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The Changing View of Neural Specificity

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Since the introduction of the "neuron doctrine"—the concept that the nervous system consists of separate cellular units interconnected by a complex axonal and dendritic network (*1*)—neurobiologists have wondered how this complex machinery is assembled. We now summarize evidence obtained from a variety of animals and neural regions that has gradually led to a major shift in the way many neurobiologists view the formation of the detailed yet stereotyped patterns of connections that characterize the nervous systems of virtually all animals.

The Classical View of Specific Nerve Cell Connections

Most neurobiologists 10 or 15 years ago thought that the explanation of neural specificity was nerve cell recognition. This consensus grew out of the pioneering work of Sperry and his collaborators in the early 1940's, work that culminated in 1963 with Sperry's definitive statement of the "chemoaffinity theory" (*2*). The essence of this hypothesis is that pre- and postsynaptic elements bear specific surface labels that recognize each other by mutual affinity during the process of axon outgrowth and synapse formation. Such labels were thought to promote both accurate axon trajectories and the formation of appropriate synaptic connections.

This idea, of course, was not entirely new—for example, S. Ramón y Cajal and J. N. Langley had suggested much the same concept at the end of the 19th century (*3*)—but Sperry supported the notion with compelling experiments on the neural connections between the eye and the brain and raised these earlier suggestions to the level of a central tenet of developmental neurobiology. Sperry's key experiment involved rotating the eye through 180° after having severed the optic nerve in amphibians (*4*). These animals, unlike mammals, have retinal axons that are able to grow back to the

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optic tectum. After recovering, the experimental animals behaved as if their visual world had also been rotated. On the basis of this outcome, and on later neuroanatomical studies (*5*), Sperry concluded that axons from different retinal regions do not compensate for eye rotation but grow back to their original sites of termination in the optic tectum. This work seemed to provide definitive evidence that pre- and postsynaptic cells are specified at a very high level of resolution, and that this identity promotes appropriate connectivity (*2*).

Summary. The generation of specific patterns of neuronal connections has usually been regarded as a central problem in neurobiology. The prevailing view for many years has been that these connections are established by complementary recognition molecules on the pre- and postsynaptic cells (the chemoaffinity theory). Experimental results obtained in the past decade, however, indicate that the view that axon guidance and synaptogenesis proceed according to restrictive chemical markers is too narrow. Although a more rigid plan may prevail in some invertebrates, the formation of specific connections in vertebrates also involves competition between axon terminals, trophic feedback between pre- and postsynaptic cells, and modification of connections by functional activity.

Revisionist Thinking on the Retinotectal System

Within a few years, other workers, especially Gaze and his associates, began to debate Sperry's proposition. The results of several experiments began to cast doubt on the idea that pre- and postsynaptic elements bear unique labels that serve to identify them in a more or less rigid way with respect to axon outgrowth and synapse formation. Particularly important were experiments in which optic nerve regeneration was allowed to occur after the retina or the tectum had been partially ablated (*6*). The questions posed in such size-disparity experiments were (i) whether the projection from a reduced population of retinal cells would expand over time to include both appropriate and inappropriate regions of the intact tectum and (ii) whether retinal axons that normally innervate a particular part of the tectum would nonetheless innervate an inappropriate region after the usual site of termination in the tectum had been removed. In both cases the new projections filled the available space in an orderly fashion. Axons from a retinal fragment would expand their projection to fill the entire tectum; conversely, axons from an intact retina ultimately compressed their projection to occupy the residual tectum after partial ablation.

A second line of investigation in the retinotectal system that belied the simplest interpretation of Sperry's chemoaffinity hypothesis concerned observations on retinotectal connections in larval amphibians and mature fish in which retinal and tectal cells are added continuously (*7*). In agreement with the flexibility implied by size-disparity experiments, these developmental studies indicated that retinotectal connections are malleable during normal growth. Thus retinal connections initially made in one region of the tectum gradually shift their location, presumably to keep pace with the changing size and geometrical relations between the eye and the brain during growth.

