

Regressive Events in Neurogenesis

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The development of the vertebrate nervous system, like the development of all other organs and tissues, consists of both progressive and regressive phenomena. The progressive phenomena (so termed because of their essentially additive character) include the proliferation of cells, their migration from the proliferative zones in which they are generated to their definitive locations, their selective aggregation with other cells of like kind, the establishment of phenotypic

their morphological or functional features—is an essential feature of all development. Yet as Glücksmann pointed out more than 30 years ago, regressive phenomena of this kind are common at some point during the development of virtually all biological systems, including the vertebrate nervous system (2). In this overview, we wish to direct attention to the rapidly growing body of evidence that neuronal cell death and the selective elimination of neuronal processes are

Summary. The development of most regions of the vertebrate nervous system includes a distinct phase of neuronal degeneration during which a substantial proportion of the neurons initially generated die. This degeneration primarily adjusts the magnitude of each neuronal population to the size or functional needs of its projection field, but in the process it seems also to eliminate many neurons whose axons have grown to either the wrong target or an inappropriate region within the target area. In addition, many connections that are initially formed are later eliminated without the death of the parent cell. In most cases such process elimination results in the removal of terminal axonal branches and hence serves as a mechanism to “fine-tune” neuronal wiring. However, there are now also several examples of the large-scale elimination of early-formed pathways as a result of the selective degeneration of long axon collaterals. Thus, far from being relatively minor aspects of neural development, these regressive phenomena are now recognized as playing a major role in determining the form of the mature nervous system.

diversity (including the acquisition by the neurons of new membrane properties, their selection of an appropriate mode of synaptic transmission, and the assumption of a characteristic morphology), and ultimately the formation of the complex patterns of connections that characterize the mature, functioning nervous system (1). So striking and so obviously important are these progressive developmental events that to a large extent they have diverted attention from the fact that some loss of previously acquired properties—be it the restriction of cellular potential, the death of cells, or the progressive elimination of certain of

both widespread and substantial during vertebrate neurogenesis, and that together they play a major role in establishing the definitive form of the adult nervous system.

Neuronal Death

That cells die during the development of the nervous system has been known for nearly 80 years (3), but it was not until the late 1940's that cell death came to be recognized as a major morphogenetic feature in neurogenesis (4). Since that time a considerable body of evidence has accumulated to show that during the development of most regions of both the central and the peripheral nervous system there is a distinct phase of neuronal degeneration during which a significant proportion of the constituent

cells die and are rapidly removed by the surrounding glial cells (5). It is not necessary here to list all the structures in which the phenomenon that has come to be known as “naturally occurring neuronal death” has been found: it is sufficient to say that it has been reported in structures as diverse as sensory and autonomic ganglia, cranial motor nuclei and spinal motoneuron pools, the retina, various brain stem nuclei, and the cerebral cortex, and that it has been observed in a wide range of species and in all major classes of vertebrates.

Indeed, in only a few regions has no significant neuronal degeneration been observed during development. These include certain pontine nuclei in the avian brain, the locus coeruleus and red nucleus, and one of the principal fields of the hippocampal formation (6). In many respects these are unusual structures (for example, their neurons have either unusually rich axon collateral arborizations or an uncommonly large range of potential targets); if we leave such structures aside, it would be fair to say that cell death is a ubiquitous phenomenon, and if for this reason alone, of considerable importance in neuronal development.

Equally importantly, where it occurs it usually accounts for the loss of a considerable proportion of the initial population of neurons. Among the areas that have been most carefully analyzed from this point of view, the magnitude of the reported cell death ranges from a low figure of about 15 percent of the initial population in one of the auditory relay nuclei in the avian brain (7) to about 75 or even 85 percent in the mesencephalic nucleus of the trigeminal nerve and one of the other auditory relay nuclei in the avian brain (7, 8). Between these extremes lie the majority of structures in which typically about half of the neurons that are initially generated die at some stage between the initial aggregation of the cells that constitute the nucleus, ganglion, or cortical layer, and its final maturation.

The phase of cell death is usually confined to a well-defined period that is distinctive for each neuronal population, as can be illustrated by reference to four different neuronal populations in chick embryos. In the motor columns of the spinal cord about 40 percent of the motoneurons die between days 5 and 9 of incubation (9); in the mesencephalic nucleus of the trigeminal nerve 75 percent of the cells die between days 9 and 13 (8); in the nucleus of origin of centrifugal fibers to the retina (the isthmo-optic nucleus) just under 60 percent die between days 13 and 17 (10); and in the ciliary

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ganglion 50 percent degenerate between days 8 and 14 (11). In most of these systems it has been possible to show that all, or at least the great majority, of the axons have reached the target field *before* the onset of cell death (12, 13). It is natural, therefore, to ask what else is happening at these critical periods in the life history of each structure. In most cases many events occur at, or just before, the phase of cell death. However, the event that seems to be most significant is that the neuronal population as a whole is beginning to establish connections in its projection field, whether forming end plates on muscle fibers, innervating sensory receptors, or establishing synapses upon other neurons. This finding has directed attention to the target field as the locus of the causative factors underlying cell death.

