innervated in such a way that the associated sensory neurone is unidirectional (Tobias & Murphey, 1979). When the directionality of individual hairs was examined it became clear that only wind directions in a plane parallel to the substrate were represented. It was demonstrated that receptors of the same directionality are grouped together in strips along the long axis of the cercus (Bacon & Murphey, 1984). Finally it was shown that receptors of different directionalities arborize in different locations (Bacon & Murphey, 1984). Some examples are illustrated in Fig. 2B where filiform receptors of different directionalities have been stained.

It is important to consider the relationship between position of a receptor on the cercus and its projection in the central nervous system. Receptors in a strip project to different regions of the cercal glomerulus. The position of receptors of common directionality along the long axis of a strip is correlated with subtle and continuous changes in arbor location (Murphey, 1981; Bacon & Murphey, 1984). Thus, this long axis is 'topographic' by the usual definition. Similarly, more than half (the ventromedial half) of the cercal circumference exhibits a continuous mapping between soma position and location of the axonal arborization in the cercal glomerulus (Murphey, 1981; W. W. Walthall & R. K. Murphey, unpublished observations). However, there are anomalies in the topography which we do not understand. For example, afferents projecting like the one labelled 'a' in Fig. 2B are found both dorsally and ventrally on the cercus. This makes good functional sense because both are tuned to the same

direction (Bacon & Murphey, 1984). But this is anomalous if position on the cercus is represented by a 1:1 map of the glomerulus. Since there are obvious examples of two locations on the cercus mapping to one location in the CNS, the map must be folded upon itself in some manner. Such a 2:1 mapping must be considered degenerate. However, there can be no doubt that position of a sensory neurone is correlated with target area in the CNS and it seems reasonable to call this a topographic map.

This orderly afferent projection has important implications for synaptic connectivity. Because each interneurone has a distinctive dendritic shape with respect to the afferent projection, this might determine receptive field shape. This idea was assessed by aiming a jet of wind at an animal from different directions while recording intracellularly from an identified interneurone (for example 10-3, Fig. 2). This

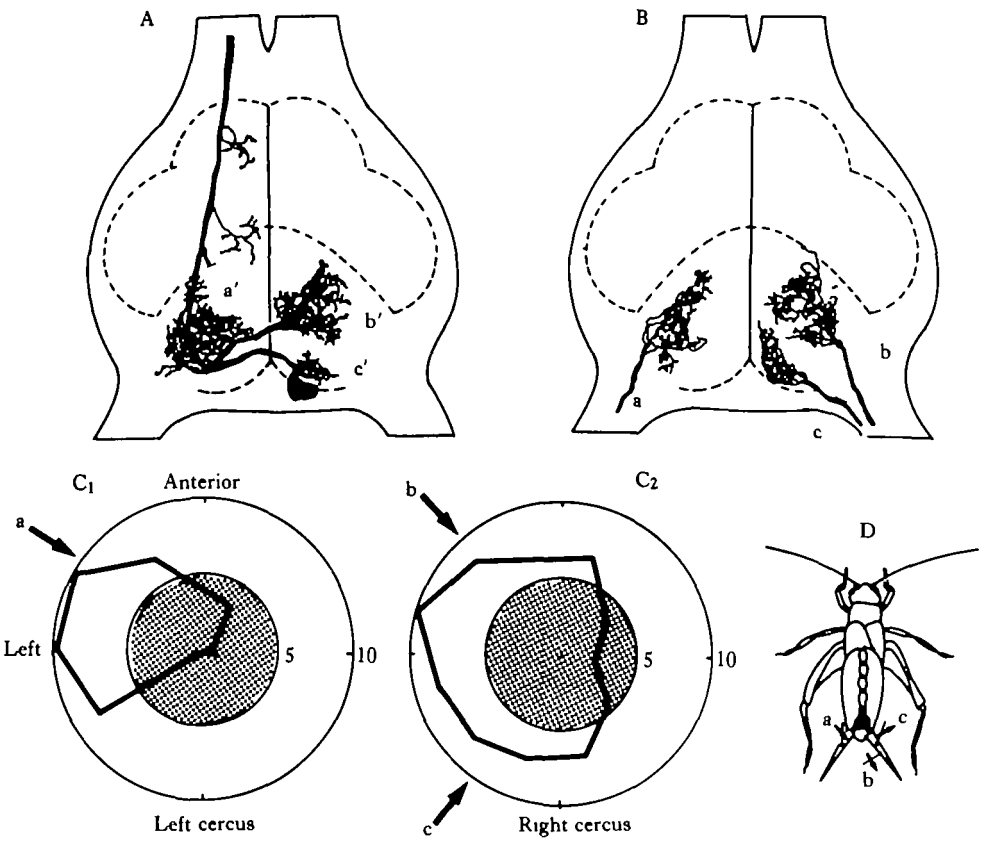


Fig. 2. Structure-function correlates for interneurone 10-3. (A) Dorsal view of interneurone 10-3. Note that it has three separate dendrites (a', b', c'). (B) The terminal arborization of three different filiform sensory neurones. Each terminal labelled a, b, c would intersect a single dendrite on interneurone 10-3a', b', c' respectively. (C) The receptive field of interneurone 10-3 when the left (C_1) or right (C_2) cercus is intact and the receptors on the remaining cercus are blocked with Vaseline. The arrows labelled a, b and c correspond to the wind direction for the three receptors shown in B and D. Receptive fields are plotted in polar coordinates; distance from the centre is action potential number per stimulus. The shading indicates spontaneous activity levels. Points which fall within the shaded area indicate inhibition. (D) Drawing of the animal to indicate the location of the terminal ganglion (darkened), the three receptors and their directionality.

interneurone has dendrites on both sides of the midline and receives excitatory synaptic input from each cercus. A receptive field for this interneurone can be determined for each cercus alone by blocking hair movement on the other cercus. When this is done, two similar though not identical receptive fields are obtained (Fig. 2C). Each receptive field implies a different set of connections. When only the left cercus is intact, the neurone responds best to wind from the left. The left dendrite is located in a region of the afferent map corresponding to anterior left. When only the right cercus is intact, the neurone again responds to wind from the left. The right side of the cell has dendritic fields in two regions – one corresponding to left/posterior (i.e. wind from the medial side of the cercus, Fig. $2C_2$, c; Fig. 2D, c) and one corresponding to left/anterior (wind along shaft of the right cercus, Fig. $2C_2$, b; Fig. 2D, b). Thus the shapes of the two receptive fields of this cell are correlated with the location of its dendrites in the afferent projection. Normally the interneurone sums these two inputs and its receptive field is tuned to the left side of the animal (see Miller & Jacobs, 1984, this volume). A similar analysis was conducted on three other neurones and provided similar results; the receptive field of a cell was predicted by the anatomy (Bacon & Murphey, 1984).

