Embryogenesis of an insect nervous system
I. A map of the thoracic and abdominal
neuroblasts in *Locusta migratoria*

By C. M. BATE[1]

From the Department of Neurobiology, Australian National University

SUMMARY

Maps of the thoracic and abdominal neuroblasts have been prepared by reconstruction from serial sections of timed *Locusta migratoria* embryos. The maps are bilaterally symmetrical, periodic and consistent for embryos of the same age, with a fundamental arrangement of 7 rows of 4–10 cells plus 1 median neuroblast per segment. A map of 60 + 1 cells is repeated in each of the three thoracic segments, with an additional median cell developing late at the anterior end of the prothorax. The arrangement in the abdomen is similar, with 56 + 1 cells per segment. Neuroblasts differentiate and subsequently degenerate in an antero-posterior sequence, but construction of the thoracic ganglia involves a delayed degeneration of part of the original set in these segments. The maps show that the neuroblasts are as reliable in their number and arrangement as the adult cells they produce. The number of neuroblasts used in constructing the relatively complex thoracic ganglia is similar to that which produces the simpler abdominal ganglia. Some motorneurons have the same parent neuroblast. The maps are intended as a first step in an analysis of the relation between the progeny of different neuroblasts and the family of neurons which each neuroblast produces.

INTRODUCTION

The embryogenesis of the insect nervous system remains an almost completely uncharted subject. The difficulties of experimenting with insect embryos and the extended period of larval development during which neurons may be manipulated with relative ease have combined to focus attention on the post-embryonic development and repair of the system (reviewed in Young, 1973). Experiments on larval and adult nervous systems indicate the kinds of properties which have to be ascribed to developing neurons. For example in both sensory and motor systems transplantation experiments reveal the limits to the alternative connexions which neurons can make among the units of the already differentiated system, and properties of specificity and serial homology are inferred (e.g. Edwards & Sahota, 1967; Young, 1972). But experiments on larval and adult nervous systems leave unanswered the question of how embryonic cells are initially organized into a network of differentiated neurons. Also,

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[1] *Author's address:* Department of Neurobiology, Research School of Biological Sciences, Australian National University, Canberra, A.C.T. 2601, Australia.

because of the incomplete view of the constraints operating during the phase of assembly, such experiments give few additional insights into the way in which the nervous system is organized.

Nevertheless, in a general sense, the cells which generate the insect nervous system, the neuroblasts (Wheeler, 1891), are well known. Because of their large size, their repetitive cycles of mitosis, and their segmental origins, they are valuable in a number of different fields – in the study of the mitotic mechanism (Carlson & Gaulden, 1964), as evidence of the segmental composition of the arthropod head (Butt, 1960; Manton, 1960) and as a source of neurons for growth in tissue culture (Seecof, Donady & Teplitz, 1973). Yet apart from the work of Panov (1963, 1966) on the origin and fate of neuroblasts in a number of different insects, the papers of Nordlander & Edwards (1969 a, b, 1970 c) on the postembryonic development of the brain and optic lobes of *Danaus* and a thorough study by Malzacher (1968) of the brain anlage in *Carausius* and *Periplaneta*, we have no more detailed knowledge now of the number and arrangement of these cells than the outline descriptions, provided by earlier workers such as Wheeler (1891, 1893), Strindberg (1913), Baden (1936) and Roonwal (1937) (reviewed by Johannsen & Butt, 1941; Anderson, 1972 a, b).

By contrast, our knowledge of the differentiated cells of the adult nervous system is steadily improving. For some insects (e.g. *Periplaneta*: Cohen & Jacklet, 1967; Young, 1969; Pearson & Fourtner, 1973; *Schistocerca*: Bentley, 1970; Burrows & Hoyle, 1973) neuron maps are available which reveal from animal to animal a relatively constant position, shape, and pattern of connexions for identified cells. Besides making the components of various neuronal circuits repeatably available for stimulation, recording, dye injection and in some cases chemical analysis, these maps pose the further question of why the cells are arranged in a particular consistent way. During development of the nervous system it appears that the individual cells budded off from the neuroblasts have access to restricted parts of the genetic programme which commit them to alternative and sometimes unique pathways of differentiation. At a later stage these cells appear at predictable places on the neuron map. So the question of arrangement can be rephrased as 'what is the system according to which prospective neurons are assigned to alternative developmental fates?'.

Maps of the adult neurons which are available for the thoracic ganglia of *Schistocerca gregaria* can be applied directly to the Australian plague locust *Chortoicetes terminifera* (Burrows, 1973) and there is no reason to doubt that they are the same for *Locusta migratoria* as well. In *Locusta* these adult cells, or their immediate precursors, are budded off from the neuroblasts during 150 h of embryonic life. The intention in this paper is to provide a complementary map of the neuroblasts as a first step in the analysis of the relation between them and their progeny at one remove (see below) the adult neurons.

MATERIALS AND METHODS

Locusta migratoria were cultured in the laboratory at 28 °C and offered tubes of moist sand in which to lay eggs. The eggs were collected within 1 h of laying and stored in sand at 30 °C. Timed embryos were either dissected from the egg shell under Ringer (Usherwood, 1968), fixed in Carnoy, stained briefly with Hansen's trioxyhaematein and mounted in Permount, or processed for serial sectioning.