Cell death as a mechanism for matching the size of each neuronal population to the magnitude of its target field. The most direct evidence for the view that the target field is critical in determining the number of projection neurons that survive has come from studies in which target fields of various neuronal populations have been experimentally manipulated. Such studies have been of three general kinds. First are those in which the entire projection field of the neuronal population was removed, some time before its axons grew out to reach their target fields; under these circumstances, massive, superadded degeneration of cells often leads to the total disappearance of the whole neuronal population (14). Since the induced neuronal degeneration always occurs over the same time period as the naturally occurring cell death, it should probably be viewed as an accentuation of that process. Second are those studies in which the target field was only partially ablated; this is followed by a roughly proportional increase in the amount of cell death in the innervating neuronal population, again occurring within the same time frame (9, 11, 14). And third, in a few instances it has been possible to expand the projection field artificially, for example by transplanting a supernumerary limb or eye. In these cases the magnitude of the cell death has been appreciably reduced, implying that some proportion of the cells that might have been expected to die, have been "rescued" and can survive for the life of the animal (15) (Fig. 1).

Collectively, the above findings have led to the view that in nearly all parts of the nervous system neurons are initially overproduced, and that in most instances the relevant target field can sup-

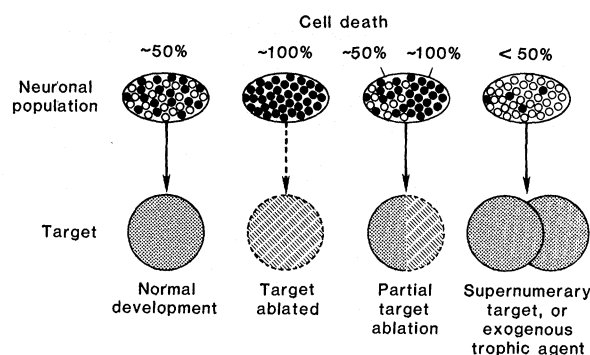


Fig. 1. Major features of naturally occurring neuronal death during development. In most regions about 50 percent of the neurons that are initially generated die at about the time the population as a whole begins to form connections within its target field. If the target is partially or totally ablated neurons are increasingly lost proportionally to the amount of target removed, over the same time period. Expanding the target field or providing an exogenous trophic factor rescues some of the neurons that might be expected to die.

port only a limited number of the cells initially generated. According to this view, naturally occurring cell death primarily matches the size of each neuronal population to the size (or perhaps, more correctly, the functional needs) of its target field. As we shall see, however, the process of neuronal cell death is not merely a random matching of cell number to target size, but also results in the selective elimination of neurons whose axons grow to the wrong target area or terminate in the wrong region within the target field, the elimination of functionally redundant neurons, and the termination of specific neuronal lineages. But before considering the latter phenomena, it is appropriate to briefly review some of the factors involved in the size-matching process as, to date, this has been the most intensively studied.

An overproduction of neurons and the subsequent elimination of a sizable proportion of the cells has many of the features of a selective process; this similarity has led to the view that axons compete with each other for some "entity" within the target field and that the success or failure of an axon in this competition determines the fate of its parent neuron. What it is that axons compete for is not clear. It was initially thought that they compete for synaptic sites (or for specialized receptors in the case of sensory neurons). In a specialized sense this may be true, but in a wider sense it is evident, from several different lines of work, that it is not innervation sites per se that are critical, but more probably the limited availability of trophic materials within the various target fields. The first direct evidence for this view was the finding that neurons from the avian ciliary ganglion can be maintained more or less indefinitely *in vitro* not only in the presence of an excess of cultured muscle cells on which they can form end plates, but also in a medium that has been conditioned

by muscle cells of the appropriate type (16). The relevant trophic factor in this case is unknown; indeed, at present only one neuronal trophic agent has been isolated and adequately characterized—the celebrated nerve growth factor (NGF) first identified by Levi-Montalcini and Hamburger in the early 1950's (17).

Since NGF has been the subject of several comprehensive reviews in the past few years (18), we need only allude to a few key points to illustrate what seems to be a general model for trophic interactions between neurons and the cells and tissues they innervate. Perhaps the most significant finding, both historically and conceptually, is that NGF is essential for the survival of two—and only two—classes of neurons, sympathetic and sensory ganglion cells (both of which are derived from the neural crest). This suggests that there may be a large number of such trophic materials, each acting on a different range of neurons, that remain to be discovered. Related to this is the finding that NGF reaches its target cells by being taken up by their axon terminals (where it may have certain local effects) (19) and then retrogradely transported to the soma (20), where it seems to exert its wider effects on cellular growth and maintenance. We shall return to this point after considering the phenomenon of process elimination, but here we should draw attention to the important recent studies of Hamburger and his colleagues, which have directly established that exogenous NGF can prevent much of the neuronal degeneration that normally occurs in the sensory ganglia of chick embryos (21).

At present the only serious challenge to the systems-matching hypothesis for naturally occurring cell death is the observation by Lamb that after removing one hind limb in *Xenopus laevis* larvae and manipulating the spinal cord to aberrantly direct the axons of the limb innervating motoneurons on both sides into

the surviving limb, the number of motoneurons that survive on the two sides is not significantly different from that seen in normal animals (22). Taken at face value this finding would suggest that the spontaneous death of motoneurons does not depend simply on the magnitude of the projection field but is determined by some other mechanism (such as the elimination of errors); the key finding that under some circumstances the muscles

in a single limb can support twice the normal number of motoneurons appears, on the surface, to contradict the generally accepted hypothesis. Purves (23) has suggested that Lamb's findings might be reconciled with the competition hypothesis if the amount of the relevant trophic factor produced by the target is regulated by the magnitude of its initial innervation.

