Systems Approaches to Developmental Neurobiology

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PREFACE

It is appropriate at the outset of this book to pose a question that was often asked -- of the organizers before the meeting took place and later among those who participated in the meeting -- "What is meant by 'Systems Approaches' in the study of developmental neurobiology?" The answer, as we originally conceived it, can be succinctly summarized by the word "interactions". That brief epithet was expanded during the general discussion portion of the meeting, where the following definition was offered:

> "Systems approaches in developmental neurobiology are unified by attention to the emergent properties of the developing system under investigation and by a focus on the aspects of development of the nervous system that depend on interactions among its various elements, be they molecular, intracellular or multicellular."

As opposed to ignoring complexity or trying to wish it away, those of us who utilize a systems approach embrace the principle that complexity is what makes the nervous system special. We have come to recognize that wherever we look, we find interactions which are to be probed and eventually understood. Even the so-called "simple systems", a term that has been used to describe many invertebrate preparations, are embraced under the above definition, since with further study it is becoming increasing clear that such systems are not as simple as once thought. We also include molecular genetics under the systems rubric. After all, genes regulate other genes which regulate others, and so it goes.

After examining the list of chapter titles and contributors included in this book a cynical observer might conclude that we used the term "Systems Approaches" as a catch-all, a flimsy umbrella, to allow us to invite a rather eclectic group of hand-picked colleagues who might otherwise be difficult to lump together into a single NATO-Advanced Study Institute. The cynic might also note that the term has a refreshing ring to it, a pleasant change from the over-used "Cell and Molecular" descriptor, and he might be aware that this aspect of novelty is not without merit in the competitive world of grantsmanship. We must confess that these crasser considerations did in fact play a role in our initial planning and choice of topic. However, the underlying validity of the scientific premise stated above -- when one is interested in interactions one is by definition using a systems approach -- was clearly substantiated during the course of the meeting. The participants were drawn from fields as disparate as molecular biology, behavior, and computer modeling, and their technical expertise was as varied as their native tongues, but in a surprising number of cases, common ideas and principles emerged. We hope the essence of these communications, the "emergent features" of this scientific interchange, if you will, have been captured in this volume.

The structure of this Advanced Study Institute was by design different from most meetings, and a brief summary of the organization is warranted here so that the reader can appreciate the organization of this book. There were 15 "Lecturers", each of whom was given a two-hour slot in which to deliver a formal presentation. One of the other lecturers was assigned as the "Discussant", whose task was to lead the one-hour discussion period that followed. Selected questions of special interest or obvious significance have been incorporated into the text of each chapter. Each question posed and the Lecturer's response is enclosed by horizontal lines to set it apart from the main body of the discourse. It was our hope that by including these exchanges in the written report, we would convey to the reader a

sense of the interactive ambience that pervaded the meeting. Additional opportunities for interaction were provided by poster displays. All participants were encouraged to bring a poster (most did), and these were viewed and discussed during several evening sessions. Abstracts of the posters presented are collected at the end of this volume.

The last half-day session of the meeting was devoted to a general discussion, in which the participants and lecturers broke into smaller subgroups and distributed themselves among four topics that the group as a whole had identified as major, recurring themes. The topics were: evolution and species differences, axon pathfinding, juvenile/adult plasticity, and competition. We then met once more as a body, and each subgroup presented a report of their discussion. Following is a very brief synopsis of what was said.

EVOLUTION AND SPECIES DIFFERENCES

Evolution is development. Evolution is no more than the cumulative ontogeny of many individuals over the epochs. Therefore, both evolution and development address the fundamental origins of life biology. The process of evolution of the nervous system, like all other parts of the organism, involves a concatenated series of slight modifications of what is already there. The theme of evolution ran as an undercurrent through many of the talks, and it was explicitly addressed in one lecture (Marois and Meinertzhagen, 1990, this volume) and in an impromptu chalk-talk by Ghysen. From studies of the phyletic diversity and the ontogenetic history of neural organization has come the growing awareness that the nervous system is built on a simple orthogonal pattern of connectives and commissures (Blagburn and Bacon, Ross and Easter, 1990, this volume) that form a scaffolding upon which more complex networks, such as the mammalian cortex (Kind and Innocenti, Shulz and Frégnac, 1990, this volume), are built by processes that include both addition and deletion. Similarly, morphologically complex synaptic structures, which by inference convey enhanced functional abilities, appear to have evolved from the sequential addition or modification of pre-existing units (LaMantia, Marois and Meinertzhagen, 1990, this volume). Common principles may govern cell determination (Ruiz-Gómez, Jimenez and Campos-Ortega, Braisted and Raymond, Metcalfe and Westerfield, 1990, this volume) and segmentation (Ruiz-Gómez, Metcalfe and Westerfield, 1990, this volume) between vertebrates and invertebrates.

