PATHFINDING BY GROWTH CONES IN THE CENTRAL NERVOUS SYSTEM OF THE GRASSHOPPER EMBRYO:

J.A. Raper, M. Bastiani,* and C.S. Goodman

In the grasshopper embryo the morphological development of individually identified neurons can be traced prior to the onset of axonogenesis on through maturity (eg., Goodman and Spitzer, 1979). The behavior of individual growth cones can be characterized in their natural environment as they extend towards their targets. Detailed observations on the growth cones of a small group of related central interneurons demonstrate that each growth cone follows a highly stereotyped and specific path in the neuropil, and, further, suggest that the growth cones of many neurons are determined to recognize and elongate upon the axons of specific earlier differentiating neurons.

The first 6 progeny of the identified neuronal precursor cell, neuroblast 7-4 (Figure 36A), have been studied in detail (Raper et al., 1982 a, b). Listed in the order of their maturation, they are the Q1, Q2, G, C, Q5, and Q6 neurons (Figure 36B). Q1's growth cone pioneers a portion of the posterior commissure as it extends across the ganglionic midline. The growth cones of Q2, G, C, Q5, and Q6 subsequently traverse the commissure upon the axon of Q1 in an orderly sequence and with a delay of about 3 hours and 50 microns between them.

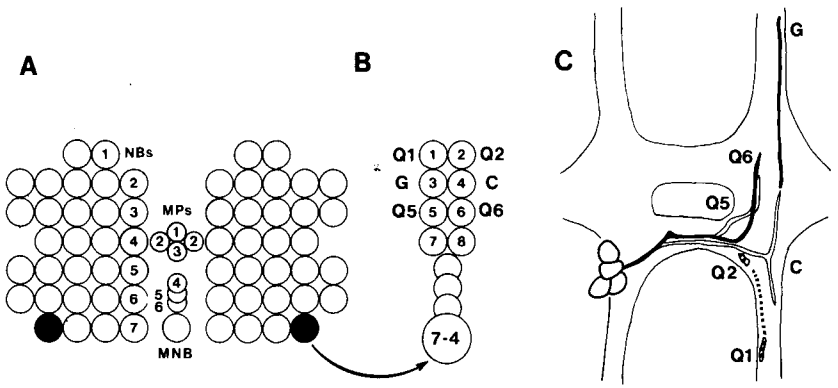

Figure 36. The first 6 progeny of neuroblast 7-4. The neurons in each segmental ganglion arise from a plate of 61 neuroblasts (NBs) and 7 midline precursor cells (MPs). A. NB 7-4 (solid) is the posterior, lateralmost neuroblast on each side of every segment. B. The first neurons arising from NB 7-4. C. The axons of all 6 siblings cross the ganglionic midline in the posterior commissure but diverge from each other at specific locations in the contralateral neuropil. [Raper, Bastiani, Goodman]

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Upon reaching the contralateral neuropil, the growth cones of Q1 and Q2 turn posteriorly in a medial portion of the ganglionic connective. First G, and then C, pass over the location where Q1 and Q2 turn posteriorly, and continue extending until they reach a lateral position in the neuropil. Here G's growth cone often pauses, allowing C's growth cone to catch up. Subsequently, G's growth cone rapidly extends anteriorly in the connective. C's growth cone first extends for short distances both anteriorly and posteriorly. Within a day of development, C extends rapidly in an exclusively posterior direction. Q5 and Q6 turn anteriorly in a medial position in the contralateral neuropil.

The growth cones of the Q1 and Q2 neurons turn posteriorly upon the axons of two identified central pioneer neurons, the MP1 and dorsal MP2 cells. The growth cones of the G and C neurons turn and extend in opposite directions upon a discrete bundle of identified axons. This bundle is initially formed by four axons. One pair, which grows anteriorly along the basement membrane that covers the dorsal surface of the ganglion, meets and fasciculates upon a second pair of axons that grows posteriorly. The resulting axonal bundle then falls off the basement membrane before G and C fasciculate upon it. G extends anteriorly in direct contact with the axons in the bundle (Figure 37, A and B). The neurons that give rise to the bundle's anteriorly extending axons have been identified and named A1 and A2; they reside in the next posterior ganglion on the contralateral side of the pathway they help to form. G's growth cone has never been observed to turn anteriorly until the A1 and A2 axons pass by it (Figure 38). C begins its rapid posterior extension only after several other axons have joined the bundle (Figure 37, C and D). During this period of rapid posterior extension, C's growth cone is found to be preceded by the growth cones of at least 2 pairs of identified cells, those of the posteriorly directed neurons that originally helped to form the axonal bundle, the P1 and P2 cells; and those of 2 other neurons, the X1 and X2 cells. (These findings are summarized in Figure 39).