This analysis, of course, rests on the assumption that the excitatory receptive field results primarily from monosynaptic afferent connections. Bacon & Murphey (1984) studied the connection of the class of afferents (Fig. 2B, c) which is presumed to synapse on a dendrite very near the soma of interneurone 10-3 (Fig. 2A, c'). Using methods shown diagrammatically in Fig. 3 they recorded simultaneously from single afferents known to overlap this dendrite and from the soma of interneurone 10-3. It was possible to show that some afferents synapsed with this neurone (Fig. 3B) while others did not (Fig. 3A). Specifically, if they chose an afferent likely to contact the interneurone based on anatomy, they often saw synaptic potentials synchronized with the afferent action potentials (Fig. 3B). It was interesting that only some afferents of this type (approximately 20%), synapsed with neurone 10-3. Thus the connection appears to be probabilistic rather than absolute. The short, constant latency of the synaptic potentials combined with the anatomy support the idea that interneurones receive monosynaptic connections from sensory cells. This is consistent with work on cockroach giant fibres (Callec, Guillet, Pichon & Boistel, 1971). If an afferent was chosen which had the 'incorrect' anatomy, one whose terminals would not overlap the dendrite, they never saw correlated synaptic potentials. In summary, the excitatory synaptic input to the interneurones appears to be a joint function of the topography of the afferent projection and the shape of the dendritic field of the interneurone.

IS THERE DIFFERENTIAL CONNECTIVITY WITHOUT AN ANATOMICAL CORRELATE?

Sensory neurones innervating clavate and filiform hairs may provide an interesting contrast to work described so far. Gravity-detecting neurones associated with the clavate hairs (Bischof, 1975) and wind-detecting neurones associated with the filiform hairs both arborize in the same area of neuropile – the cercal glomerulus (Fig. 1). The clavate receptors respond to displacements of the animal's body with respect to the gravity field (Bischof, 1975). They mediate reflexes which compensate for such

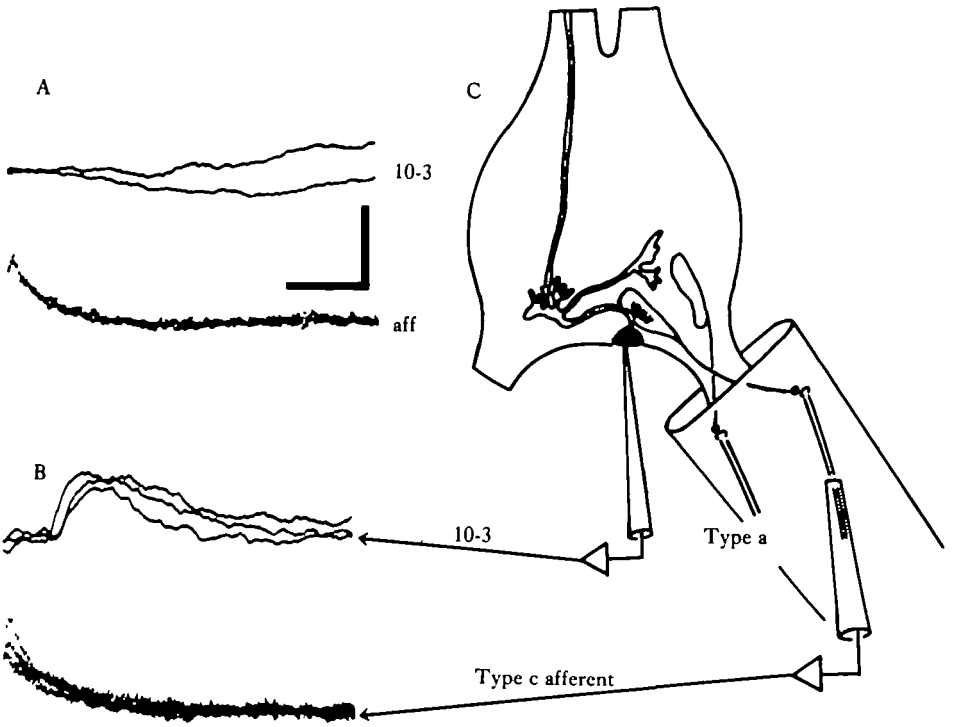


Fig. 3. Further characterization of the synaptic connection between afferents and interneurone. (A) An afferent (aff) which provides no detectable input to interneurone 10-3, consistent with their non-overlapping arborizations in the CNS (bilateral homology of the type a afferent shown in Fig. 2B). (B) Simultaneous recording from interneurone 10-3 and a type c afferent. (C) Schematic view of the anatomy of interneurone 10-3 in relation to the projection from the two classes of afferent. The afferents shown are types c and the bilateral homology of type a from Fig. 2B. The location of type a's arbor has been exaggerated to emphasize that it does not touch the dendrite of interneurone 10-3. Scale, horizontal, 4 ms; vertical, 2 mV. From Bacon & Murphey, 1984.