We have recently attempted to test

Purves's notion in a different neural system from that used by Lamb. Often when one eye rudiment in chick embryos is removed the optic chiasm is disrupted, and subsequently most of the axons from both isthmo-optic nuclei (that is, the nuclei of origin of the centrifugal projection to the retina) are aberrantly misrouted into the surviving eye. Whenever this occurs, neuronal degeneration in both nuclei is greatly accentuated, and the total number of cells that survive in the two nuclei never exceeds the number normally found in a single nucleus (24). Thus, unlike Lamb's finding, in the isthmo-optic system doubling the centrifugal innervation of the retina does not increase the survival of isthmo-optic neurons, and we may infer that it does not increase the amount of trophic material available to their axons.

One of the most convincing examples of the importance of competition in the regulation of neuronal cell death has come from recent studies of the development of the retino-collicular projection in rats and hamsters (25). In both of these rodents, as in most mammals, the retina projects predominantly to the contralateral superior colliculus, but early in postnatal life there is a widespread projection to the ipsilateral colliculus. In the immediate postnatal period this projection extends across the entire colliculus, but over the course of the next 2 weeks it becomes progressively restricted to the medial and rostral parts of the colliculus. If one eye is removed on or shortly after the day of birth, the early widespread projection to the ipsilateral colliculus persists indefinitely (Fig. 2A). We have recently been able to show that (i) the progressive restriction of this projection that normally occurs is largely brought about by the selective death of ganglion cells that project outside the rostromedial part of the colliculus, and (ii) essentially all of these neurons are spared by enucleating the contralateral eye (26, 27) (Fig. 2B). Evidence now suggests that the competition between the relatively small number of ipsilateral and the much larger number of contralateral retino-collicular fibers is mediated in some way by electrical activity, since reducing activity in the contralateral eye with the sodium channel blocking agent tetrodotoxin also preserves the widespread distribution of the ipsilateral retino-collicular projection and apparently "rescues" a substantial proportion of the ganglion cells that would normally die during the topographic restriction of this projection (28) (Fig. 2C).