The routes upon which these central growth cones extend, the locations at which they turn, and the directions in which they turn are highly stereotyped and precise. They do not grow in arbitrary directions or rely upon the subsequent pruning of inappropriate neurites to assume their basic morphology. It is unlikely that these growth cones are passively directed by mechanical guidance cues. Although they all enter the contralateral neuropil in the same location, they diverge from each other at reproducible, cell-specific choice points. The growth cones of the G and C neurons grow past the location at

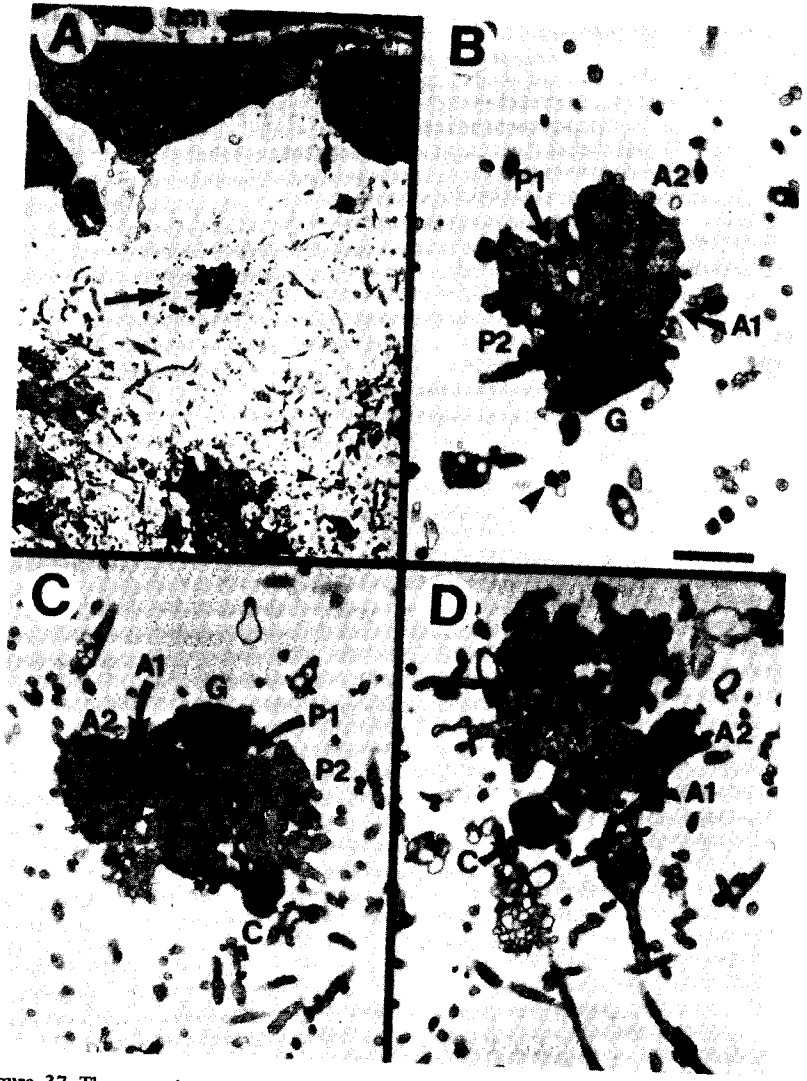


Figure 37. The axon bundle upon which G and C fasciculate and diverge. Cross sections of embryonic neuropil; dorsal is to the top of the figure and the midline is to the left. A. Low magnification view of the axon bundle (large arrow) in which G extends anteriorly and C extends posteriorly. G is the ventral axon profile filled with reaction product. bm=basement membrane; gl=glial cell. B. Higher magnification of the bundle. C. View of another, older preparation in which both the G (dorsal axon) and C (ventral axon) were filled with HRP. The A1, A2, P1, and P2 axons are indicated. There are an additional 5 axons belonging to unidentified neurons. D. The same bundle sectioned further posteriorly. The P1, P2, and G profiles have dropped out. Scale bar: A=5 microns; B to D=1 micron. [Raper, Bastiani, Goodman]