displacements (Horn & Bischof, 1983) and they excite interneurones with dendrites in the cercal glomerulus (Sakaguchi & Murphey, 1983). The filiform receptors, as described above, respond to wind (Palka & Edwards, 1974; Tobias & Murphey, 1979), mediate escape movements and also excite interneurones with dendrites in the cercal glomerulus (Bacon & Murphey, 1984). The interneurones that respond best to the two modalities are different neurones. For example, Murphey, Palka & Hustert (1977) could find no evidence for gravity effects on the wind-sensitive giant interneurones and D. S. Sakaguchi (unpublished) confirmed this for neurones 7-1 and MGI using intracellular methods. Sakaguchi & Murphey (1983) could find no consistent response of their position-sensitive interneurones to wind or tones. While this is relatively weak evidence, it suggests differential connectivity, even though the anatomy is similar; the two receptors and both sets of interneurones have dendrites in the cercal glomerulus. Other possibilities for producing these apparent differences in connectivity, such as phasic *vs* tonic synapses, or interneurones tuned to phasic *vs* tonic stimuli, remain to be assessed. However, these results bring out the possibility of even more refined types of specificity than those discussed so far.

THE ORIGINS OF ANATOMICAL ORDER

The developmental origin of the anatomical relationship between sensory neurones and their target interneurones is fundamental to the synaptic connectivity already described. Examining and manipulating factors responsible for the shape of the afferent arborization has provided a significant insight into the source of order for the afferents. We have probed the sensory neurone's patterned axonal projection by transplanting tissue from one position to another on the cercus. The logic of the experiments has its origins in the classical literature on the differentiation of insect epidermis and a theoretical treatment of those results (Wolpert, 1969; French, Bryant & Bryant, 1976). This theory, called the 'Theory of Positional Information', suggests that epidermal cells are determined to differentiate in particular ways by their location in a field of cells. For example, in the wing of a butterfly, epidermal cells in different positions, all initially identical, produce patterns composed of different coloured scales. Extensive evidence for this type of positional control exists for the production of many cuticular patterns. Since there is an obvious correlation between the peripheral position of a sensory cell and its region of termination in the CNS, we began working on the hypothesis, first clearly enunciated by Bate (1973), that a sensory neurone's terminal arbor is determined by a mechanism similar to the one responsible for the pattern in the butterfly wing (e.g. positional information). Palka has made a similar argument for the cricket cercal system (Palka & Schubiger, 1975; Palka & Olberg, 1977). There are two parts to our evidence linking positional information to the patterned neural projection and ultimately synaptic connectivity.

The first is to determine whether a neurone's fate can be altered by transplanting it to an unusual position. Tissue from a black cricket, *Gryllus bimaculatus*, was transplanted to a tan cricket, *Acheta domesticus*. The different colours of the epidermis allow one to keep track of the transplanted cells. The epidermal colour is cell autonomous and thus the transplanted tissue and all cells descended from it are marked. To determine that transplanted *Gryllus* neurones behaved normally when grafted to an *Acheta*, small strips of tissue containing identified clavate receptors were transplanted from donor to a homologous position on the host (the sensory axon is severed and the soma is moved along with the surrounding epidermis). Tissue containing clavate neurones was used because it is much easier to re-identify the clavate receptors. Since the two receptor types, clavate and filiform, have the same afferent projection, anything learned about one is applicable to the other. When the afferent projection of these transplanted neurones was assessed, they were found to be identical to the normal *Acheta* neurones that previously occupied that place. They regenerated into the host nervous system and arborized in positions typical for their peripheral location. This result serves as the control for experiments aimed at assessing the positional information hypothesis.

When fully differentiated sensory neurones were transplanted to a non-homologous position on the cercus (Fig. 4, inset), the regenerating cell arborized in the appropriate part of the nervous system, as if it had never been transplanted (Fig. $4A_2$). This is consistent with other reports which showed that the fate of a differentiated neurone cannot be altered by transplantation (Anderson & Bacon, 1979). Thus

Transplanting terminally differentiated neurones cannot tell us anything further about the specification process.

The second and most important aspect of our evidence lies in determining the fates of newly generated sensory neurones that arise as a result of rapid mitosis at the border between an ectopic graft of epidermis and the host tissue. The positional information theory predicts that new cells generated near the graft and host border will form cells typical of neither host nor graft, but will have intermediate properties. On the left side