Not all of those present were convinced of the usefulness of studying evolution, however, and this prompted a lively discussion. For one used to designing experiments it is frustrating not to be able to manipulate phylogeny. Nevertheless, the point was made that an awareness of the building-block process of evolution and the links between ontogeny and phylogeny may provide insights that will prove helpful in gaining a better understanding of how the nervous system develops.

AXONAL PATHFINDING

Many of the chapters address this topic, which is *au courant* in developmental neurobiology. The discussion focused on three principles governing axonal outgrowth:

Substrates. These mediate the "stick and let go" of axon growth. Guidance by the substrate can be a facilitatory or an inhibitory phenomenon (Allsopp and Bonhoeffer, 1990, this volume). The question of how axons know which way to grow has not been resolved, but an enthusiastic search for polarity cues continues (Taylor and Gaze, Hankin and Lund, Gaspar and Sotelo, 1990, this volume). The role of chemotactic factors released by the target is another area of hot pursuit (Hankin and Lund, 1990, this volume).

Guidepost cells. These special features of the cellular environment through which an axon grows have been documented in invertebrates, where they appear to play a significant role in directing the growing axon, thereby establishing the basic architecture of axon pathways. Experimental studies in which guidepost cells have been ablated have given contradictory results, so just how important the guidepost cells are remains unclear, as does the issue of whether there are homologues in vertebrates.

Pioneer fibers. These are the first axons that grow out, thereby establishing the future pathways over which later cohorts of axons will travel. The first axons that colonize virgin territory (the pioneers) must be responding to exogenous guidance cues, whereas those that follow have the much easier task of fasciculating with their predecessors. Although the concept of pioneer neurons was first developed in invertebrates (Blagburn and Bacon, 1990, this volume), recent work suggests that it is valid for vertebrates, too (Ross and Easter, Metcalfe and Westerfield, 1990, this volume).

The usual models for studying axonal guidance have all involved axons that travel long distances (e.g. the retinotectal system, Taylor and Gaze, Hankin and Lund, 1990, this volume). What about short distance axons involved in generating the complex organization of the synaptic neuropil? What cues guide growth of those processes? Essentially nothing is known about this important aspect of axonal outgrowth, and it is not clear how it can be addressed experimentally in a meaningful way.

JUVENILE/ADULT PLASTICITY

The issue that consumed the participants in this discussion was the principle of critical periods. What is a "critical period"? The definition may vary from one system to another (Marois and Meinertzhagen, Stanford and Sherman, Shulz and Frégnac, 1990, this volume). With regard to the mammalian visual cortex, where the volume of work on critical periods justifies designating it as the archetype, two types of critical period have been recognized:

1. The developmental period during which the cortex can adapt to the environment.
2. The period during which environmental features are necessary for normal development.

Questions related to evolution were also posed. How did critical periods evolve? They have not been seen in phylogenetically old systems, although perhaps no one has looked carefully enough. What is the purpose of a critical period? If it conveys some advantage, why does it end?

COMPETITION

Here again, there was a struggle to come up with a meaningful and consistent definition. There were many opinions, including:

1. Elements searching to obtain limited resources.
2. An interaction among axonal or dendritic processes to establish a balance in the parcellation of territory and/or an optimal axonal or dendritic size.