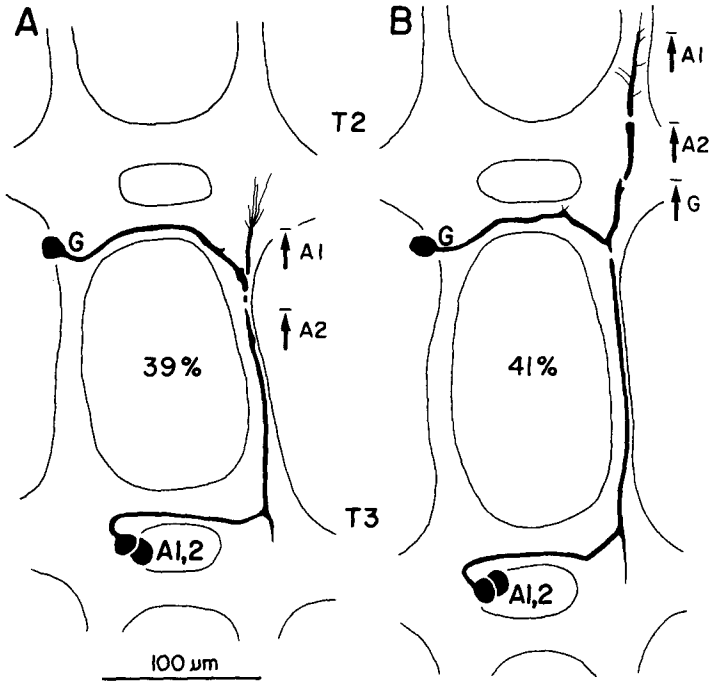
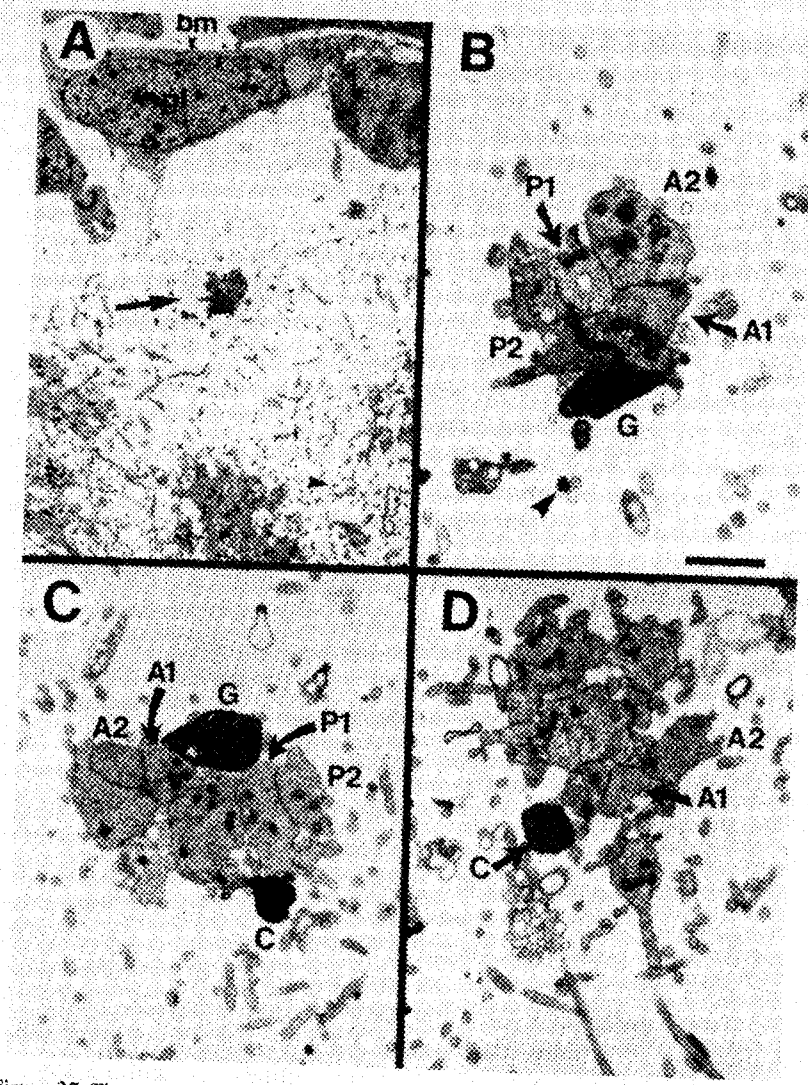