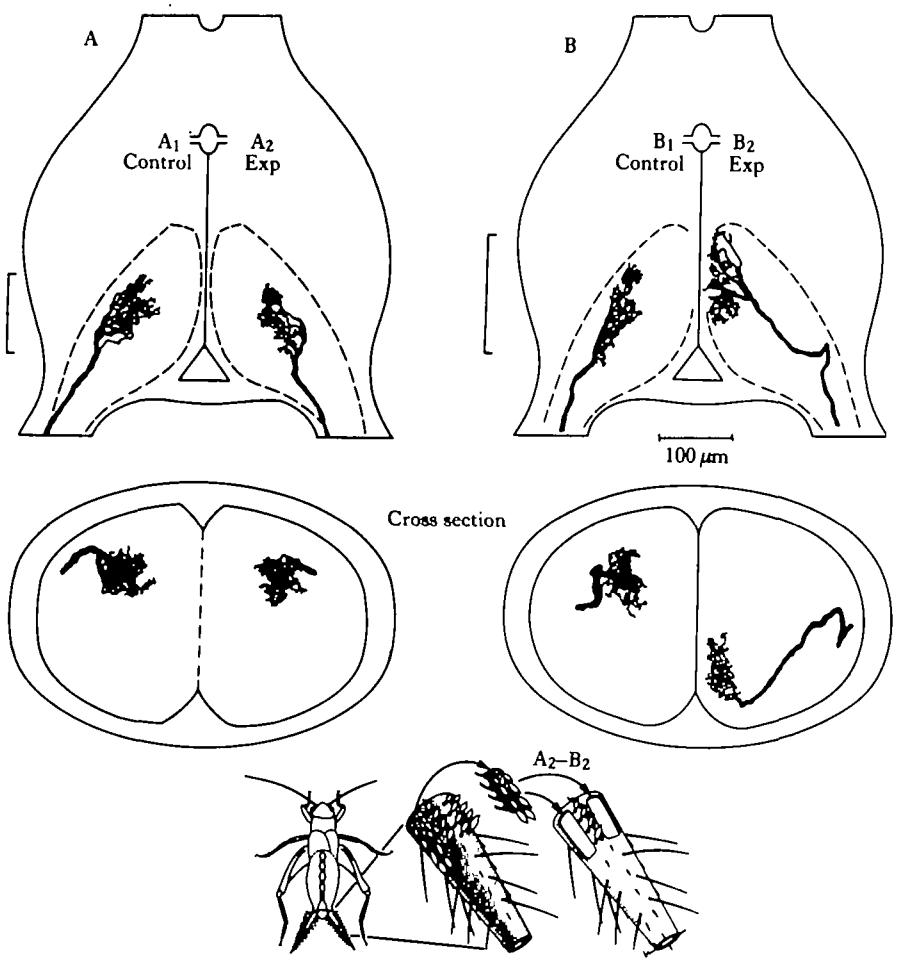


Fig. 4. Positional information and the differentiation of sensory neurones. (A) The result when a fully differentiated neurone is transplanted to a non-homologous position on a second cricket. The transplanted neurone regenerates into the host tissue and arborizes in its usual location in spite of the transplant (A_2). The axonal arborization of the homologous neurone on the pristine cercus serves as a control (A_1). (B) A new neurone derived from host tissue after the transplant arborizes in a new site in the CNS. This neurone, which is recognized as graft-derived because of the colour of the hair it innervates, arborizes in a location atypical for neurones usually produced by the transplanted tissue (B_2). A neurone homologous to those normally produced by the transplanted tissue is shown in B_1. The lower panels in A and B are cross-sectional views of the neurones taken from the region of ganglion indicated by the bracket. Inset: Two types of cross species transplant have been performed: one moves tissue from one species to a homologous position on another species; a second moves a similar piece of tissue to an atypical location. Only the results for this second type of graft are shown in A_2 and B_2.

of the ganglion in Fig. 4B a cell was stained that is the homologue of the transplanted cell nearest the graft border. This cell serves as a control for the type of neurone normally formed by the transplanted tissue. On the right is a newly generated cell near the graft margin. Clearly the two are different – the control arborizes dorso-laterally and the experimental ventro-medially. This is quite distinct from the result of Fig. 4A where fully differentiated transplanted cells are identical to pristine homologues. The areas to which the newly generated sensory neurones project correspond to positions that normally separate the graft and host tissue. (Conceptually identical results have also been obtained for filiform hairs.) This result is strong evidence for a positional control in the differentiation of these sensory neurones. Since this mechanism controls the location of the terminal arborization, it must also influence synaptic connectivity – a fact we have preliminary evidence for (Walthall, Bacon & Murphey, 1983). These results suggest that an important step in the assembly of normal afferent projections is the result of interactions amongst the neuronal precursors (the epidermal mother cells). These interactions of necessity are of peripheral origin and emphasize the fact that positional influences, long the subject of embryologists, are crucial to the formation of selective synaptic connections.

MODULATION OF SYNAPTIC STRENGTH

The formation of synapses is not a digital event. The relative strength of a synapse is also controlled. Much of the work on the cricket cercal system was stimulated by the results of Palka & Edwards (1974), who first showed that cercal synaptic inputs to a giant interneurone were modulated by rearing an animal with only one cercus. Palka & Edwards (1974) assessed the responsiveness of the largest interneurones to input from the remaining cercus in chronically and acutely cercectomized animals. Their experiments demonstrated quite clearly that an interneurone (or interneurones) was more sensitive to the remaining cercal inputs in chronic than in acutely treated specimens. The original experiment was repeated using intracellular methods and the results for three interneurones are summarized in Fig. 5A. The number of action potentials elicited in two of the three chronically treated neurones was several times greater than that seen in acutely treated specimens (Murphey & Levine, 1980; Levine & Murphey, 1980*a*).

There were many ways in which this result might have come about. The first to be demonstrated was a loss of inhibition impinging on the deafferented interneurones. This release from inhibition allows an interneurone to respond better to the intact afferent inputs from the remaining cercus. For example, interneurone 10-3, which is subject to considerable inhibitory control, both pre- and postsynaptically (Levine & Murphey, 1980*b*), exhibits less inhibitory input in chronically treated specimens than in controls (Fig. 6). As Fig. 2 illustrates, numerous excitatory inputs (e.g. from afferents b and c) would remain on interneurone 10-3 even after removing the left cercus. Presumably these remaining inputs are released from inhibition by the treatment and lead to the enhanced excitatory response.

Palka & Edwards (1974) hypothesized that afferents which did not normally cross the midline do so in chronically treated specimens and such a change in anatomy could account for the observed change in response. A determined search for such a change