Another issue was the question of what the neurons are competing for. Again, several alternatives were suggested, including trophic factors, postsynaptic "space", presynaptic inputs, and economical representation in the target field. Although the term "competition" is heavily used, and the principle has been demonstrated in several different systems, the underlying meaning and mechanisms are proving difficult to pin down. This has not dissuaded many from continuing their efforts, however, and several of the chapters dealt with competitive phenomena (Kind and Innocenti, Stanford and Sherman, Shulz and Frégnac, 1990, this volume).

Finally, a word on authorship of the chapters. Each Lecturer selected in advance a Scribe, whose job was to take notes during the lecture and discussion, then to produce a manuscript. In some cases the Scribe was a colleague and associate of the Lecturer, and the work the Lecturer described was in part attributable to the Scribe. In other instances, the Scribe had not participated in the work, and played the role of a reporter only. Lecturers all

had the opportunity to comment on the manuscript produced by the Scribe, and some played a more active role in the actual writing. Therefore, the issue of authorship has been left up to each Lecturer/Scribe team, with the general stipulation that if both are to be authors, the Scribe is first author. In some cases, the Lecturer chose not to be an author. A footnote on the title page of each chapter indicates the authorship for the purpose of citation.

October, 1989

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CONTENTS

NEURAL DEVELOPMENT IN INSECTS: NEURON BIRTH, PATHFINDING, SYNAPTOGENESIS, COMPETITION *

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This lecture provides an introduction to the development of the insect nervous system. Insect nervous systems are particularly useful for investigating the cellular and molecular mechanisms which give rise to neuronal specificity, because they are made up of relatively small numbers of neurons, many of which can be reliably identified as individuals. The first sections deal with the development of the CNS, focussing on how the pattern of neuroblasts and their progeny of identified neurons is set up, and the way in which axons fasciculate in the initial orthogonal array of axon tracts. The next section examines genetic studies of synapse formation between identifed neurons. The last, and longest, sections address the development of the sensory neurons of the insect's peripheral nervous system (PNS), and its usefulness as a model system for studying the rules by which sensory axons grow into the CNS, and establish and modify synaptic connections in the CNS.

ESTABLISHMENT OF NEURONAL IDENTITY

The basic pattern of the arthropod CNS consists of a chain of cephalic, thoracic and abdominal ganglia; the three thoracic ganglia contain approximately 2000 neurons each, and the abdominal ganglia, about 500 (Thomas et al., 1984). Many studies have utilized Orthopteroid insects (locusts, grasshoppers, crickets and cockroaches) because their large, individually identifiable neurons permit a detailed cellular analysis of developmental events. The powerful techniques of molecular genetics can more easily be applied to the Dipteran, *Drosophila melanogaster*, allowing a molecular analysis of neuronal development. Despite the fact that Orthoptera and Diptera are not closely related phylogenetically, the pattern of neuroblasts in each segment, and even the stereotyped way in which identified neurons extend their axons and form the basic tracts and commissures of the CNS, appear to have been conserved throughout the 300 million years during which the various groups have diverged (Thomas et al., 1984). The existence of a common developmental plan has recently been corroborated at the molecular level by the finding that homologous genes are expressed in the developing nervous systems of both locust and *Drosophila* (Zinn et al., 1988).

The insect CNS develops from a sheet of neuroectodermal cells running along the ventral surface of the embryo. There are two steps in this process: first, ectoderm cells enlarge into neuroblasts (NBs); second, each NB generates a specific family of neurons. Out of approximately 150 undifferentiated neuroectoderm cells per hemisegment, 25 - 30 enlarge to become NBs, while others form epidermal cells and the less numerous midline precursors, glial precursors, or the neuroblast support cells (Doe et al., 1985). The pattern in which these cells develop is repeated (with minor variations) from segment to segment. The neuronal precursors can be identified not only according to

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* This chapter to be cited as: Blagburn, J. M., and Bacon, J. P., 1990, Neural development in insects: neuron birth, pathfinding, synaptogenesis, competition, in: "Systems Approaches to Developmental Neurobiology," P. A. Raymond, S. S. Easter, Jr., and G. M. Innocenti, eds., Plenum Press, New York.