Cell death as a mechanism for eliminating erroneous projections. The first

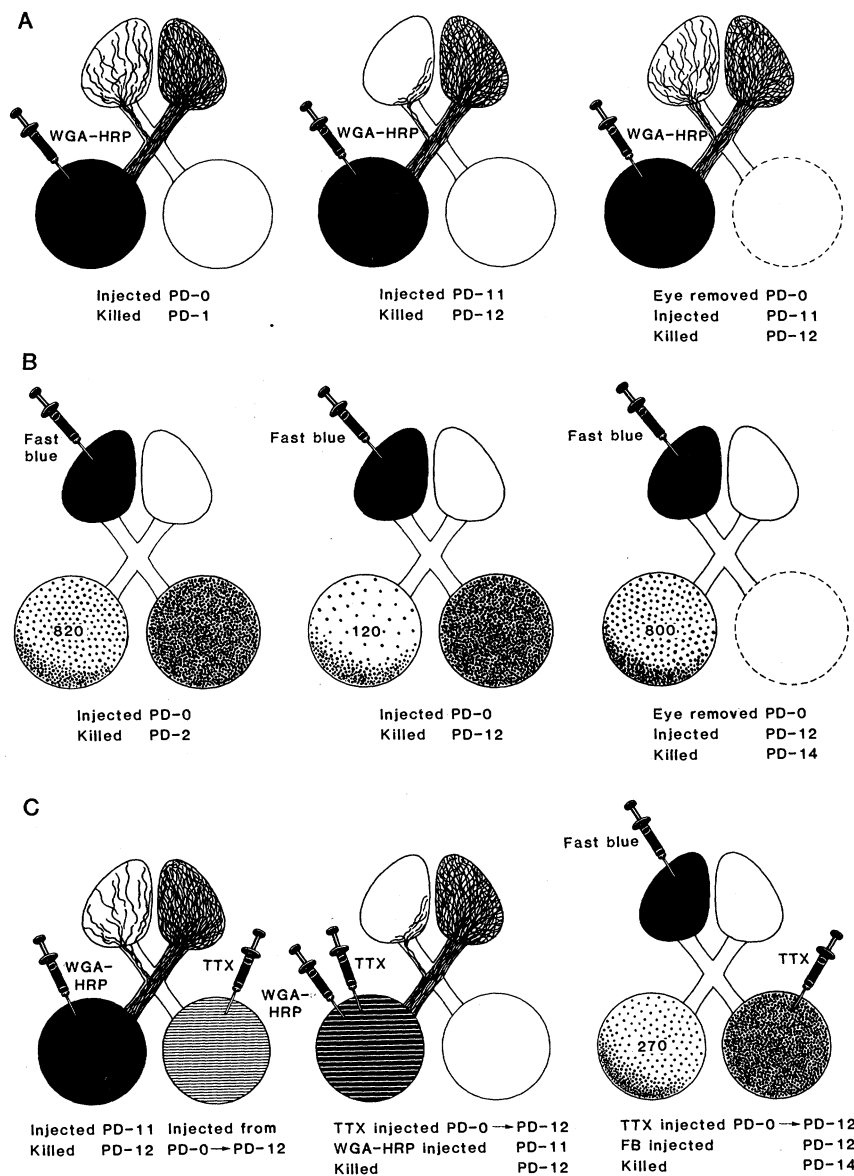


Fig. 2. (A) Schematic diagram of the early widespread ipsilateral retino-collicular projection in rats and its restriction to the rostromedial part of the colliculus by postnatal day 12 (PD-12). If the contralateral eye is removed on the day of birth (PD-0) the projection does not become restricted (25). Abbreviations: WGA, wheat germ agglutinin; HRP, horseradish peroxidase. (B) Retrograde labeling experiments with fluorescent dyes like fast blue establish that the restriction of the ipsilateral retino-collicular projection is due to the preferential death of ganglion cells. Injections on PD-0 label just over 800 cells outside the inferior temporal retina (which is the source of the persistent projection to the rostromedial colliculus). If the animals are allowed to survive until PD-12, only about 120 cells are labeled outside the temporal crescent, indicating that more than 80 percent of the cells have died. These cells are rescued by removal of the contralateral eye on PD-0 (26). (C) Blocking electrical activity in ganglion cells in the contralateral retina [by injecting the sodium channel blocker tetrodotoxin (TTX)] prevents the restriction of the ipsilateral retino-collicular projection (left panel) and rescues a substantial number of ganglion cells that might be expected to die (right panel). Injecting tetrodotoxin into an eye virtually eliminates the ipsilateral projection from that eye (middle panel) (28).

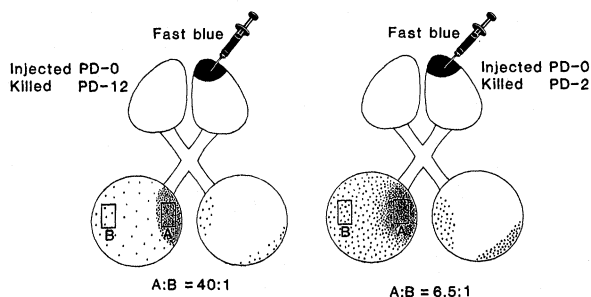
suggestion that naturally occurring cell death may be important for the elimination of neurons that have made erroneous connections came from studies of the development of the isthmo-optic nucleus. Early in the development of this nucleus a small number (~ 0.1 percent) of the neurons project erroneously to the ipsilateral, rather than to the contralateral, eye. During the phase of naturally occurring cell death in the nucleus (which normally occurs between days 13 and 17 of development) essentially *all* of the ipsilaterally projecting cells within the nucleus are eliminated, whereas fewer than 60 percent of the population as a whole degenerate (12, 29).

Until recently it has been difficult to test this error-elimination hypothesis in other systems (30), but in the past year we have obtained evidence in support of this notion in our studies in the development of the chick visual system and the developing retino-collicular projection in mammals. In the developing chick visual system a number of transient projections are seen at mid-embryonic stages. These include a sizable projection from the retina of one eye to the retina of the opposite side and a projection from the retina to the ipsilateral tectal lobe (31). Both of these targeting errors are completely eliminated at later stages during a large-scale phase of retinal ganglion cell death (32).

In mature rats the retino-collicular projection is highly ordered in the sense that each region of the retina projects to a topographically restricted region of the contralateral superior colliculus. This can be shown anatomically by making relatively small, localized injections of an appropriate marker into the colliculus and subsequently mapping the distribution of the retrogradely labeled retinal ganglion cells. If such an injection is made into the caudal part of the colliculus in a young adult animal, one finds a focus of intense labeling in the nasal part of the retina and relatively few labeled ganglion cells outside this region. However, if a comparable injection is made in a newborn animal, one finds not only an intense focus of labeled cells in the nasal retina, but also many labeled ganglion cells scattered throughout the rest of the retina (33). This difference is attributable to the preferential death of the erroneously projecting ganglion cells outside the nasal retina during the first 2 weeks after birth.

We have shown this experimentally by making small collicular injections on the day of birth with one of the retrogradely transported fluorescent dyes (fast blue) that can persist within neurons for sever-

Fig. 3. An experiment to show that early in the development of the rat retino-collicular projection large numbers of ganglion cells make targeting errors and that the great majority of these are eliminated during the process of naturally occurring cell death (between birth and PD-12) (33).



al weeks without affecting their growth and survival. If the animals are killed within 2 days of the injection there is, as mentioned, an intensely labeled region in the related nasal retina, but also a large number of labeled ganglion cells scattered across the remainder of the retina. However, if the animals are allowed to survive until postnatal day 12—by which time counts of the number of fibers in the optic nerve, and of ganglion cells, indicate that phase of cell death in the retina is essentially over (34)—one again finds many fewer labeled cells outside the primary focus in the nasal retina (Fig. 3). This is strong presumptive evidence that a majority of the labeled cells lying outside the primary focus in the nasal retina (which, on topographic grounds, must be regarded as having aberrant axonal projections) are eliminated during the phase of ganglion cell death.