Embryos for use in serial reconstructions were dissected out under a phosphate-buffered mixture of paraformaldehyde and glutaraldehyde (pH 7·4), fixed for 2 h at room temperature, washed in buffer, postosmicated, dehydrated with methanol and embedded in TAAB resin. Serial 1 μm sections were cut with glass knives on an ultramicrotome, mounted on slides and stained with toluidine blue. Every third section in the series was projected through an inverted microscope under a × 40 objective and traced. Before reconstruction every section in the series was checked under a × 100 objective to correct errors made at the lower magnification. A two dimensional map of the neuroblasts was then constructed in the horizontal plane using Pusey's (1939) method.

RESULTS

A general outline of the embryology of *Locusta* is to be found in Roonwal's papers (1936, 1937) and will not be repeated here. According to most authors the neuroblasts differentiate among the cells of the epidermis by enlargement, but according to Malzacher's careful study of *Carausius* and *Periplaneta* (1968) by a process involving an equal division by the central cell of a neurogene cell group to give two sister neuroblasts. The neuroblasts are arranged in rows and columns on either side of the midline with a single median cell in each segment (Roonwal, 1937). Typically, they are large cells with pale nuclei (Fig. 1) whose increasing size displaces the surrounding cells of the epidermis. These interstitial cells form a dorso-ventrally elongated envelope between adjacent neuroblasts. By repeated asymmetric divisions perpendicular to the surface, the neuroblasts generate inwardly directed, radial columns of cells (Fig. 1). According to Baden (1936) these cells do not divide again in the related orthopteran *Melanoplus differentialis*, and Roonwal (1937) in his account of *Locusta* leaves the question of subsequent divisions undecided. However, it is clear (Fig. 1) that divisions do occur among the daughter cells of the neuroblasts in *Locusta*. The alternative destinies of the family of cells produced by a single neuroblast are unknown – some of them may become glial cells which undergo repetitive mitoses. None the less, the daughter cells from which neurons are derived divide once, equally, with a variable orientation before contributing axons to the embryonic neuropile.

As development proceeds, the ganglia enlarge and the medio-lateral, antero-

posterior curvature of the anlage increases. In this way the original flattened sheet of epidermis is converted to a corrugated surface, whose periodic bulges correspond with the segmental ganglia. At the same time a process of degeneration overtakes the neuroblasts within the ganglia. At any one time therefore,
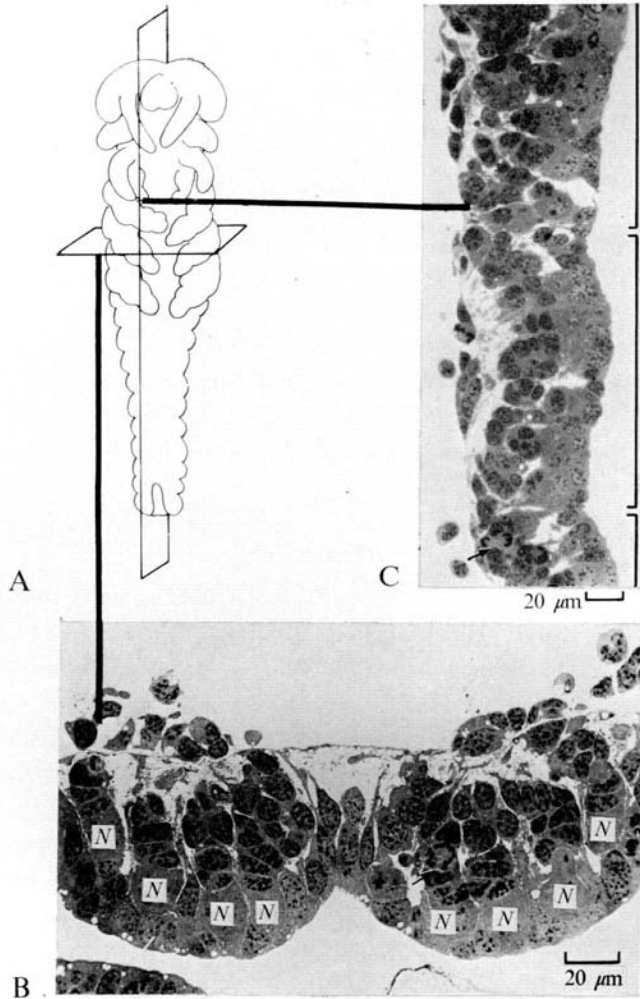

Fig. 1. A, 100-h embryo to show the alternative planes of section used in constructing neuroblast maps. B, A transverse section of the mesothorax at 100 h. One preganglion cell (arrowed) and one of the neuroblasts (N) can be seen in mitosis. C, A longitudinal section of the mesothorax at 100 h, with the limits of the mesothorax and adjacent segments indicated by brackets. One preganglion cell (arrowed) is in mitosis.

the number of neuroblasts depends on the number of cells in the epidermis which were originally committed to form neuroblasts and the extent of the later process of selective degeneration. The arrangement depends on the points at which cells were committed to form neuroblasts and the subsequent displace-

ment of these cells by the increasing size and curvature of the surface of the ganglion.