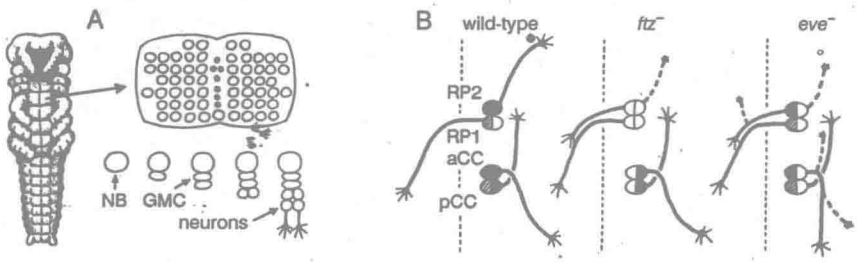

Fig. 1. A. Drawing of a grasshopper embryo (ventral view) at 32% of embryogenesis, showing the metameric arrangement of cephalic, three thoracic, and eleven abdominal segments. A diagrammatic representation of the neuroepithelium of the prothoracic segment, showing the characteristic pattern of neuroblasts (open circles) and midline precursors (filled, small circles). Below is shown the temporal sequence (left to right) of the production of neuroblast progeny (NB, neuroblast; GMC, ganglion mother cell). B. Cartoon of the RP1, RP2, aCC and pCC neurons, in normal embryos, embryos without neural *ftz* expression (*ftz*$^-$), and embryos without functional *eve* protein (*eve*$^-$). The relative strength of gene expression is indicated by the shading of the neuron cell body: the left half represents *ftz* expression and the right half, the presence or absence of functional *eve* protein. Black indicates a high level of expression, hatching a low level. The solid axons represent the most frequent pattern of growth, while broken lines indicate other pathways taken by the axon. The vertical dotted lines indicate the midlines of the embryos. (Based on results in Doe et al., 1988 a, b.)

their position within the array, but also according to the family of identified neurons which they generate. Each neuroblast divides asymmetrically to form a chain of ganglion mother cells (GMCs) which in turn divide once to form families of neurons (Fig. 1A). Midline precursors divide once only, to form two neurons each, while the glial precursors form a family of glial cells which migrate through the segment (Doe and Goodman, 1985).

The identity of a neuroblast appears to be determined by the position in the array in which it enlarges. Prior to this, any one of a cluster of undifferentiated neuroectoderm cells is capable of becoming the NB, but only one of them does so. Cell interactions then prevent neighbouring cells from differentiating into NBs. Laser ablation experiments in grasshoppers have shown that neuroectoderm cells are pluripotent; i.e., cells within a cluster can regulate for a missing NB, and furthermore, if a whole cell cluster is ablated, nearby neuroectoderm cells (that would normally differentiate into other neuroblasts) can differentiate into the missing NB (Doe and Goodman, 1985). Transplantation experiments in *Drosophila* also indicate that interactions take place between neuroectoderm cells during early neurogenesis, and analysis of mutants in which overproduction of neurons occurs has provided an insight into the molecular basis of these interactions (Jimenez and Campos-Ortega, 1990, this volume).

Neuroectodermal cells can regulate for missing near neighbors, but over a longer range, are neuroectoderm cells determined to produce anterior or posterior NBs before they differentiate?

"Determination" is an operational term that depends on transplantation (Slack, 1983). To answer this question, therefore, it would be neccessary to carry out the technically very difficult experiment of removing ectodermal cells from one end of a segment, and transplanting them to the other end, and then determining the identity of the NBs which they produced. It is not known if a neuroectoderm cell from the posterior region of a segment would be capable of forming an anterior NB if transplanted to an anterior position.

The identity of a neuron appears to be determined firstly by its lineage, and secondly by interactions with its sibling. Thus a particular mitotic division of a NB will always produce the same ganglion mother cell. The daughters of that GMC are born equivalent and their respective fates are determined by cell interactions, with one fate preferred and dominant over the other (Kuwada and Goodman, 1985). Recent evidence suggests that one way in which *Drosophila* neurons assume their characteristic fates is via transcription of segmentation genes.