One can show that the death of ganglion cells outside the nasal retina is disproportionately great by estimating the relative densities of labeled cells in the nasal retina and in a comparable area in the temporal retina. In animals receiving injections on the day of birth and killed 2 days later, there are only about seven times as many labeled cells in the primary (nasal) focus as in a mirror image position in the temporal retina. In the retinas of animals allowed to survive until day 12, the corresponding ratio is about 40:1 (33). Experiments like this, and others done to analyze the elimination of the ipsilateral retino-collicular projection, indicate that whereas the overall loss of ganglion cells in the first 2 weeks of postnatal life in rats is about 60 percent, about 90 percent or more of the cells with aberrantly projecting axons degenerate.

It would be a mistake to imply (as some have assumed) that cell death occurs primarily to eliminate early errors in the formation of connections. The elimination of most errors of this type appears rather to be one of the "dividends" associated with the larger process of population matching, but we must admit that at present we know relatively little about mechanisms that lead to the pref-

erential death of certain neurons during a general phase of cell death.

One possible mechanism is illustrated in Fig. 4. The ideas underlying this are drawn from a hypothesis put forward several years ago by Hebb to account for learning at the cellular level and later adopted by Stent to account for the effects of selective sensory deprivation (35). According to this schema, although a trophic factor may be generally available within the target area, the amount available to any given neuron may be increased by the near synchronous activity of a group of presynaptic fibers and the postsynaptic cells they contact (on the reasonable assumption that postsynaptic cells are a major source of trophic materials). In the case of the central projections of the retina, a mechanism like this would clearly favor the survival of neighboring ganglion cells at the expense of more distantly located cells whose probability of simultaneous activation may be expected to be low. An activity-dependent mechanism of this kind has been thought to be responsible for the "fine-tuning" of connections in the visual system (36) and to explain the formation of eye dominance stripes in animals in which both eyes innervate a single tectal lobe (37).

Hormone-dependent neuronal death. A third known type of cell death during neural development relates to the selective responsiveness of certain neurons to circulating hormones. This has been especially well documented in invertebrates such as the moth *Manduca sexta*, in which a number of neurons consistently die with each successive molt. As Truman and Schwartz have shown, the deaths of these cells are related to the available concentrations of the hormone ecdysone and can be prevented by the exogenous administration of the hormone (38).

A comparable pattern of neuronal atrophy and cell death has been reported recently in the avian forebrain. Apparently in certain songbirds a number of forebrain structures that are critically involved in the elaboration and expression of the song initially develop

in the same way and to the same degree in the two sexes. But in females (which are songless), the relevant nuclei atrophy, and by the time the animals are sexually mature they show marked cell loss. Again the selective degeneration of the relevant neurons can be arrested by appropriate hormone treatment (39). Although cell deaths of this type point to the potential importance of diffusible factors for neuronal survival, they do not permit broad generalization. Recent progress in our understanding of the role of steroid hormones in the death of malignant cells, however, may provide a useful model for future work on hormone- and trophic-factor-dependent neuronal death. Apparently the intracellular receptors for corticosteroids are DNA-binding proteins that seem to activate the so-called lysis genes, which, in turn, lead to the prompt disintegration of the cell (40). Conceivably neuronal trophic factors might suppress lysis gene activity; conversely, after their removal the lysis genes may be activated.

Cell death and the control of the neuronal lineages. A final class of early neuronal deaths are those that occur very shortly after the cells' generation.

Although to date this phenomenon has not been extensively studied in the vertebrate nervous system, it has been carefully analyzed in the nematode *Caenorhabditis elegans*. Because of their small size and translucent body wall it has been possible to examine the development of each somatic cell directly and, in doing so, to establish the lineages of all the identified neurons. As Horvitz and his colleagues have shown, in wild-type organisms certain daughter cells in a given lineage always die shortly after mitosis (41). A number of lineage mutants have also been identified; in some of these, cell deaths do not occur and the affected lineage is therefore extended to give rise to an abnormally large number of neurons. In view of the unusually tight genetic control of such neuronal deaths, they are correctly regarded as "programmed"; the neurons that are "destined to die" cannot be rescued by experimentally eliminating the sister cell shortly after the last mitotic division. In this respect they are quite different from early cell deaths in the vertebrate nervous system that seem to be both fortuitous (in the sense that no given cell is predestined to die) and regulative (in the

sense that they can often be prevented by the appropriate experimental manipulation).

Whether or not genetically determined cell lineage terminations also occur in the vertebrate nervous system is not known. Degenerating cells have been observed in the dorsal root ganglia of chicks within just a few hours of their last round of DNA synthesis [as judged by ^3H -labeled thymidine uptake (42)]; and although it is conceivable that some of these may be due to lineage terminations (or conceivably metabolic or genetic abnormalities), the fact that many of these early occurring cell deaths can be prevented by the administration of exogenous NGF (43) suggests that in most instances this is not the case.