Onset of neuroblast divisions

The consistent and unusual orientation of the mitotic spindle, perpendicular to the surface makes the onset of divisions easy to observe in whole mounted embryos (Fig. 2). In Fig. 2 the appearance of dividing neuroblasts is mapped
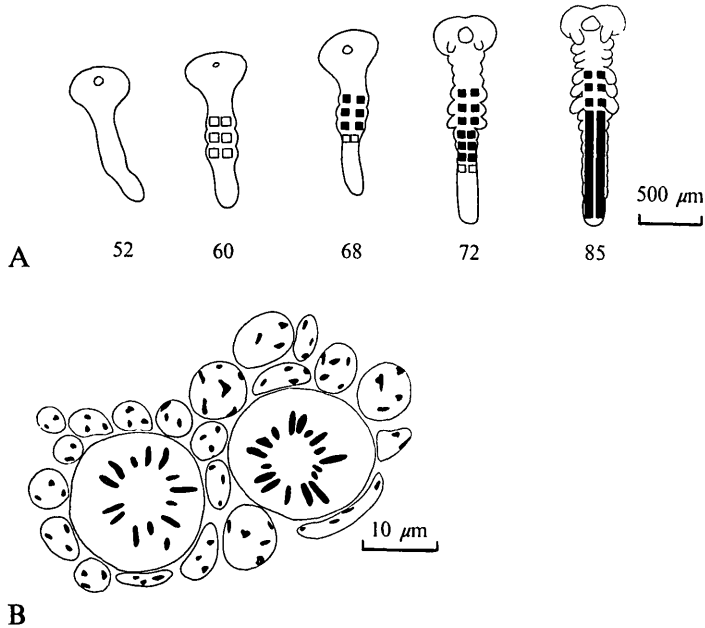

Fig. 2. A, Onset of neuroblast divisions in the thorax and abdomen at the indicated stages. □, Dividing neuroblasts present in some embryos; ■, dividing neuroblasts present in all embryos. B, Camera lucida drawing of two neuroblasts in division, showing characteristic mitotic figures. Ventral surface view of a whole mounted embryo at 85 h; the surrounding interphase nuclei are epidermal.

from the prothorax to the last abdominal segment. As reported by many authors the process of neuroblast differentiation moves antero-posteriorly. At 52 h none of the characteristically oriented mitotic figures can be seen, but by 60 h division has begun in some animals in the pro-, meso- and metathorax. Division begins in the abdomen at about 68 h and by 72 h has spread to the first three ganglia. By 85 h neuroblasts are dividing in all segments from the prothorax to the eleventh abdominal. In each case the single median neuroblast appears later than the lateral ones.

Neuroblast maps

The criteria used by Malzacher (1968) were also used here to identify neuro-blasts for inclusion in the maps, that is to say cells which contribute to the

C. M. BATE

nervous system by unequal divisions and differ clearly in size and structure
from their daughter cells.

(a) 85 *hours*

Reconstructions from serial transverse sections (Fig. 1) produce a simul-
taneous map of the cells on both sides of one segment (Fig. 3). In both the
thorax and the abdomen the arrangement of the neuroblasts is bilaterally
symmetrical and periodic. The fundamental plan in both is the same, with, on
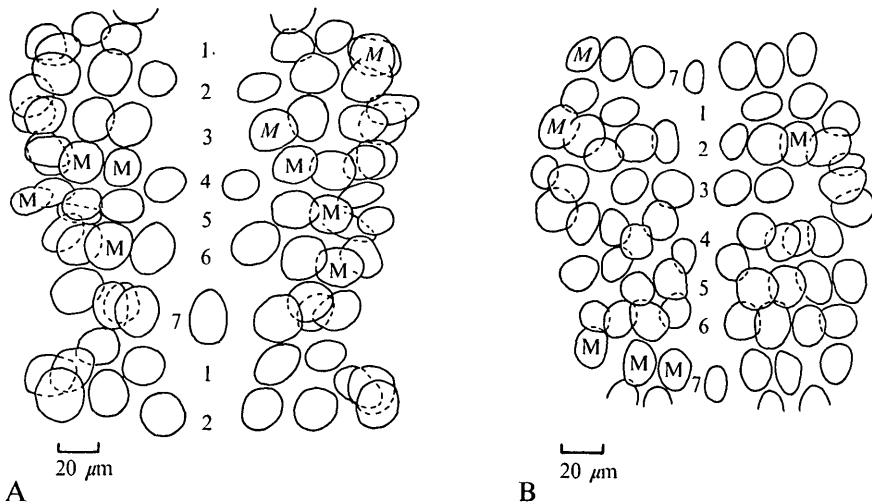either side of the midline, seven rows of neuroblasts consisting of from two to

Fig. 3. A, A map of the mesothoracic neuroblasts at 85 h. B, A map of the neuro-
blasts in the third abdominal segment at 85 h. Both ventral views. *M*, Neuroblast
in mitosis. The numbering of rows is arbitrary.

five cells per row. The repeat number for each side of the thorax is 30, whereas
in the abdomen it is only 28. Several reconstructions of this kind were made and
the patterns shown in Fig. 3 are consistent apart from minor variations in the
packing of individual cells. The distribution of mitoses does not agree with
Poulson's (1950) finding in *Drosophila* that equivalent cells on opposite sides
divide in synchrony.