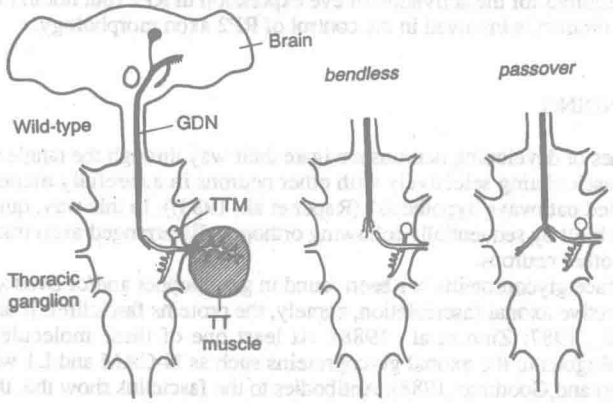

Fig. 2. Cartoons of the *Drosophila* escape system in the wild-type (left), ***bendless*** (center) and ***passover*** (right) mutants. The giant descending neuron (GDN) sends an axon from the brain to the thoracic ganglion, where it normally forms an electrical synapse with the tergotrochanteral motoneuron (TTM). The latter innervates the tergotrochanteral (TT) muscle which extends the leg. In the mutants, the GDN - TTM synapse is absent, possibly due to disruption in the system by which the neurons recognize each other. (Based on results in Thomas and Wyman, 1983.)

CONTROL OF NEURONAL FATE BY SEGMENTATION GENES

Segmentation genes are transcribed in the early embryo, and their products delineate progressively finer subdivisions of the embryo. The first to be transcribed are the gap genes (for example, *Krüppel* and *hunchback*), then the pair-rule genes, such as *even-skipped (eve)* and *fushi tarazu (ftz)* which are expressed in alternate parasegments, and finally the segment polarity genes, such as *engrailed (en)* which is expressed in 14 blastoderm stripes, a few cells wide. With segment number and polarity established, the expression of homeotic selector genes, such as *Ultrabithorax (Ubx)*, then determines segment identity (Akam, 1987).

After playing their cardinal role in blastoderm pattern formation, many segmentation genes are again expressed transiently later in development, in the embryonic CNS. However, the pattern of CNS expression is not merely an inheritance of the blastoderm pattern. For example, the *ftz* gene is first expressed in 7 blastoderm stripes, in alternate parasegments, but later is expressed in small, segmentally repeated sets of GMCs, neurons and glial precursor cells (but not neuroblasts). The patterns of *ftz* and *eve* expression have been examined in detail in two pairs of sibling neurons, aCC and pCC, and RP1 and RP2. Both aCC and RP2 express *ftz* strongly, but pCC and RP1 express the gene weakly. The aCC and pCC express *eve* strongly, as does RP2, but *eve* protein is absent from RP1 (Fig. 1B; Doe et al., 1988a).

It is difficult to examine these neurons in embryos homozygous for a *ftz* null allele because segmentation and subsequent development of the nervous system is severely disrupted. Fortunately, the *ftz* gene contains separate control elements for expression of the striped blastoderm pattern and for the later expression in the CNS. This enabled the neurogenic element to be deleted, and transformant mutant lines were made which express *ftz* normally in the blastoderm and so establish a normal segmentation pattern, but do not express *ftz* in the CNS. In such *ftz* $^-$ embryos, aCC, pCC and RP1 are all normal (judged by the criterion of axon morphology) but the RP2 axon, instead of extending anteriorly and then laterally to follow the intersegmental nerve, follows the RP1 axon. RP2 sometimes also extends a second axon anteriorly. In the *ftz* $^-$ mutants, RP2 also fails to express *eve* (Fig. 1B). In the absence of ftz, RP2 behaves similarly to its sibling, RP1, in terms of axon path and lack of *eve* expression (Doe et al., 1988a).

As with *ftz*, it is not possible to study the CNS of embryos with no *eve* expression because of the disruption of segment formation. The existence of a temperature-sensitive allele of *eve* permitted *eve* function to be disrupted in the CNS after the embryos were raised at a temperature which permitted normal blastoderm pattern formation. RP2 is also abnormal in these *eve* $^-$ embryos, even though *ftz* expression is normal. In the absence of functional *eve* protein aCC axon morphology is aberrant whereas the pCC axon is normal (Fig. 1B; Doe et al., 1988b). It seems reasonable to

conclude that ftz is required for the activation of eve expression in RP2 (but not in other neurons such as aCC) and that *eve* product is involved in the control of RP2 axon morphology.