Figure 38. The relative timing of G's anterior extension relative to that of A1 and A2. A. The relative positions of the G, A1, and A2 growth cones in a 39% embryo. A1 has grown past G's growth cone. B. G extends anteriorly upon the axons and behind the growth cones of the A1 and A2 neurons. [Raper, Bastiani, Goodman]

which the Q1 and Q2 growth cones turn posteriorly, and the G and C growth cones are found to be in nearly identical positions in the lateral neuropil before they extend in opposite directions.

In many instances, growth cones are observed to extend upon the axons of specific, earlier differentiating neurons, giving rise to the "labeled pathways" hypothesis (Goodman et al., 1982; see also Ghysen and Janson, 1980, for a similar proposal). The hypothesis states that (1) a small number of early differentiating neurons pioneer a stereotyped array of axonal pathways; (2) these axonal pathways are differentially labeled, most likely on their cell surfaces; and (3) the growth cones of later differentiating neurons are programmed to choose between and elongate upon these specifically labeled pathways. This hypothesis is consistent with studies in both vertebrates and invertebrates that demonstrate that axons entering the CNS in abnor-



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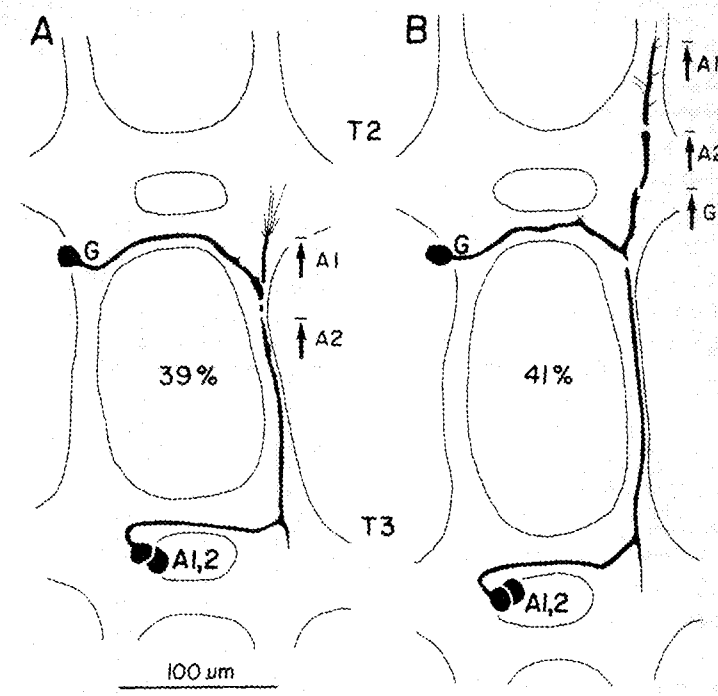