Reconstructions from transverse sections do not allow segmental boundaries
to be included in the map. To determine at which point the boundary falls it
was necessary to make further reconstructions from longitudinal sections. In
sections of this kind (Fig. 1) it is clear to which segment the daughter cells of
any neuroblast are assigned and segment boundaries can be drawn between
adjacent rows of cells. Further reconstructions were confined to sections in this
plane once the bilateral symmetry of the system was established.

In both the thoracic and abdominal ganglia the segmental boundary falls
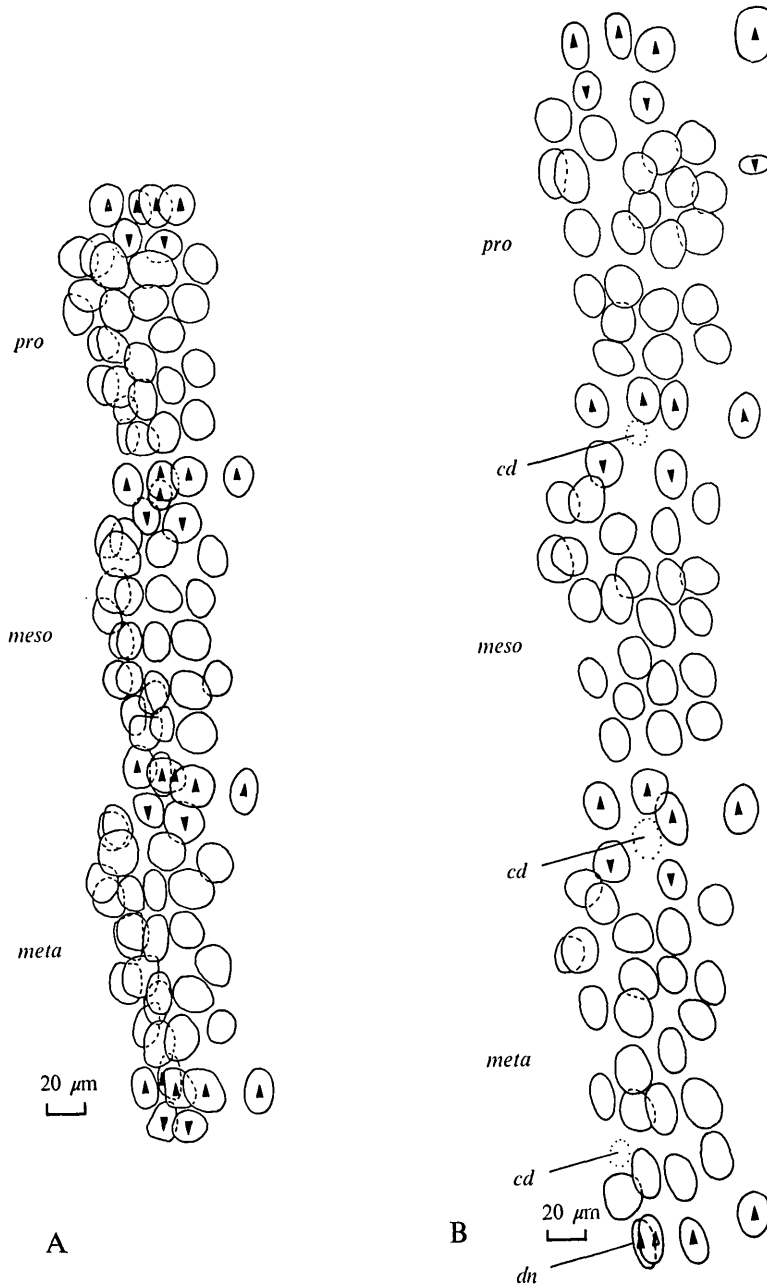between a posterior row of three or four cells and an anterior row of two cells

Fig. 4. A, A map of the left segmental neuroblasts in the pro-, meso- and meta-thorax at 85 h, reconstructed from longitudinal sections. B, The same segments at 110 h. The number of neuroblasts is reduced by cell death (*cd*, chromatic droplets; *dn*, neuroblast at an early stage of degeneration). Both ventral views, with arrows indicating the segmental contribution of boundary neuroblast rows. Note the additional median cell in the prothorax at 110 h.