Process Elimination

Because of the widespread occurrence of neuronal death, it has been tempting to interpret most structural reorganizations during the development of the nervous system as being due to a phase of cell death. Neuronal death leads, of course, to the elimination of all process-

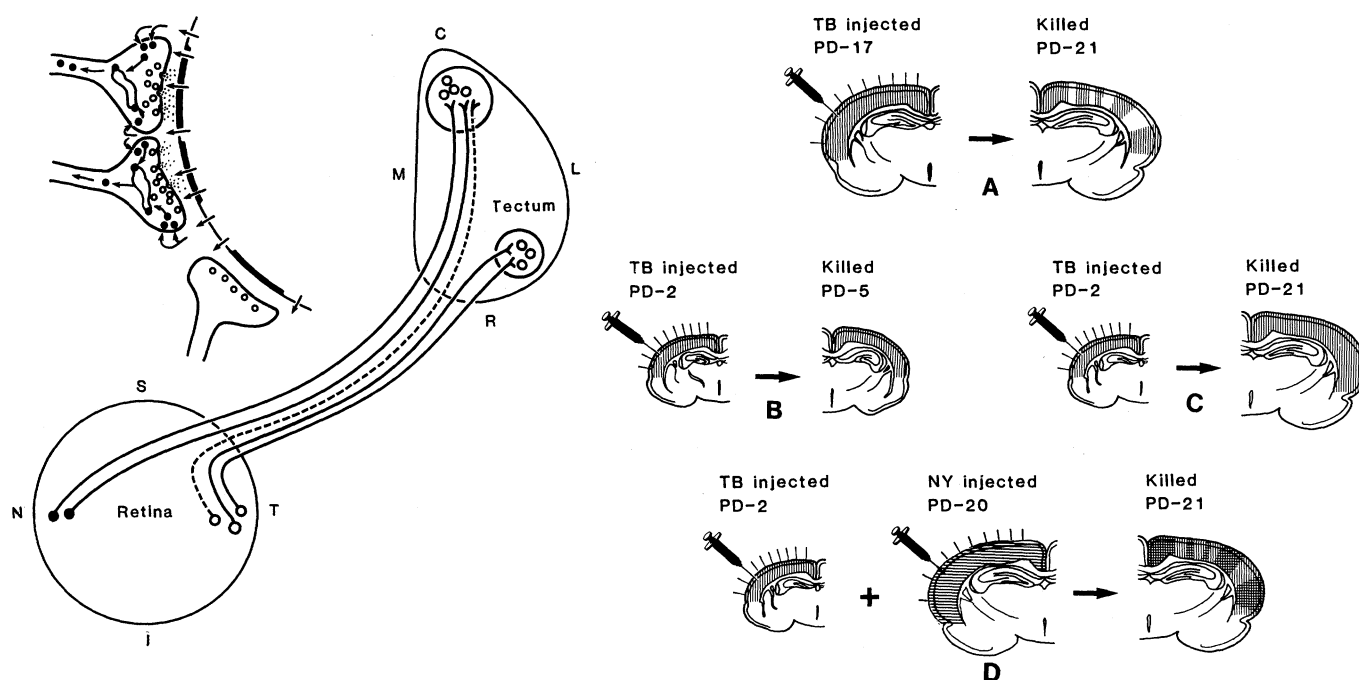


Fig. 4 (left). Cells that send their axons to an inappropriate region (like that shown by the broken lines) are usually eliminated at some stage during development. One possible explanation is that such cells are less likely to fire synchronously with other inputs to the target cells and hence are less likely to activate postsynaptic cells than are neurons that are near neighbors. If the survival of neurons depends on the uptake of trophic materials released when the target cells are active, axons that fire synchronously and activate the target cells will be at a competitive advantage, since it seems likely that the exocytotic release of neural transmitters is related to the endocytotic uptake of trophic materials by presynaptic axon terminal. Fig. 5 (right). Retrogradely transported fluorescent dyes true blue (TB) and nuclear yellow (NY) have been used to show that the restriction of the callosal projection in animals is due to the selective loss of collateral branches. (A) The normal patchy distribution of callosally projecting cells in the somatosensory cortex of young adult rats. (B) The widespread distribution of these neurons in newborn animals. (C) The persistence of this pattern in animals labeled just after birth (PD-2) and allowed to survive until after the normal restriction of the callosal projection (PD-21). (D) Labeling with a second dye, in the same animal but after the restriction, shows the characteristic patchy distribution of callosal cells at this stage and establishes that the cells that initially gave rise to this projection have survived even though many have lost their callosal collaterals. [Modified from figure 1 in (58)]

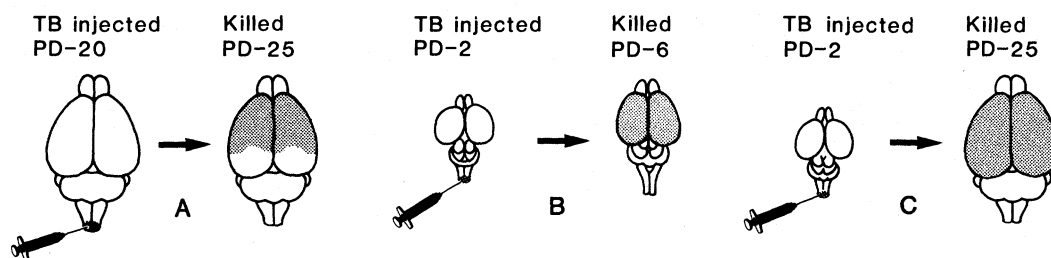


Fig. 6. The postnatal restriction of the rat corticospinal projection, which initially involves layer V pyramidal cells over the entire rostrocaudal extent of the hemisphere (stippling) and later becomes restricted to its rostral two-thirds, is due to the selective loss of "spinal" collaterals of corticopontine and corticotectal neurons. [Modified from figure 1 in (62)]

es of the degenerated cells, but there is now evidence for a different type of regressive phenomenon—one that leads to the selective removal of individual neuronal processes without the death of the parent cell.