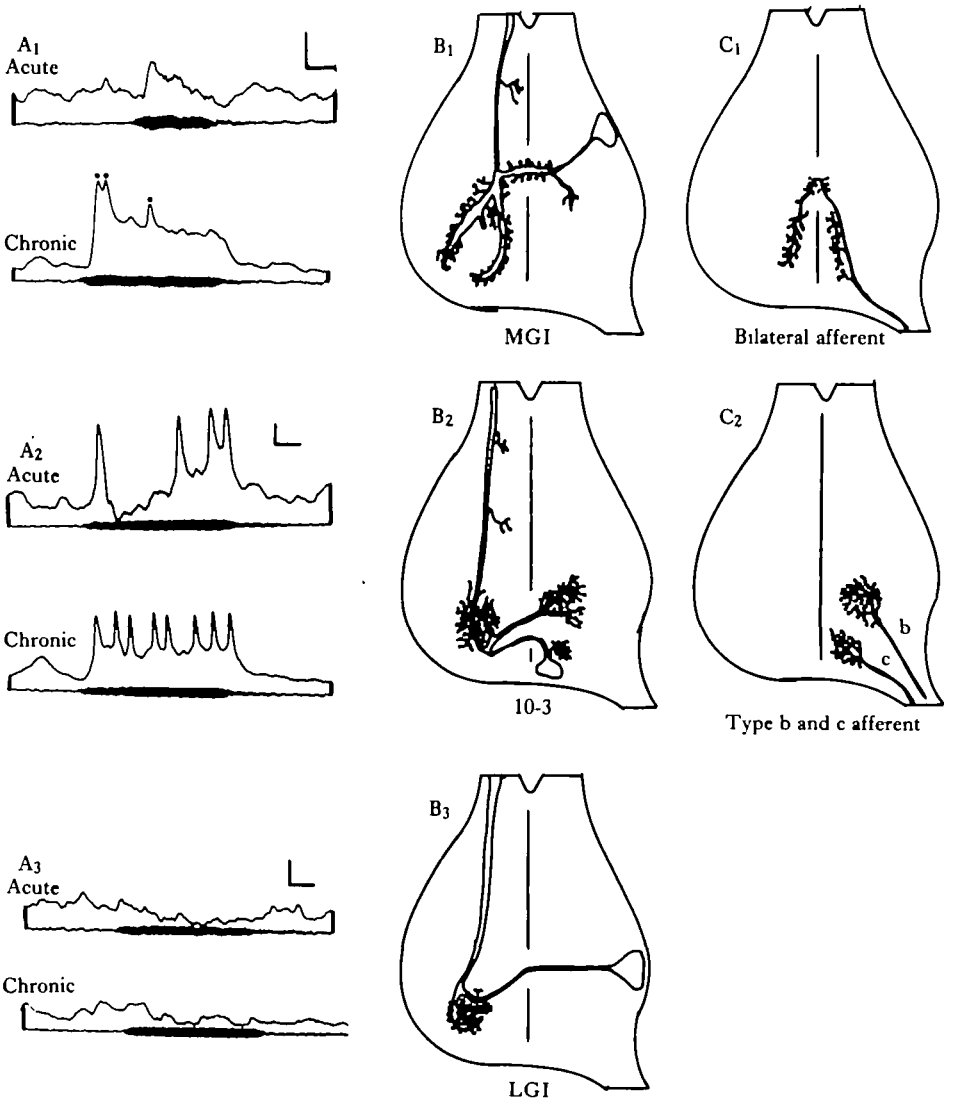
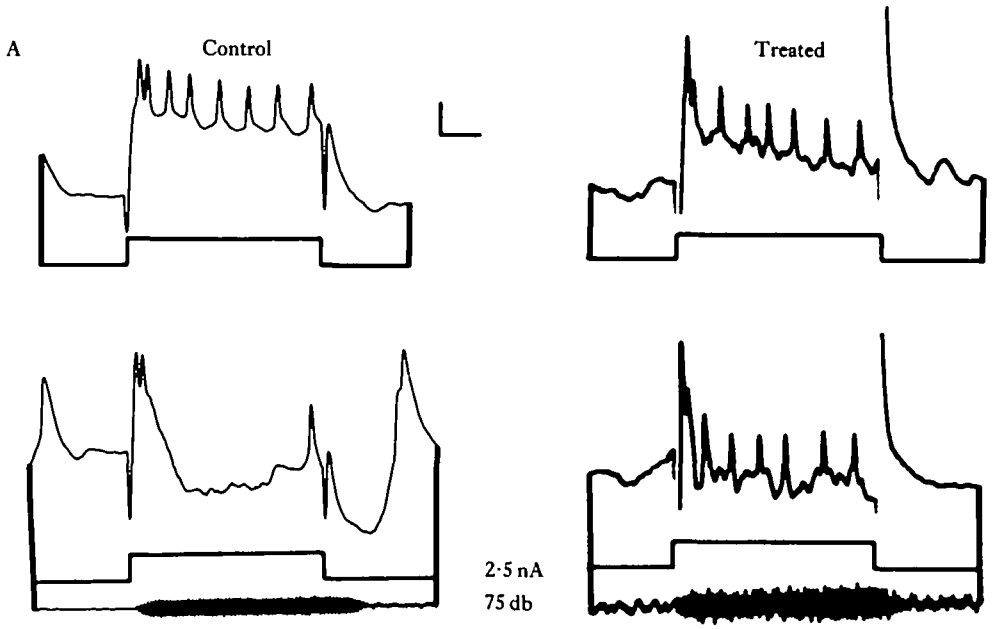


Fig. 5. Modulation of synaptic input by unilateral cercal removal. (A_1–A_3) The response of three interneurones in chronically and acutely treated specimens. Note that MGI and 10-3 are altered in their excitatory response to a standard 85 db tone (which activates filiform hairs on the remaining cercus). LGI is not altered (from Levine & Murphey, 1980*a*; Murphey & Levine, 1980). (B_1–B_3) The structure of the three interneurones. (C_1), (C_2) Schematic views of the afferents whose inputs are probably modulated. (C_1) A representative sensory neurone which arborizes bilaterally and is modified by the treatment (see Fig. 7B). The left side of this terminal arbor is wrapped around the large medial dendrite of MGI. It does not intersect dendrites of either 10-3 or LGI. (Cross-sectional views are not shown but would corroborate this statement.) (C_2) Representative axonal arbors which would contact interneurone 10-3, but not MGI or LGI.

failed (Murphey & Levine, 1980). Recently this idea was reassessed using the single cell staining techniques now available. Two classes of afferents were examined; those which normally terminate ipsilateral to their point of entry and arborize very near the midline, and a small number of recently discovered afferents which normally cross the