In sum, this work refuted the idea that neural specificity in the retinotectal system of lower vertebrates is generated solely by assembling lock-and-key molecules that restrict permissible axon trajectories or synaptic connections. On the other hand, virtually all experiments in the retinotectal system indicate the operation of some form of recognition during axon outgrowth and synapse formation. For example, regulation of the appearance of cell adhesion molecules may provide binding forces for the selective fasciculation of growing axons (*8*). The molecular basis of adhesion between axons within the developing optic tract and tectum remains an area of active research (*9*).

Evidence from Other Regions of the Central Nervous System

Several aspects of the development of other regions of the central nervous system have also been difficult to reconcile with the original concept of chemoaffinity. For example, during outgrowth, fibers fail to retain neighbor relations with adjacent axons as they approach their target (*10*). Furthermore, initial projections from two or more sources to a single target structure are often diffuse and intermixed before they begin to segregate into appropriate territories (*11*). During this phase of widespread connections, more axons project to the target structure than are present in the adult, and many of these supernumerary axons are eliminated during the phase of segregation (*12*). The transient overproduction of axons occurs throughout the developing central nervous system including the mammalian forebrain, where the number of axons exceeds by several-fold the number present in the adult (*13*). Finally, in the infant forebrain (*14*) and cerebellum (*15*) the distribution of synapses is more diffuse and their density is higher than in the adult. The elimination of these supernumerary axons and synaptic terminals depends on competition between various inputs rather than on rigid axon-target recognition. For example, in the developing visual system the removal of one afferent pathway often influences the size and pattern of terminal fields (as well as the number of axons and synapses) in the projections from the remaining afferents in the target structure (*16*). Ablation experiments performed in the developing and adult central nervous systems in mammals also show that connections can be substantially changed when one input to a given structure is deleted (*17*) or when the target structure is surgically diminished (*18*). Here again, synaptic connectivity seems to depend on a balance between inputs.

The knowledge that function can directly influence the pattern of neuronal connectivity provided some insight into the usefulness of this apparent flexibility during synaptogenesis. Hubel and Wiesel (*19*) deprived one eye of patterned vision and showed that visual experience at critical ages profoundly changes the size of terminal fields in the visual pathway. The importance of neural activity in the formation of appropriate connections in the vertebrate visual system has been confirmed in studies in which tetrodotoxin has been used to block the inward sodium current and thus inactivate neurons (*20*); blocking impulse activity prevents or reduces rearrangement of axonal connections. A similar approach in the goldfish has also implicated activity in the formation of retinotectal connections (*21*).

Taken together, these examples support the view that the development of neuronal connections in the central nervous system of vertebrates is a dynamic process of rearrangement of connections rather than simply a wiring of rigidly identified elements.

The Revised View and Observations in the Peripheral Nervous System

At the same time that these experiments were encouraging reevaluation of the chemoaffinity hypothesis in the central nervous system, scientists studying the peripheral nervous system were exploring recognition during synapse formation in individual nerve cells. For instance, the highly patterned connections in mammalian autonomic ganglia arise in part through intercellular recognition during axon outgrowth and synapse formation (*22*). However, the detailed connections between preganglionic spinal neurons and the ganglion cells they innervate indicate the operation of continuously graded preferences between pre- and postsynaptic elements rather than rigid cellular distinctions (*23*). Moreover, as in the central nervous system, connectivity in the autonomic system is malleable during normal development (*24*); even in maturity, the preferences normally expressed between synaptic elements can be overridden by altering the availability of different classes of pre- and postsynaptic elements (*25*). Evidently synapse formation in this part of the nervous system proceeds on the basis of relatively weak preferences rather than sharp restrictions.