(Figs. 4, 5). The median neuroblast lies just anterior to the segmental boundary. The maps of the three thoracic segments are very similar at this stage, but it was surprising to find that it requires much the same number and arrangement of cells to generate the far simpler ganglia of the abdomen. As it might be argued that the first three abdominal ganglia are a special case (they migrate anteriorly to fuse with the metathoracic ganglion later in embryonic life) further reconstructions were made of abdominal ganglia four and five. At 85 h these ganglia
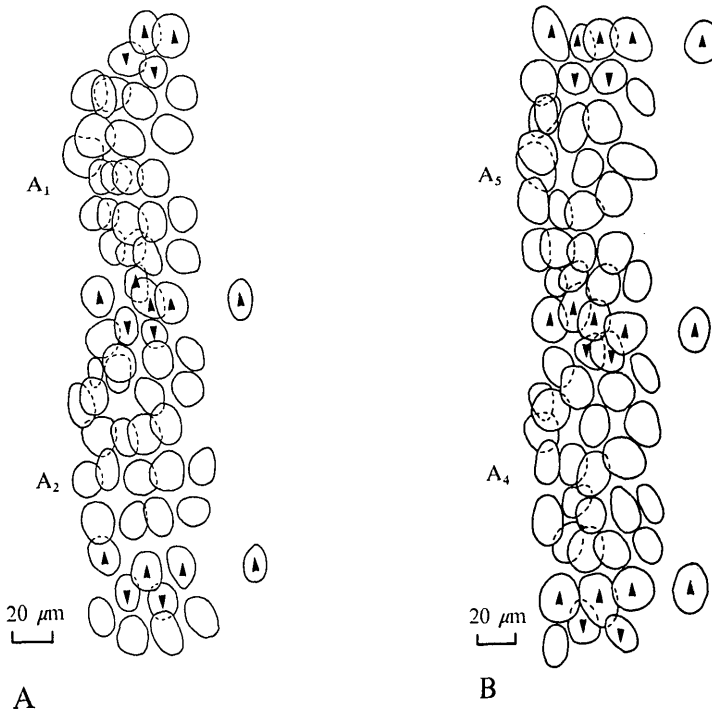

Fig. 5. A, A map of the left segmental neuroblasts in the anterior two abdominal ganglia at 85 h. B, A map of the left segmental neuroblasts of the fourth and fifth abdominal ganglia at 96 h. Both ventral views.

are so small as to be difficult to reconstruct with certainty, so maps were made of the ganglia at 96 h (Fig. 5), and at this stage it becomes clear that the arrangement in the fourth and fifth abdominal ganglia resembles that in the first two at 85 h and is also similar to the pattern found in the thorax.

(b) 110 hours

By 110 h there are signs of cell death in the form of chromatic droplets (Wigglesworth, 1942) scattered among the neuroblast progeny, in the neuroblast layer and among the interstitial cells. Reconstructions of the thoracic and first abdominal ganglia (Figs. 4, 6) show a reduced number of neuroblasts. Of the thirty cells originally present in each half segment of the thorax, the pro- and

mesothorax each have 27, the metathorax 28. The additional cell in the meta-thorax appears to be in the second most posterior row. However the presence of a large group of chromatic droplets just anterior to this cell and a comparison with the 85 h map suggest that this cell may have been displaced anteriorly from the posterior row of the segment. In that case the supernumerary cell is
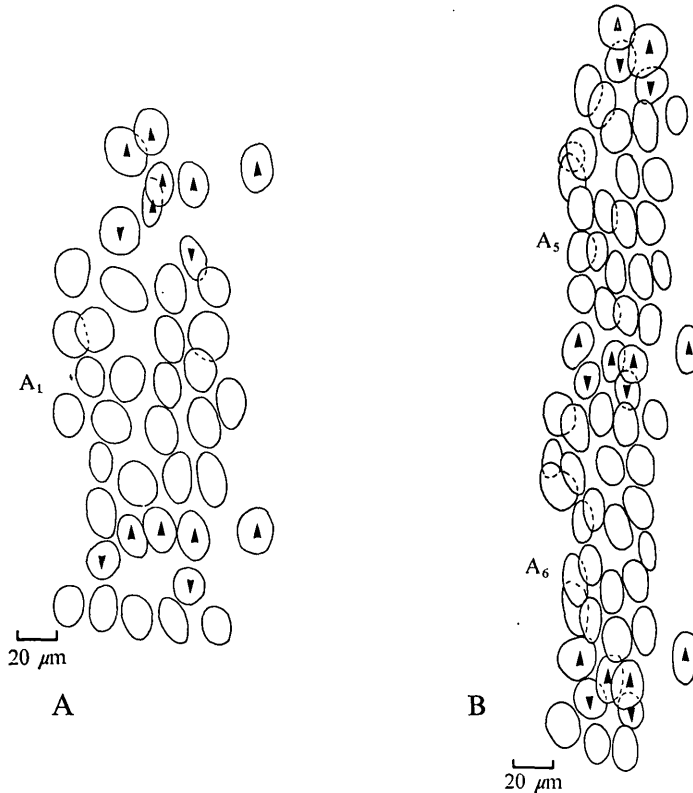

Fig. 6. A, A map of the left segmental neuroblasts in the first abdominal ganglion at 110 h. The number of neuroblasts is now reduced by degeneration (compare figure 5A). B, A map of the left segmental neuroblasts in the fifth and sixth abdominal ganglia at 110 h. The neuroblast count in each half ganglion remains at 28 (+1) cells. Both ventral views.

one of the median cells in the posterior row. This cell has the clumped chro-matin of a cell about to degenerate and coincides with chromatic droplets and cell fragments at equivalent positions in the pro- and mesothorax. This suggests that a selective wave of neuroblast degeneration is passing along the segments antero-posteriorly. In line with this idea, the number of neuroblasts in the first abdominal segment is also reduced (Fig. 6) but in segments five and six the number remains, as before, 28, and in these ganglia there are no signs of degenerating neuroblasts.