A
Control
Treated
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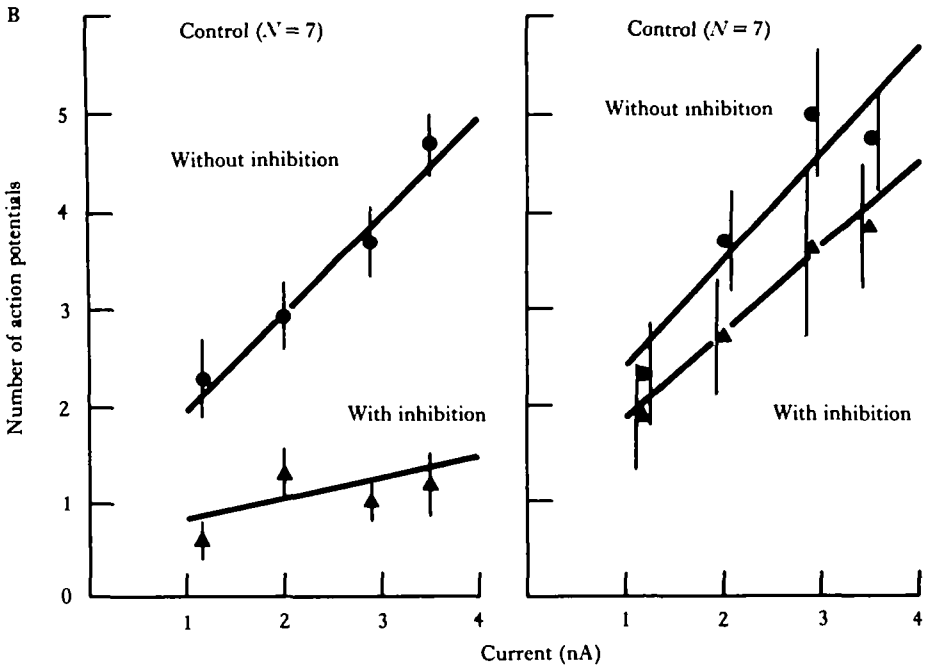
B
Control (N = 7)
Without inhibition
With inhibition
Control (N = 7)
Without inhibition
With inhibition
Number of action potentials
Current (nA)
0
1
2
3
4
5

Fig. 6

midline and arborize bilaterally. In chronically cercectomized specimens, afferents which do not normally cross the midline were not altered by the treatment – in particular they did not cross the midline to innervate the deafferented region of the ganglion (Fig. $7A_2$). Thus this possibility, first suggested by Palka & Edwards (1974), cannot account for the physiological changes. However, an afferent which normally possessed a bilateral axonal arbor (neurone X) was dramatically altered by chronic removal of the opposite cercus. Qualitatively they appear to shift most of the terminal to the deafferented side of the nervous system (Fig. $7B_2$). The number of varicosities on a single identified afferent (neurone X) ipsilateral to the surgery nearly doubled, from an average of 150 ± 53 in 10 control specimens to 269 ± 36 in 10 chronically treated specimens (Fig. $7B_3$, Murphey & Lemere, 1984).

The shift in distribution of varicosities within neurone X's arbor is the result of two changes. First, the arbor adds varicosities at an abnormally rapid rate on the same side as the amputation. This was shown by examining the juvenile arborization at the time of the amputation. The juvenile neurone X is very similar to the adult in shape, but it contains only about 60 % of the normal adult number of varicosities. At the time of amputation there are 91 ± 23 (mean $\pm$ standard deviation) varicosities to the left of the midline, and under normal conditions this grows to 150 ± 53 in the adult. In experimentals, this number increases to 269 ± 36. This means the arbor grows faster than normal in this region.

Second, there is a compensatory decrease in the number of varicosities on the side opposite the amputation. In the juvenile, the number of varicosities in this region is 131 ± 35, and normally, this would increase to 222 ± 57. In the experimental animals, this number decreases to 52 ± 20. Therefore, as a result of the amputation of one cercus, sensory neurone X has retracted parts of its arbor contralateral to the amputation.

The total number of varicosities in these experimental neurones is not significantly different from controls. This suggests that total arbor size is constant and an intrinsic property of the cell. Since the experimental neurone X adds varicosities in one area and retracts them from another area, apparently it is able to detect the relative number of neighbours locally and shift its resources to the area containing fewest neighbours. These changes in growth of the axonal arbor of neurone X fit the concept of competition as it is presently defined. This result is one of the first pieces of evidence for competitive interactions in an invertebrate nervous system.

It had not been possible to establish the existence of competition in previous experiments on insects (Ghysen, 1980; Murphey, Johnson & Sakaguchi, 1983*a*) even though it is a ubiquitous phenomenon in the vertebrate nervous system (see Wiesel

Fig. 6. Influence of inhibition on current-evoked trains of action potentials in interneurone 10-3. (A) Examples of current-evoked trains of action potentials in interneurone 10-3, recorded *via* a bridge circuit, in the absence (upper) and presence (lower) of a tone which activates inhibition. Calibration: vertical, 5 mV; horizontal, 20 ms. (B) Graphic summary of the effect of inhibition on trains of current-evoked action potentials. The number of action potentials (±S.E.) evoked during the 20–70 ms following the onset of a 100-ms current pulse. Abscissa: current intensity. Each point represents the mean from 10 trials of each of seven animals. In controls, the number of action potentials evoked by a current pulse (circles), was reduced by concurrent sound activation of the inhibitory pathway (triangles). In treated siblings, the reduction was negligible, indicating that inhibition was less effective. (From Levine & Murphey, 1980*a*.)