Nor is there much evidence of strict target recognition during the formation of the neuromuscular junction. In most vertebrates, innervation of muscle by foreign nerves can be produced by surgical rerouting; such experiments show little sign of neural preference for the normal target (*26*). The accuracy of neuromuscular innervation in development is evidently generated largely by accurate initial projections rather than by target discrimination; even this ability is lost in mature mammals (*27*). On the other hand, some recognition of different muscles is apparent in lower vertebrates during limb reinnervation (*28*), and in mammals, weak preferences during synapse formation can be demonstrated under special circumstances (*29*). Thus the innervation of muscle also argues against the operation of highly restrictive chemoaffinity molecules during the development of synaptic connections.

A different line of work in the peripheral nervous system indicates extensive rearrangement of synaptic connections in early life; as in the central nervous system, such evidence is at odds with the chemoaffinity hypothesis. In the innervation of muscle, for example, each target cell is innervated by more axons early in development than at maturity (*30*). Over a period of several weeks, competitive interactions between the axons innervating the same muscle cell lead to a stereotyped one-on-one arrangement which characterizes neuromuscular innervation in mature mammals. This competition is modulated by neural activity; the rate at which synapses are eliminated is increased by augmented activity and decreased by diminished activity (*31*). A similar phenomenon occurs during the innervation of autonomic ganglion cells (*32*) and has been described in brainstem nuclei (*33*), and in the cerebellum (*34*).

The central and peripheral nervous systems of vertebrates are thus similar with respect to neural specificity; in both regions recognition seems to provide only an initial bias to the formation of appropriate connections. Moreover, competitive rearrangement of synaptic connections in early life is evidently commonplace.

Work in the peripheral nervous system has indicated that many of these competitive rearrangements are probably based on the acquisition of trophic support from target cells. The gradual appreciation of the role of target-derived synaptogenic factors in the formation of neural patterns began in the late 1940's with the work of Hamburger and Levi-Montalcini (*35*) that, over a period of several decades, established the significance of developmental interactions in which target cells affect the neurons that innervate them by producing specific trophic molecules that are taken up by the innervating cells (*36*). It is now widely accepted that nerve growth factor (NGF), a small, well-characterized protein, is produced by the targets of postganglionic sympathetic neurons (and probably dorsal root ganglion cells) (*37*). The axon terminals of these nerve cells bear specific receptors for NGF that take up the molecule and retrogradely transport it to the cell body, where its major influence in the course of development is to promote the survival of the presynaptic cells during the normal period of neuronal cell death (*38*). The widespread occurrence of neuronal death in parts of the nervous system that are not sensitive to NGF suggests the existence of trophic agents subserving an analogous function in other systems. Less widely recognized is an effect of NGF on the formation and maintenance of synaptic connections through local modulation of terminal arborizations (*23, 39*). Decreased availability of nerve growth factor causes a local retraction of axon terminals in sensitive neurons, and, conversely, an excess of this agent induces sprouting and the formation of new synaptic connections. Since retraction and expansion of axon terminals in response to a variety of experimental manipulations is a general phenomenon in the nervous system, many neurobiologists have again surmised that functionally analogous agents in other parts of the nervous system may influence terminal arborizations much as NGF does.

Cell Recognition in Invertebrates

Most classical experiments on both interneuronal recognition and competitive interactions between nerve cells during synapse formation have been carried out in vertebrates. The question arises whether similar interactions operate in invertebrates, where patterns of connections may be less variable than in the vertebrate nervous system because one or a few neurons in simpler invertebrate nervous systems carry out functions undertaken by many neurons in more complex animals.

Research on the nervous systems of invertebrates has generally exploited the fact that many of these species have relatively few neurons; invertebrate neurons can often be uniquely identified in different individuals of the same species. Given the small numbers of cells in these animals, it is plausible that labels might identify individual nerve cells. By the same token, the detailed similarity of nervous systems in individuals of the same species makes it somewhat unlikely that competition between populations of nerve cells, feedback, and prolonged periods of neural malleability play an important role in establishing patterns of invertebrate neural connections. In general, these expectations have been substantiated.