Synapse elimination. The first evidence for the local elimination of neuronal processes came from studies of the developing neuromuscular junction. Although in mature rats (and other mammals) most muscle cells are innervated by a single axon, shortly after birth they are usually innervated by as many as five or six separate axons (44). The progressive elimination of the supernumerary axons generally occurs relatively late (in mammals during the first 2 or 3 weeks after birth) and usually long after the period of naturally occurring cell death in the motoneuron pools of the spinal cord (45). It soon became evident that this phenomenon is not limited to the neuromuscular system but occurs widely in both the peripheral and the central nervous system. It has been especially well documented in the rat submandibular ganglion, where again, most of the cells are initially innervated by several separate axons, and then, during the first 2 weeks after birth, all but a single input are withdrawn (46). While some preganglionic axon terminals are being eliminated the persisting axon continues to form additional synapses on the postsynaptic cell. The substantial body of literature bearing on this subject has been reviewed by Purves and Lichtman (47) and will be only summarized here.

The elimination or withdrawal of axon terminals, like neuronal death, is a widespread phenomenon during neural development. It generally occurs appreciably later than the phase of cell death in the same neuronal system and is not confined to those special situations in which each mature target cell is innervated by only a single presynaptic fiber; in mature sympathetic and ciliary ganglia, for example, each cell can be innervated by several presynaptic axons, but even here there are many more axons innervating each ganglion cell during early postnatal life (48). For technical reasons it has

been more difficult to demonstrate the phenomenon in the central nervous system, but in at least three systems—the cerebellar Purkinje cells, the visual system of the cat, and the avian cochlear nuclei—the phenomenon has been convincingly shown with anatomical and physiological techniques (49). For example, each Purkinje cell in adult animals receives a number of different types of input but is innervated by only a single climbing fiber from the inferior olivary nucleus. In the immediate postnatal period each Purkinje cell receives several distinct climbing fiber inputs (49), which can be induced to persist by eliminating other inputs (50).

At the neuromuscular junction, neuronal activity has been implicated in the reduction of polyneuronal innervation (51). However, perhaps the most striking demonstration of the role of activity in eliminating processes has been obtained in the mammalian visual system. In layer IV of the visual cortex of cats and monkeys, the inputs from the relevant layers of the lateral geniculate nucleus, which are connected with the two eyes, overlap extensively at first, but in time become progressively separated into distinct eye dominance columns or stripes (52–54). In normal animals the stripes connected with each eye are of the same width, but if, during a critical period in postnatal life, one eye is deprived of form vision by suturing the eyelids closed, the stripes in layer IV associated with the deprived eye become significantly reduced in width while those connected to the non-deprived eye are correspondingly enlarged (53, 54). This system retains a degree of plasticity for a considerable time, so that if the originally deprived eye is opened and the eyelids of the other eye are sutured closed, the initially reduced eye dominance columns now expand and those that had expanded now shrink (54, 55). This suggests that the relative absence of activity in the deprived eye somehow places it at a competitive disadvantage. Furthermore, in cats, the segregation of eye dominance stripes depends on activity and can be prevented, or at least delayed, by bilater-

ally blocking impulse conduction in retinal ganglion cells or by bilateral eyelid suture (56).

The elimination of long axon collaterals. In the past 4 or 5 years it has become clear that process elimination without cell death is not limited to the fine-tuning of neuronal circuits; in some situations it is responsible for the complete removal (or the substantial reorganization) of entire neural pathways. The first indication of this came from studies of the development of callosal projections in the mammalian brain (57–59). In the brains of most adult mammals, callosal connections are restricted to certain well-defined areas, such as the region of representation of the vertical meridian in the visual cortex, and the trunk, head, and neck representations in the somatosensory cortex. So it came as something of a surprise when in 1977, Innocenti *et al.* reported that callosally projecting neurons were distributed throughout the visual cortex in young kittens and that the characteristic adult distribution only gradually emerged over the first few weeks after birth (60). Similar observations were reported for the somatosensory cortex of rodents (61). At the time these studies were carried out it seemed reasonable to assume that the progressive elimination of such large segments of the callosal projection was brought about by the selective death of many of the callosally projecting neurons. However, when the retrogradely transported fluorescent dyes became available for neuroanatomical studies, it was shown by three independent groups (57–59) that the major (and possibly the sole) factor in the restriction of the callosal projection is the selective elimination of callosal collaterals without the death of their parent cells.

Our own studies in the rat on this issue (58) are summarized in Fig. 5. If one makes multiple injections of one of the persistent fluorescent dyes into the somatosensory cortex on one side shortly after birth, callosally projecting neurons in the opposite hemisphere are labeled more or less uniformly throughout the mediolateral extent of the hemisphere.