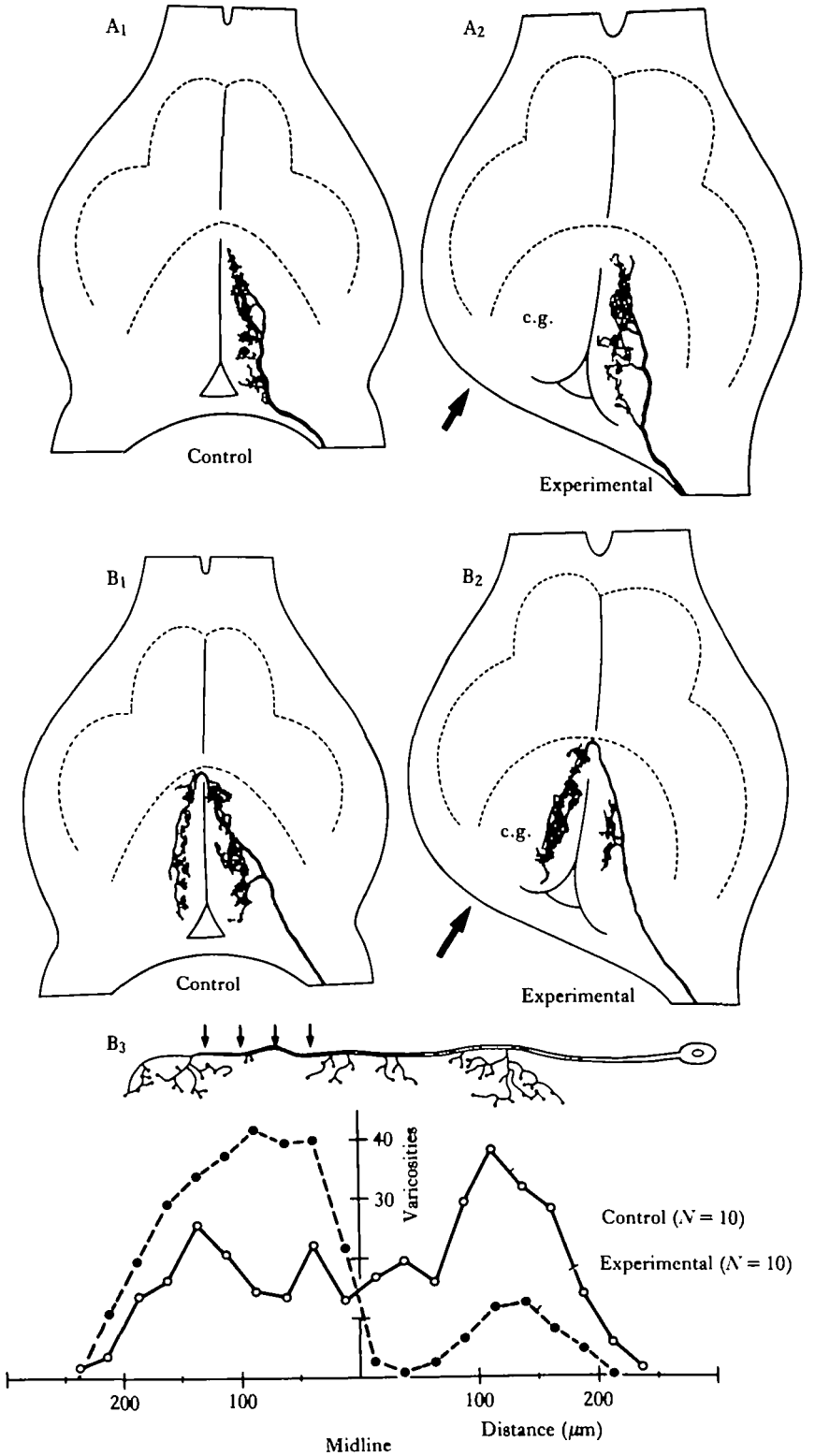
A1
A2
c.g.
Control
Experimental
B1
B2
c.g.
Control
Experimental
B3
40
30
Varicosities
Control (N = 10)
Experimental (N = 10)
200
100
100
200
Distance (μm)
Midline

Fig. 7

Hubel, 1965; Guillery, 1970; and review by Purves, 1980). The results documented in Fig. 7B were obtained because one neurone was treated differentially in different parts of its axonal arbor. Previous experiments simply increased (Murphey *et al.* 1983*a*) or decreased (Ghysen, 1980) the total number of afferent neurones interacting in the CNS. In the cricket, the total number of afferents in one glomerulus was increased by creating supernumery limbs, but each individual arbor remained normal. In fact, the ganglion was slightly enlarged. This suggests that central neurones were induced to grow larger and simply accommodated extra terminals of normal size. Competitive interactions could not be detected. Results on *Drosophila* provided a complementary case where the total number of afferents was decreased by mutation; the terminal arbors of remaining neurones were not detectably altered (Ghysen, 1980). In spite of these failures, the success obtained by studying the bilaterally projecting sensory neurones (Fig. 7) provides a powerful clue to the interactions which guide assembly of the orderly afferent projection.

This type of structural alteration could account for some of the physiological changes which have been observed after cercetomy. For example, the axonal arbors of the bilateral neurones are wrapped around the large medial dendrite of the MGI (Fig. $5B_1$, C_1). This puts them in an ideal position to excite the MGI. If we assume that the observed changes in axonal arborization will be reflected in the strength of the connection with the MGI then we would expect the deafferented MGI to receive a stronger crossed synaptic input, which it does (Fig. $5A_1$). This idea also predicts that the other MGI will lose synaptic input, but this has never been examined. At the present time this is purely correlative. However, tests for these ideas are readily designed. The most important experiment will be to record unitary excitatory potentials from the MGI in acute and chronically treated specimens and determine whether the strength of the connection is altered.

Each of the interneurones studied in these experiments is probably changed by different combinations of the two mechanisms: release from inhibition and enhanced excitation. All of the interneurones have less than the normal amount of inhibition impinging on them. In the case of interneurone 10-3, this releases the remaining inputs from inhibition and an enhanced response is seen. The MGI loses inhibition as well, but it is also in a position to receive a strengthened excitatory input from the bilaterally arborizing sensory cell. Thus the MGI's enhanced input is likely to be a combination of enhanced afferent input from the remaining cercus and loss of inhibition. Finally, the LGI, while it loses inhibition, is not detectably altered in