AXONAL PATHFINDING

The growth cones of developing neurons navigate their way through the tangle of the developing nervous system by fasciculating selectively with other neurons in a carefully orchestrated temporal sequence (the "labelled pathways hypothesis" (Raper et al., 1983)). In this way, quite complex axon morphologies can be built by sequentially following orthogonally arranged axon tracts that have been laid down earlier by other neurons.

A family of surface glycoproteins has been found in grasshopper and/or *Drosophila* that appear to be involved in selective axonal fasciculation, namely, the proteins fasciclin I, II and III (Bastiani et al., 1987; Patel et al., 1987; Zinn et al., 1988). At least one of these molecules, fasciclin II, is homologous and analogous to the axonal glycoproteins such as N-CAM and L1 which are found in vertebrates (Harrelson and Goodman,1988). Antibodies to the fasciclins show that they are expressed on different but overlapping subsets of axon bundles; in locust some axons transiently express fasciclin I within the commissures but then express fasciclin II in the longitudinal pathways. In *Drosophila*, fasciclin III is expressed on a subset of axons in the commissures and the intersegmental nerve, and the axon of RP1 stains strongly for the protein while the RP2 axon shows no staining (Patel et al., 1987). A possible explanation for the aberrant pathway choice by the RP2 axon in the absence of functional *eve* protein (see above) is that *eve* may normally suppress the expression of the fasciclin III gene. In its absence, both RP1 and RP2 may express fasciclin III, causing them to fasciculate together.

Deleting the *Drosophila* fasciclin III gene causes some disruption in the developing CNS, including broader (less tightly fasciculated) commissures, and abnormalities in the trajectory of the RP1 axon (Jacobs et al., 1987). Blocking fasciclin II molecules with antibody partially interferes with the ability of an identified grasshopper neuron, MP1, to recognize and selectively fasciculate with a longitudinal axon pathway (Harrelson and Goodman, 1988). The fact that axonal growth is not as drastically affected as when individual pathway neurons are laser-ablated (Raper et al., 1984), suggests that there are several adhesion or recognition molecules which serve to "label" a particular pathway, so that blocking only one of them causes little disruption.

SYNAPTIC RECOGNITION

Having navigated to the appropriate region of the developing CNS, neurons must then recognize their correct synaptic partners. Less is known about this aspect of neuronal specificity, although it is possible that recognition between potential synaptic partners may be a process similar to selective fasciculation of axons. One approach to investigating synaptic recognition mechanisms is to isolate *Drosophila* mutants in which pairs of identified neurons fail to make synaptic connections.

The largest neurons in the *Drosophila* CNS are those which make up the escape system, and these are present also in larger Diptera (Thomas and Wyman, 1983; Bacon and Strausfeld, 1986). The giant descending neuron (GDN, also known as GF) conveys visual and mechanosensory information from the brain to the fused thoracic ganglia, where it forms (among other connections) an electrical synapse with the tergotrochanteral motoneuron (TTM) of the midleg extensor muscle (Fig. 2). Thus the fly is able to jump away rapidly at the approach of a predator. It is relatively simple to assay for the presence of escape system mutants in mutagenized *Drosophila*. Two mutants (*bendless* and *passover*) were isolated because of their inability to escape in response to a visual stimulus. In these animals the latency between GDN and TTM spikes is 2.2 ms, instead of the normal 0.8 ms, suggesting that the electrical synapse between the neurons fails to develop normally.

In the wild type animal the GDN exhibits a characteristic bend at the end of the axon where it contacts the TTM. In *bendless (ben)* mutants, the GDN bend is missing and the axon does not contact the TTM, but instead sometimes forms filopodia-like extensions as though searching for its postsynaptic target (Fig.2). In the *passover (pass)* mutants, the GDN anatomy appears normal but the TTM continues growing on past the ganglion midline, as though failing to recognize and therefore failing to synapse with GDN (Fig. 2). It seems that both these mutations affect the recognition system between the two cells at the time during pupal development when synaptogenesis between these cells would normally occur (Thomas and Wyman, 1983). The *ben* and *pass* genes are currently being cloned.