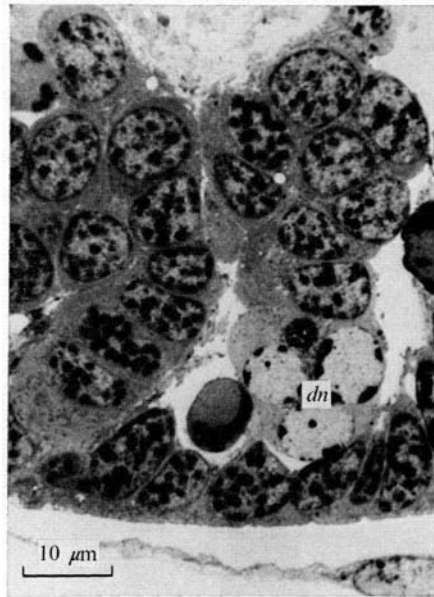At 85 h the complement of cells in each of the three thoracic segments is

Fig. 7. Degenerating neuroblast (*dn*) with a column of daughter cells above it. First abdominal ganglion at 120 h.
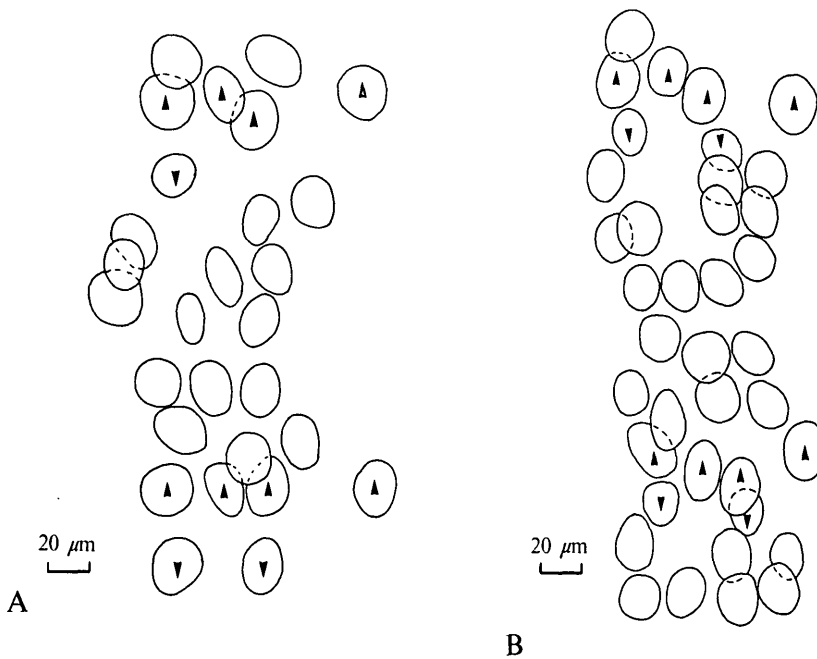

Fig. 8. A, A map of the left segmental neuroblasts in the first abdominal ganglion at 120 h. B, A map of the left segmental neuroblasts in the fifth abdominal ganglion at 120 h. Both ventral views.

identical, but between 100 and 110 h a single additional cell appears uniquely on the midline at the anterior margin of the prothorax (Fig. 4). This neuroblast buds off daughter cells in a postero-dorsal direction, mirroring the activity of the median cell at the other end of the segment.
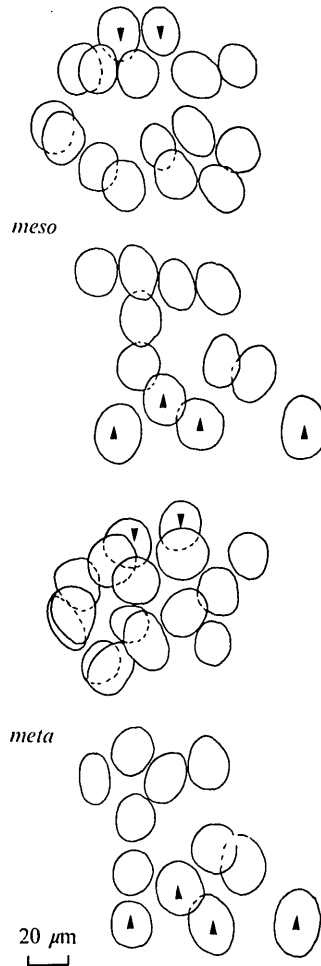

Fig. 9. A map of the left segmental neuroblasts in the meso- and metathorax at 120 h. Ventral view.