Fig. 7. The effect of unilateral cercal removal on the structure of afferents belonging to the intact cercus. (A_1), (A_2) The terminal arborization of an identified filiform neurone on normal and chronically cercectomized specimens. There is no obvious change in morphology. In particular the cell in A_2 does not cross the midline. (B_1), (B_2) The bilateral terminal arborization of an identified filiform neurone in control and chronically treated specimens. Note the shift in the arbor *toward* the deafferented side of the CNS. The dashed lines indicate the neuropil cell body boundary and the cercal glomerulus (c.g.). (B_3) Quantitative analysis of the differences between the experimental and treated arborizations of the bilateral sensory neurone shown in B_1 and B_2. The bend in the bilateral neurone's axon has been straightened and the midline taken as a reference point. Distance from the midline is plotted on the x coordinate. The average number of varicosities located in 25 μm wide strips, arranged perpendicular to the axon, was plotted on the y coordinate. Note the experimental terminal is quite asymmetric; it has more varicosities than normal at every point along the arbor ipsilateral to the amputated cercus and fewer than normal at every point contralateral to the amputated cercus.

excitatory input. This is consistent with two details of its structure. First, its dendrites are confined to the side of the deafferentation and thus it lacks input from non-crossing afferents on the remaining cercus. Second, its dendrites do not intersect the axonal arbor of the bilateral afferents and it thus receives no direct excitatory input from the remaining cercus *via* this pathway. In summary, there are strong structural correlates to the modulation of excitatory synaptic connections.

THE CERCAL SENSORY SYSTEM IN THE CONTEXT OF PREVIOUS STUDIES OF NEUROSPECIFICITY

Historically, four basic mechanisms have been thought to guide the assembly of afferent projections and thereby determine connectivity. These mechanisms are cues that neurones might use to locate their proper targets. They will be referred to here by the shorthand terms of 'mechanical', 'temporal', 'positional' and 'competitive' cues.

Mechanical cues

One simple mechanism for creating an organized projection would be to arrange the mechanics of growth and the location of cells such that the axons of cells are automatically directed into appropriate areas of the CNS (e.g. see Harrison, 1910; Weiss & Taylor, 1944; Horder & Martin, 1978; Macagno, 1979; Scholes, 1979; Bodick & Levinthal, 1980). At a gross anatomical level, simple mechanics do play a role since displaced cercal afferents will arborize in whatever ganglion is available to them. For example, when transplanted to the thorax they arborize in thoracic ganglia and make synaptic connections there (Murphey *et al.* 1983*b*). Such a mechanism might also be expected to exist on a more local level within the cercal glomerulus system since location in the periphery, in the nerve, and ultimately in the ganglion are fairly simply related. However, in a variety of transplant situations the expected tight coupling between axon trajectory and target area has been disrupted in the cercal system; most neurones arrive at their proper target regions even by circuitous routes (Murphey *et al.* 1983*a*; W. W. Walthall & R. K. Murphey, in preparation). This leads us to conclude that mechanical guides or cues, a very reputable source of guidance in some other species, while important at a gross level play no role in guiding the assembly of detailed connections within the cercal glomerulus.

Temporal cues

A second mechanism, often discussed in tandem with mechanical cues, is birth order or time of arrival of the target area. By this mechanism, timing might be crucial in determining a neurone's fate as in the visual system of some crustacea (Macagno, 1979). The role of temporal cues has been tested explicitly in the cercal system and there is no evidence for either birth order or time of arrival of an axon in the CNS, having a crucial role in connectivity (Murphey, Jacklet & Schuster, 1980; Murphey, Johnson & Walthall, 1981; Murphey *et al.* 1983*a*). Thus these two passive cues, mechanical and temporal cues, seem to be relatively weak forces in the assembly of the cercal system.

Positional cues

Sperry's (1963) chemoaffinity model for the assembly of the retino-tectal system suggests a more active mechanism, one which assigns neurones an identity based on their position. This allows them to seek out partners in a corresponding array even if their location or time of arrival is disturbed. The results of Fig. 4 provide very strong evidence for such a mechanism in the cricket cercal system.

Competitive cues

One commonly reported observation in vertebrates is that neighbouring terminal arborizations interact with one another in a manner which restrains their growth and this is called competition. Removal of neighbouring terminals can release a cell from this restraint, allowing its arbor to expand or invade the neighbour's territory (Schmidt, Cicerone & Easter, 1978). This is reminiscent of the changes demonstrated in Fig. 7B where a cricket sensory neurone expands its terminals in regions normally occupied by absent neighbours. Thus it appears that competition of the type commonly seen in vertebrates is also present in crickets.

Competitive interactions may depend on neural activity in the competing elements. A role for neuronal activity was first suggested by Wiesel & Hubel's (1965) work on the visual cortex. The evidence for such an activity-dependent synaptogenesis has been confirmed and extended in both higher (Archer, Dubin & Stark, 1982) and lower vertebrate visual systems (Meyer, 1982). In essence, the suggestion is that neural activity can stabilize active synapses at the expense of less active or less synchronous synapses. This mechanism has yet to receive solid support in the cercal system. Although there is some evidence of a role for neural activity in the function of the cercal system (Matsumoto & Murphey, 1977), the evidence for an effect on specificity *per se* is non-existent. In summary, two important mechanisms, chemoaffinity and competition, used to conceptualize the neurospecificity issue seem to have an important role in assembling the cercal sensory system.

CONCLUSION

In some respects, the study of the anatomy of the cercal sensory system is complete. The rules for the assembly of the afferent projection and the correlation between the afferent projection and connectivity with postsynaptic cells are clear. Numerous physiological experiments can now be designed to test the implications of these observations. However, as powerful as the anatomical techniques are, we feel we have probed the limits of this approach to the neurospecificity question. These anatomical techniques are incapable of taking the next step and providing an answer to the question, how do neurones recognize one another? There are at least two parts to the problem. First, are the cellular interactions in the periphery which direct sensory neurones to different areas in the CNS to synapse with selected target neurones. Second, they must be able to recognize their neighbours in order to compete with them centrally. Since these are similar to the phenomena which have been used classically to define neurospecificity, it should be possible to take advantage of the

short life cycle of insects, the genetics available for some insects and the high antigenic nature of insect tissues to take the next step and analyse neurospecificity at the molecular level.

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