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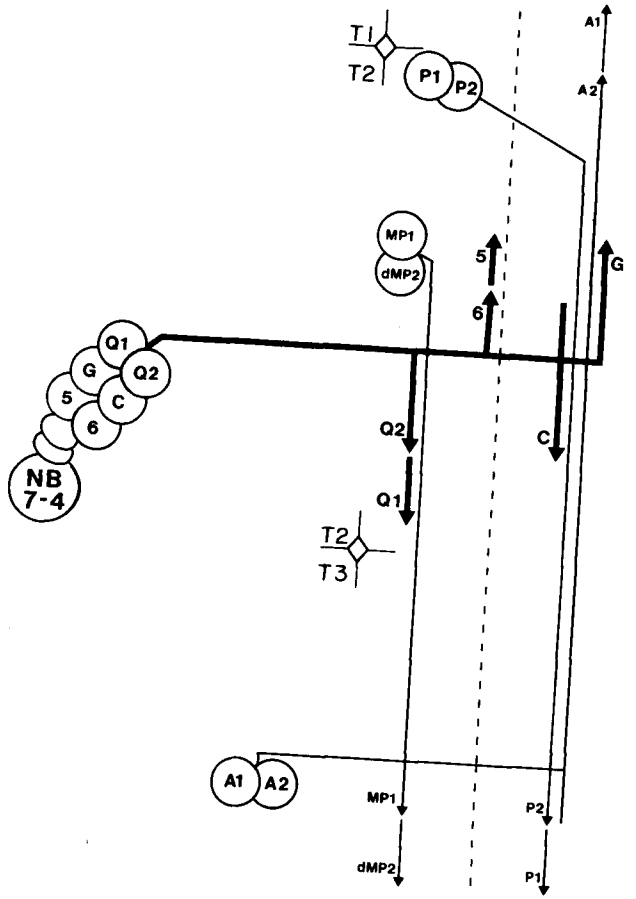

Figure 39. The growth cones of the first 6 progeny of NB 7-4 extend upon specific axonal pathways in the developing neuropil. After crossing the ganglionic midline, the Q1 and Q2 axons turn posteriorly upon the MP1 and dMP2 axons. The subsequent progeny of NB 7-4 cross the posterior commissure upon the axons of Q1 and Q2. G extends anteriorly upon the A1, A2, P1, and P2 axons. C extends posteriorly upon several axons including those from the P1 and P2 neurons. Q5 and Q6 extend anteriorly near a different, unidentified axon bundle (dashed line). [Raper, Bastiani, Goodman]

mal locations can find and grow in specific axonal tracts (e.g., Constantine-Paton and Capranica, 1975, 1976; Ghysen, 1978; Katz and Lasek, 1979, 1981; Anderson, 1981). The "labeled pathways" hypothesis should be directly testable in the embryonic grasshopper by lesioning specific identified axons and looking for a subsequent effect upon the behavior of identified growth cones.

DEVELOPMENT OF THE *DAPHNIA* VISUAL SYSTEM:

E.R. Macagno, M.S. Flaster,* and R.S. Schehr*

The aim of this short review is to consider answers to two questions in light of our studies of the development of the *Daphnia* visual system: (1) How does a photoreceptor axon get from the array of origin (the compound eye) to the topographically appropriate region of the target array (the optic lamina)? (2) What is the role of cell interactions in the formation of the pattern of synaptic connections between the afferent axons and the target cells? Since most of the data has been published and recently reviewed (Flaster et al., 1982), only a brief background is provided below for the discussion.

Background

The parts of the *Daphnia* visual system of interest here are the single, bilaterally symmetric, compound eye and the optic lamina, the target region of the optic axons in the bilaterally symmetric optic ganglion. The 176 photoreceptors are organized into 22 units (ommatidia), each with 8 cells. The 110 laminar cells are organized into 22 clusters of 5 cells called optic cartridges. Each optic cartridge receives inputs from the 8 optic axons of a particular ommatidium, the 8 axons traveling in a bundle from eye to lamina. The projection is ordered topographically: lateral ommatidia connect to lateral cartridges, medial ommatidia to medial cartridges (Macagno et al., 1973).

The growth of optic axons is organized into an invariant temporal and spatial pattern. Fibers from a single ommatidium grow out as a bundle and maintain this discrete organization within the optic nerve even when mature. By reconstructing many specimens from serial electron micrographs (LoPresti et al., 1973) at a number of times throughout the course of development, it has been possible to demonstrate that the details of bundle growth are the same for all ommatidia, regardless of position within the eye. The only difference found between developing ommatidia is the time their axons begin to grow, which is correlated with their mediolateral positions. The fiber bundles of the most lateral rank of ommatidia on each side of the eye reach the laminar anlage first, while fiber bundles from the medial rank reach the lamina last (see Figure 40). As fiber bundles enter the lamina primordium, they encounter undifferentiated laminar cells that have become postmitotic a few (1 to 3) hours earlier (Flaster

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