Could the changes in the GDN-TTM synapse in these mutants simply be due to the presence of barriers to growth which prevent the GDN and TTM from reaching each other?

It will be important to eliminate possible explanations for the lack of connectivity, such as the existence of barriers, by studying the development of this synapse in the pupa, or by creating mosaic animals in which a mutant neuron grows into a normal ganglion. It is interesting to note that in these mutants many defects occur throughout the nervous system, implying that the putative recognition molecules which are used in the formation of the escape system are also used in the development of other neuronal circuits.

THE CERCAL SENSORY SYSTEM

The development of the insect peripheral nervous system is physically separate from that of the CNS. Individual epidermal cells undergo two mitotic divisions to produce a clonally-related group of 4 cells which then differentiate into the various components of a sense organ or sensillum. The determination of the positions of these sensilla is dealt with in detail by Ruiz-Gómez (1990, this volume). The 4 cells differentiate into a sensory neuron, a sheath cell which wraps the neuron, a cell which secretes the hair or bristle (trichogen) and a cell which secretes the socket (tormogen). The axons of sensory neurons grow towards the CNS, usually following the pioneer axons which, together with motoneurons, establish the pattern of the peripheral nerves at an early stage when the distances from the periphery to the CNS are short.

The cercal sensory system of Orthopteroid insects, in particular the cricket, *Acheta domesticus*, and the cockroach, *Periplaneta americana*, has become a popular model system for studying questions relating to synapse formation and synaptic plasticity, because both the pre- and postsynaptic cells are identifiable and amenable to anatomical and physiological study. The cerci, conical appendages on the caudal end of the animal, bear long, thin (filiform) hairs, innervated by single sensory neurons which send axons to the terminal abdominal ganglion, where they arborize and form synapses. A filiform hair is free to move in response to air movements, but its elliptical socket imposes on it a single plane of movement. However, the underlying sensory neuron is excited by hair movement in only one direction. In the cricket, there are two major planes of hair movement, and thus 4 main classes of receptor, each of which is sensitive to a different wind direction. These 4 receptor types are segregated according to their circumferential position of the cercus, for example, transversely-mobile hairs (T hairs) are found on the dorsal and ventral aspects of the cercus; while longitudinally-mobile hairs (L hairs) are situated on the medial and lateral aspects. Within these regions are strips of sensilla with different directions of excitation (Bacon and Murphey, 1984). The projections of the sensory neurons within the terminal ganglion were revealed by staining single cells with cobalt chloride, by placing a Co^{++}-filled micropipette over cut hairs. It was found that a kind of "cercotopic" projection of hair afferents exists within a defined region of neuropil of the terminal ganglion, the so-called cercal glomerulus. Each physiological receptor type arborizes in a different region of the glomerulus, thus dividing the neuropil functionally, according to wind direction (Fig. 3A).

Within the terminal ganglion of crickets and cockroaches are a set of interneurons often termed "giant interneurons" (GIs) because of the large diameter and length of their axons. These neurons form dendritic arborizations within the terminal ganglion and some of them receive monosynaptic input from the cercal sensory axons (Shepherd and Murphey, 1986; Blagburn, 1989). The GI axons extend up the nerve cord to the thoracic ganglia where they excite interneurons, and in turn, leg motoneurons, thus triggering the animal's escape response. In crickets, the projection of a GI's dendritic branches within the cercal glomerulus is correlated with the synaptic inputs it receives from the wind afferents, and thus with its directional sensitivity (Bacon and Murphey, 1984).