Comparisons of the several invertebrates and vertebrates that have been studied in this regard indicate that early developmental programs, at least in some species, may be more rigidly programmed in relatively simple animals. For example, the development of individual neurons in the roundworm *Caenorhabditis elegans*, in the grasshopper, and in the leech occurs largely by a stereotyped set of cell divisions in which there is relatively little place for feedback and consequent adjustment (*40*). Although much more is known about cell lineage than about the generation of synaptic connections in invertebrates, there is little evidence that the nerve cells of these animals depend on trophic support

from their targets, as they apparently do in birds and mammals. Furthermore, motoneurons can survive independently of their targets in at least some invertebrates (*41*). While competition can influence the growth of axonal arbors in invertebrates (*42*), competitive interactions seem to be less obvious in shaping neuronal connections. Thus, the question of whether trophic interactions in invertebrates are a source of appropriate synaptic connectivity in the style of vertebrates merits detailed exploration. On the basis of what is known, however, it seems fair to say that the neuronal development of simpler animals is preordained to a greater degree than neural development in higher animals. This biological strategy is in keeping with the relative simplicity of the behavior and life cycle of such animals, as well as the relatively limited ability of their nervous systems to change in response to experience.

In accord with this general view, invertebrate neurons—at least those in the few species that have been examined so far—seem to be recognized during the establishment of neural connections in a manner more akin to the restrictive sort of chemoaffinity originally advocated by Sperry. Evidence for the importance of surface-mediated cell-cell interactions in invertebrates has come from observing—in living tissue and in fixed tissue prepared for light or electron microscopy—the behavior of identified growth cones in insects. Identified neurons send their growth cones along stereotypic paths, where they meet and react to a predictable set of cells (*43*). The growth cone filopodia may actually penetrate and induce the formation of coated pits and vesicles in the plasma membranes of other axons (*44*). The formation of specific connections is also predictable at the level of individual neurons in some insects and in the nematode *C. elegans* (*45*).

Finally, the conspicuous rearrangements of initial projections in the nervous system of higher vertebrates is not obvious in invertebrates, even though overproduction of synapses and axonal branches has been observed in at least some regions of arthropod nervous systems (*46*). Nor has much evidence from invertebrates been presented for the role of experience or neural activity in the formation of synaptic connectivity—a hallmark of competitive interactions in vertebrates. Although some features of the development of higher vertebrate nervous systems have been described in invertebrates (*47*), they are less prominent.

Conclusion

In recent years, neurobiologists have shifted their thinking about the generation of detailed patterns of connections in the vertebrate nervous system. Although the notion of patterns based on cell recognition is supported by a great deal of evidence, experiments in a variety of neural systems indicate that such recognition is probably a relatively weak force in the generation of connections, a force that can be overcome by various experimental and naturally occurring perturbations. Restrictive recognition of surface labels is therefore unlikely to be the sole, or even the decisive, mechanism for the establishment of appropriate connectivity. At the same time, the importance of competitive interactions in the formation of neural connections in vertebrates has become increasingly apparent. In some instances, such interactions seem to be based on the secretion of trophic and synaptogenic molecules by target cells that determine both (i) the survival of innervating neurons early in development and (ii) the formation of detailed patterns of axonal aborization in target structures. The prolonged synaptic malleability generated by these long-term competitive interactions may be the basis of the extraordinary ability of the human nervous system to adapt to an ever-changing external environment.

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48. This article is based on five workshops organized by P.R. at the Neurosciences Institute of the Neurosciences Research Program in New York during 1983–84. Each session was moderated by one of the four authors, who are listed alphabetically. The following scientists participated in the workshops: F. Bonhoeffer, D. Bray, M. Constantine-Paton, G. Edelman, E. Frank, J. Freeman, E. Gall, C. Goodman, Z. Hall, W. Harris, M. Hollyday, R. Lund, E. Macagno, R. Murphey, J. Nicholls, J. Palka, E. Rubel, G. Stent, M. Stryker, D. Trisler, H. Van der Loos, and D. Willshaw. The workshops were supported by the Neurosciences Research Foundation.