(c) 120 *hours*

By 120 h there are signs of degenerating neuroblasts throughout the thorax and abdomen and occasional columns of daughter cells can be seen with a degenerating neuroblast at their base (Fig. 7). In the first abdominal segment the number of neuroblasts is reduced to 19, while in the fifth abdominal segment the number has fallen in 10 h from 28 to 23 (Fig. 8). By contrast in the thorax the numbers are relatively stable: 26 (+1) in the prothorax, 27 each in
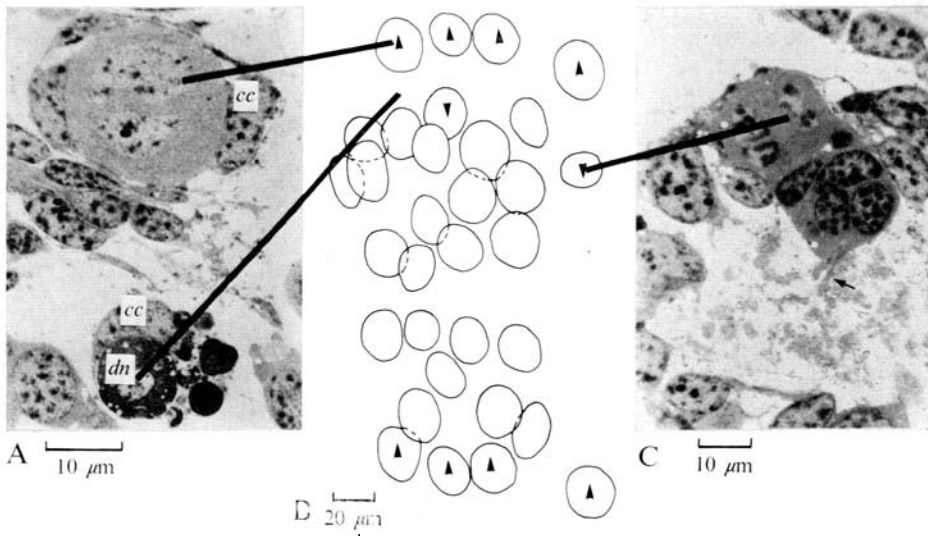
Fig. 10. B, Map of the left segmental neuroblasts in the prothorax at 120 h to show:
A, a degenerating neuroblast (*dn*) with cap cell (*cc*), found at the indicated position
on the map plus the next anterior neuroblast and C, the anomalous median neuro-
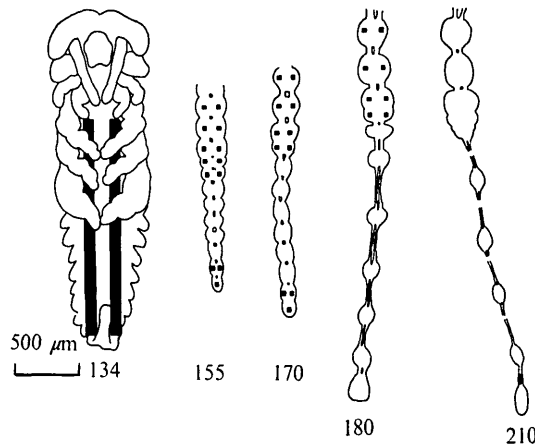blast and its progeny contributing axons (arrowed) to the embryonic neuropile.

Fig. 11. The distribution of persistent neuroblasts at the indicated stages. 134 h:
whole embryo, the rest dissected abdominal and thoracic nervous system. ■, Neuro-
blasts still present in this ganglion.

the meso- and metathorax (Fig. 9). The missing cell in the prothorax allows a
precise correlation to be made between cell death and the loss of neuroblasts.
The expected cell, present in the meso- and metathorax, absent from the pro-
thorax is the more lateral of the two cells of the anterior row. At the point
where this cell is expected to be, there are instead the obvious fragments of a
degenerating neuroblast together with an accompanying cap cell (Kawamura

& Carlson, 1962). At the same time, the progeny of the anomalous median cell in the prothorax are by now contributing axons to the embryonic neuropile (Fig. 10), so confirming that this cell is a source of neurons which are, presumably, peculiar to the prothorax.

Disappearance of the neuroblasts

By 120 h the size and separation of the remaining neuroblasts is such that it is possible to observe them fairly simply in whole mounts. After blastokinesis (details in Roonwal, 1937) and the elongation of the embryo to fill the whole egg, it is necessary to dissect out the thoracic and abdominal ganglia to follow the fate of the neuroblasts in whole mounts. At 134 h (Fig. 11) neuroblasts are still present in all segments, but by 155 h they have disappeared from abdominal ganglia three to seven. At 170 h neuroblasts are still present in the thorax, in the first abdominal ganglion which has fused with the metathoracic ganglion, and in the last, compound, abdominal ganglion. They persist in the first abdominal ganglion and the thorax at 180 h, but by 210 h (150 h after their first appearance in the thorax) they have disappeared from the thorax and abdomen completely.

DISCUSSION

Like the adult neurons the neuroblasts, apart from minor variations in packing, are arranged in a reliable way which allows the same cell to be identified in different embryos. With the exception of the additional anterior median cell which appears in the prothorax, the resemblance between the maps for the three thoracic ganglia is striking. What is more surprising is to find that the arrangement is very similar to the abdomen as well. Almost the same number of neuroblasts is required to produce each of the abdominal ganglia as to produce the relatively larger and more complicated ganglia of the thorax. In each the fundamental plan appears to be the same, and this suggests that apparent homologies between neurons in the thorax may extend for some cells to the abdomen as well. The additional complexity of the thoracic ganglia is associated with a delayed degeneration of a proportion of the neuroblasts with which they start.