This is in striking contrast to the distribution seen after a comparable series of injections in a young adult brain: here the callosally projecting neurons are confined to a number of distinct patches, between which there are few, if any, labeled neurons. However, if one makes the injections shortly after birth and allows the animal to survive until some time after the restriction of the callosal projection normally occurs (after the second postnatal week) the labeled cells are again found throughout the mediolateral extent of the hemisphere. This suggests that although the projection has become restricted, all (or at least most) of the neurons that initially projected to the opposite side are still present. Direct evidence that the restriction of the callosal connections has taken place under these circumstances can be obtained by making multiple injections of one dye (like true blue) into the somatosensory cortex shortly after birth and comparable injections with a second dye that has a different emission spectrum (such as nuclear yellow) a day or two before the animal is killed. The cells labeled by the first series of dye injections are distributed uniformly throughout the mediolateral extent of the hemisphere as before, but those labeled with the second dye (including many doubly labeled neurons) have the patchy distribution characteristic of the adult callosal projection. It can also be shown, by making dye injections elsewhere in the contralateral hemisphere, that many of the cells that initially have a crossed projection but subsequently lose it (by the selective elimination of their callosal collaterals) give rise to association projections within the ipsilateral hemisphere (59).

That the elimination of long collateral projections during development is not a peculiarity of the callosal system has now been well documented; essentially the same phenomenon occurs during the development of the corticospinal projection in rodents (62, 63). As the stippled areas in Fig. 6 show, in adult rats the corticospinal projection arises from pyramidal cells in layer V of the cerebral cortex over much of the rostral two-thirds of the hemisphere. However, when this projection is mapped by appropriate dye injections shortly after birth, the retrogradely labeled neurons are found throughout the entire rostrocaudal extent of the hemisphere, including the visual cortex near the occipital pole (Fig. 6B). Again long-term dye labeling experiments have established that the disappearance of the early corticospinal projection from the occipital region (which occurs during the first 2

weeks of postnatal life) is due to the selective loss of corticospinal collaterals without the death of the parent cell (62) (Fig. 6C).

We have been able to establish the existence of an early corticospinal projection from the visual cortex by other methods and also to identify the persisting projections of the neurons that give rise to the transient spinal collaterals. By making very small injections of the anterogradely transported markers horseradish peroxidase conjugated to wheat-germ agglutinin or tritiated proline into the visual cortex of neonatal rats and hamsters, we have been able to trace labeled fibers through the pyramidal decussation into the dorsal funiculus of the spinal cord, as far as midthoracic levels (63, 64). And by using double-dye labeling procedures it has been possible to show that at least some of the cells in the occipital cortex that initially project to the cord, have persistent projections to the superior colliculus, the pontine nuclei, or both, but none give rise to callosal or ipsilateral associational connections (65).

Although it is not yet known how widespread selective collateral elimination of this type may be, nor what factors are responsible for the removal of certain branches of an axon while others persist, it seems reasonable to suggest that in the developing cerebral cortex a mechanism like this substantially reduces the

amount of genetic information required to encode for all the connections that have to be made. Thus, instead of separately encoding for the associational and crossed connections of layer III pyramidal cells, or the spinal and brainstem connections of layer V pyramids, it seems sufficient to provide the relevant cells with rather general instructions (for example, "form callosal and associational connections" or "project to the brainstem and spinal cord") and then to allow for the epigenetic selection of those connections that are appropriate for neurons of a particular phenotype and in a given region. It remains for further work to determine the nature of the epigenetic selection mechanisms, but at least in the case of the callosal projections from the visual cortex, functional factors seem to be critical. It has been possible, for example, to rear animals with expanded callosal projections from the visual cortex by removing one or both eyes, or by artificially creating a strabismus in one eye (66).

Conclusion

It is interesting to speculate that both neuronal cell death and selective collateral elimination may be brought about by the same or a closely related mechanism—namely, competition for trophic factors that are normally available in only limited amounts. Once again we turn to NGF as a model. In an ingenious set of in vitro experiments on sympathetic ganglion cells (which depend on NGF for their survival), Campenot (67) has been able to show that the withdrawal of NGF can lead to either the death of the entire neuron or the selective elimination of individual processes while the cell body and other processes of the cell persist (Fig. 7). If this should prove to be true for other trophic factors, it may be possible to provide a parsimonious explanation for the two major regressive phenomena that play such an important role in shaping the mature nervous system.

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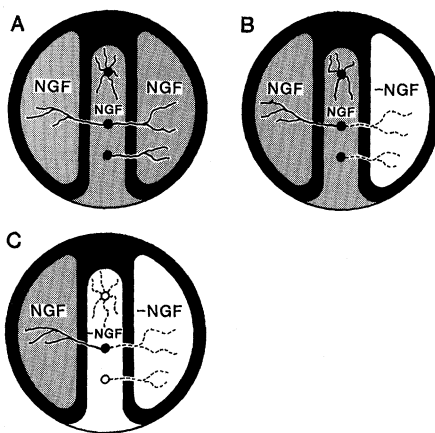


Fig. 7. (A) In the presence of NGF, dissociated sympathetic neurons can extend processes from the middle compartment of these specially designed culture dishes into the lateral compartments (67). (B) If NGF is removed from one lateral compartment the processes that extend into it from the cell bodies in the middle compartment degenerate. (C) If NGF is available to neurons only through some of its processes those processes and the parent cell body survive; if the NGF is removed from all compartments the cells die (67). A single mechanism of this type could account for both cell death and selective process elimination. [Based on data presented in (67)]

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