RESEARCH ARTICLE

Bidirectional SV40 Transcription Mediated by Tandem Sp1 Binding Interactions

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Abstract. *The 21–base pair repeat elements of the SV40 promoter contain six tandem copies of the GGGCGG hexanucleotide (GC-box), each of which can bind, with varying affinity, to the cellular transcription factor, Sp1. In vitro SV40 early RNA synthesis is mediated by interaction of Sp1 with GC-boxes I, II, and III, whereas transcription in the late direction is mediated by binding to GC-boxes III, V, and VI.*

The pattern of gene expression in mammalian cells requires thousands of genes to be turned on and off in a temporally and spatially regulated manner. The critical conditions suitable for regulating the expression of a gene product often occur at the level of transcription. To understand the mechanisms of transcriptional regulation in animal cells, we have used DNA tumor viruses such as SV40 because they provide a relatively simple and valuable model for studying transcriptional specificity. Important *cis*-regulatory elements of the SV40 early promoter have been mapped, and reconstituted in vitro transcription reactions have allowed us to identify and isolate specific cellular factors that recognize and bind to the viral promoter. We now report our analysis of the interaction of a sequence-specific DNA binding protein that activates bidirectional transcriptional initiation from the SV40 promoter region.

The early genes of simian virus 40 (SV40) are expressed shortly after infection, whereas the late genes are maximally activated only after the onset of viral DNA replication and repression of viral early transcription by T antigen (*1, 2*). Analysis of viral promoter mutants both in vivo and in vitro have established that a region of approximately 300 base pairs (bp) adjacent to the origin of DNA replication contains multiple *cis*-regulatory elements responsible for directing transcription of both early and late viral messenger RNA (mRNA) synthesis. Mutational analyses of the viral transcriptional control sequences have revealed that the major early promoter consists of three 21-bp repeated elements preceded by a stretch of AT-rich sequences, and early transcription has been shown to initiate predominantly from distinct sites located 20 to 30 nucleotides downstream from the AT-rich region (*3–11*). In addition, enhancer elements that stimulate SV40 early transcription in vivo are located within the 72-bp repeated sequences, which lie 110 to 250 bp upstream from the early transcription start sites (*4, 7, 12–15*). Late viral transcription appears to be under the direction of multiple regulatory elements and exhibits a heterogeneous population of start sites scattered throughout the control region with a major initiation site at nucleotide 325 and several minor ones located at various positions (*16*). The 21-bp repeats that constitute a major promoter element for early transcription also appear to be a component of the late promoter (*7, 9, 11, 17–19*). In particular, a minor late transcript initiating at nucleotide 170 is strongly dependent on the 21-bp repeated sequences in vitro (*9, 11, 19*). Transcriptional analysis of various plasmid templates containing the 21-bp repeats in an inverted orientation relative to the AT-rich TATA homology confirm the observation that this promoter sequence can potentiate transcription in a bidirectional manner (*10, 11, 20, 21*).

To understand the relation of these various *cis*-acting regulatory sequences to the cellular transcription machinery that must recognize and interact with them, we previously identified the protein factors responsible for activating SV40 RNA synthesis in a cell-free transcription system (*22, 23*). Fractionation of crude HeLa cell extracts resulted in the identification of a transcription factor, Sp1, that binds specifically to a hexanucleotide sequence, GGGCGG (GC-box), that is tandemly repeated six times in the 21-bp repeats of SV40 (*23, 24*). Recently, Sp1 has been shown to activate transcription and bind to the GC-box sequences present in several other viral and cellular promoters, in-

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