The evidence for neuroblast degeneration is unequivocal despite the fact that in some other insect systems it does not occur (e.g. Malzacher, 1968). In *Locusta* neuroblast elimination begins earlier than reported in other insects and 48 h before the onset of degeneration found by Roonwal in his study of *Locusta*. It is likely that Roonwal, using wax histology and without knowing the exact positions of the expected neuroblasts, missed the early stages of the process. Neuroblasts in all phases of degeneration can be observed once the process is initiated and the map allows terminal degenerative stages to be positively identified as blanks in the expected arrangement (Fig. 10). The onset of degeneration reflects the antero-posterior sequence in which the neuroblasts first appear (Fig. 2), and by 155 h neuroblasts have disappeared from the

abdomen apart from the first two ganglia and the terminal compound ganglion. In the thorax however, the process of degeneration is not completed until 210 h. Selective retention of neuroblasts is a simple method of increasing their contribution in particular ganglia. In view of the shorter life span of the abdominal neuroblasts, the production of preganglion cells in these segments is presumably far less than in the thorax. Nevertheless, the rate at which the neuroblasts divide is also important, and this together with the exact pattern of degeneration deserve further study.

In view of the apparent similarity of all the ganglia, it was surprising to find an additional median neuroblast, which does not occur in any of the more posterior segments (an examination of whole mounted embryos suggests that it does not occur in the next two anterior segments either) developing at a relatively late stage in the prothorax. The family of neurons which this cell produces is presumably unique and should therefore be relatively easy to identify in the adult ganglion.

The intention in preparing these maps has been to provide the basic information necessary for a further analysis of the relation between the neuroblasts and their descendants, the adult neurons. At present there is no explanation for the distribution of motorneurons in insect ganglia. According to Bentley (1970) the layout of the somata of fast motorneurons innervating flight muscles in the mesothorax of *Schistocerca* is a rough projection of the position of their target muscles in the periphery. On the other hand Burrows (1973) and Burrows & Hoyle (1973) did not find such a scheme for the distribution of the limb motorneurons in the metathorax of *Schistocerca*, there being no correlation between the position of the soma and the innervated muscle in the leg. There is however some evidence for a functional grouping with a tendency for cooperative fast and slow neurons to the same muscle and synergistic neurons to lie together in the ganglion in both the cockroach (Young, 1972) and the locust (Burrows & Hoyle, 1973). The significance of such a grouping is not clear however, since the position of the soma which has no synapses and is often far removed from synaptic sites within the neuropile, may be physiologically trivial.

The distribution of the adult neurons depends in part on the way in which prospective neurons are parcelled out from among the progeny of the embryonic neuroblasts, despite the fact that the simple columnar pattern of these cells is disrupted by the changing shape of the ganglion and the increasing number of cells, which together generate the form and composition of the adult system. In so far as the final arrangement of neurons depends on the initial distribution, it reflects the hierarchy according to which neurons of different classes are produced by neuroblast divisions. That is to say there may be rules according to which daughter neurons with the same or different target muscles, similar or different patterns of connexion within the neuropile, the same or different sets of enzymes can be produced from the alternative neuroblasts of the available

set, and the layout of the adult cells will in part depend on these rules. Neurons which are necessarily the progeny of different neuroblasts might be expected to cluster separately in the adult ganglion. It is particularly interesting therefore to find that in both the cockroach (Pearson & Fourtner, 1973) and the locust (Burrows, 1973) there is an apparent separation of the excitatory and inhibitory motorneurons with the inhibitory cells clustering about the midline, posterior to and separate from the excitatory neurons. Emson, Burrows & Fonnum (1974) have shown that the enzyme glutamate decarboxylase responsible for the synthesis of the likely inhibitory transmitter gamma aminobutyric acid, is found only in the inhibitory cells and not in the excitatory neurons. Inhibitory and excitatory neurons cluster separately in the lobster as well and the suggestion that this distribution is a result of their separate embryological origins is not a new one (Otsuka, Kravitz & Potter, 1967; Kandel & Kupfermann, 1970).

Clearly some of the excitatory motorneurons have the same parent neuroblast. The number of motorneurons in locust ganglia is not known but the available data set a lower limit. Bentley (1970) identified 19–20 fast motorneurons innervating flight muscles in the mesothorax in *Schistocerca*. By an indirect method Young (1969) found 10 motorneurons innervating the mesothoracic leg of *Periplaneta*, but these results should be seen in the light of a comprehensive study of the same ganglion made by Gregory (1974). Gregory, using silver stains and reverse diffusion of Procion yellow through cut nerve trunks into the ganglion, found a total of 151–155 efferent axons (i.e. excitatory and inhibitory motorneurons plus any neurosecretory fibres) in mesothoracic nerve roots two to six, with 36 efferent neurons leaving by nerve five, the main supply to the mesothoracic leg. In *Schistocerca* Hoyle & Burrows (1973) and Burrows & Hoyle (1973) have so far identified 23 excitatory motorneurons innervating the metathoracic leg. Allowing for differences between the meso- and metathoracic legs, this incomplete catalogue of neurons makes it certain that the number of excitatory motorneurons in each half ganglion of the mesothorax in *Locusta* is greater than 30 and probably very many more. Since the number of neuroblasts per half ganglion is $30+1$, some neuroblasts produce more than one motorneuron.

The maps show that the neuroblasts in the embryo are as consistent in their number and arrangement as the adult neurons which they ultimately produce. It is likely that the neurons are generated to a coherent scheme which influences the way in which the adult ganglia are organized. Experiments are now planned to analyse this scheme by exploring the relation between the progeny of the different cells in the map and between the family of cells produced by a single neuroblast.

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