Cross-species transplantation experiments have shown that the cercal receptor cells are programmed according to the position at which they arise on the cercal circumference. This positional information appears to determine the plane of hair movement, the directionality of the sensory neuron, and also guides formation of synaptic contacts with the correct set of interneurons (Kämper and Murphey, 1987). In some of these transplants cercal axons enter the terminal ganglion via the wrong nerve yet still arborize in the correct region of neuropil, thus ruling out the possibility that they simply follow their neighbors within the cercal nerve to the correct destination. Also, the results of these experiments are inconsistent with the hypothesis that the temporal order in which the axons grow into

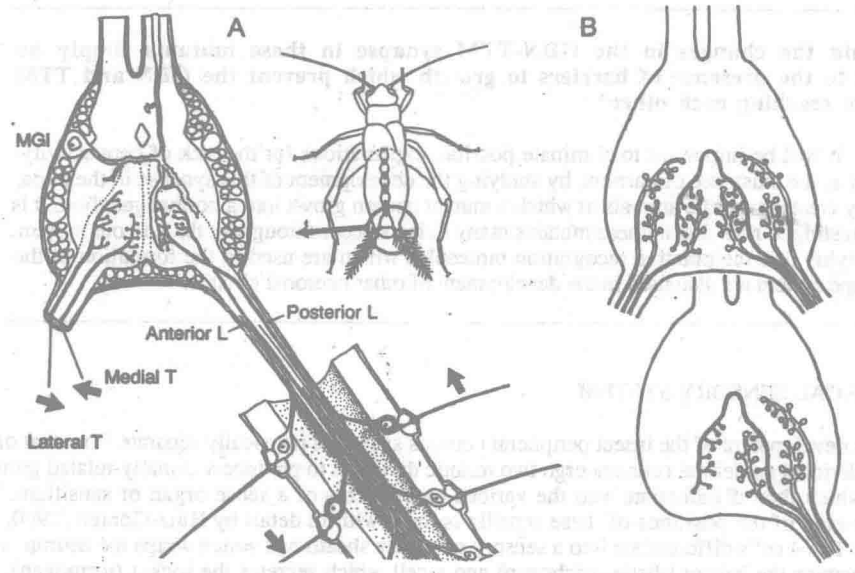

Fig. 3. A. The cricket cercal - to - giant system. A cricket is shown with the abdominal CNS exposed. The main panel is a horizontal section of the terminal abdominal ganglion showing the projections of the four types of afferents as well as one interneuron, MGI. Note that the posterior L afferent is in contact with the MGI, but the anterior L afferent is not. Arrows indicate the major excitatory wind direction of each of the 4 receptor types. B. The axon of the X neuron (shown in black) before (upper) and after (lower) deafferentation. Removal of afferents from the left side of the ganglion results in the redistribution of the arborization of X to the deafferented side. (Based on results in Bacon and Murphey, 1984; Murphey, 1986.)

the terminal ganglion determines their synaptic connectivity. There is no evidence for the existence of gradients of diffusible molecules in this system. Instead, the available experimental evidence supports the idea that the axons are guided by cell surface labels to the appropriate areas.

PLASTICITY OF INSECT SYNAPSES

Despite the apparent "simplicity" of these systems, insect synaptic connections are not entirely genetically hard-wired; they also depend upon interactions between neurons during development. In the cricket, a small number (25 out of 1000) of filiform hair afferents, the "X neurons", cross the midline and synapse with GI dendrites in both cercal glomeruli (Fig. 3B). Counts of the number of putative synaptic boutons showed that normally the arborization of the X neuron is more or less equally distributed on either side of the midline. Unilateral cercotomy removes approximately 90 - 95% of the X neuron's contralateral neighboring axons within the defined region of glomerulus to which the X neuron is restricted, whereas only 5 - 10% of the ipsilateral neighbors are removed. It was found that the X neuron shifted boutons from the afferented to the deafferented side of the ganglion, in which the number of neighboring axons was greatly reduced (Murphey and Lemere, 1984). Quantal analysis of EPSPs in the medial GI supports the idea that the number of X neuron synapses is increased on the deafferented side (Shepherd and Murphey, 1986).

The X neurons responded most effectively to cercotomy when it was carried out at early stages, when the rate of growth of the arborizations is normally at its fastest (Murphey, 1986). It is interesting to note that (1) the X neuron arborization retained its position within the neuropil, and did not sprout into other deafferented regions; and (2) that the total size of the arborization appears to be intrinsically limited. These results demonstrate that interactions between afferent axons regulate the number of synaptic contacts that they can form with postsynaptic cells. It is possible that these axons are competing for a limited resource, such as postsynaptic sites on the target interneurons.