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Cell Biology of Synaptic Plasticity

Carl W. Cotman and Manuel Nieto-Sampedro

Summary. The nervous system of mammals retains throughout the animals' lifespan the ability to modify the number, nature, and level of activity of its synapses. Synaptic plasticity is most evident after injury to the nervous system, and the cellular and molecular mechanisms that make it possible are beginning to be understood. Transplantation of brain tissue provides a powerful approach for studying mechanisms of synaptic plasticity. In turn, understanding the response of the central nervous system to injury can be used to optimize transplant survival and integration with the host brain.

The nervous system is specialized to mediate the adaptive response of the organism to environmental changes or changes in the organism itself. To this end the nervous system is uniquely modifiable, or plastic. Neuronal plasticity is largely the capability of synapses to modify their function, to be replaced, and to increase or decrease in number when required (Fig. 1). Neuronal plasticity is maximal during development and is expressed after maturity in response to external or internal perturbations, such as changes in hormonal levels, environmental modification, or injury.

In this article we review the types of changes that occur in the mature central nervous system (CNS) of mammals, where they occur, what type of neurons are most plastic, and the stimuli that evoke synaptic growth. We will discuss lesion-induced synaptic plasticity in the adult together with recent work on the integration of transplanted embryonic CNS tissues into neonatal and adult hosts. Transplantation is a powerful means of eliciting the potential plasticity of the host CNS and studying selected aspects of its mechanism. Furthermore, transplants have therapeutic potential to alleviate the behavioral deficits that result from neuronal death caused by injury, degenerative disease, and aging. Most studies on synaptic plasticity have been carried out at the cellular and subcellular levels, but molecular mechanisms are beginning to emerge that are leading to new insights on the control of neuronal growth in the normal and injured CNS.

We have found it helpful to introduce the term "synapse turnover" to discuss synaptic plasticity and its mechanisms in the mature CNS (*1*). The turnover of a synapse is defined as its loss and replacement; the turnover time of a synapse population is the period required for the disconnection of old junctions and the initiation, establishment, and maturation of the new connections (Fig. 1). The term synapse turnover unifies in one concept the increasingly evident common features of synaptic plasticity in the mature CNS.

Synaptic Growth Evoked by Partial Denervation

The most extensive evidence of synaptic growth in the CNS comes from studies in which lesions serve as the stimulus. Liu and Chambers (*2*) first described axon sprouting in the cat spinal cord. After severing adjacent dorsal roots, the residual root sprouted new axons into the denervated zones. Raisman (*3*) showed that new synapses can form in the rodent septal nucleus in response to lesions. An extensive literature now exists on the extent of lesion-induced synaptic replacement in the CNS, its rate, and the type of growth evoked. The most complete studies have been carried out on the rodent hippocampal formation; accordingly we will make use of this system to illustrate key concepts.

The hippocampal formation consists of two major subdivisions, the dentate gyrus and the hippocampus proper with its subdivisions (CA1, CA2, CA3, and CA4). Granule cells are the major cell type of the dentate gyrus, and pyramidal cells are the major type in the hippocampal subfields (Fig. 2). The major extrinsic input to the hippocampal formation originates from a special subdivision of the cerebral cortex, the entorhinal cortex (*4*).

The response to unilateral ablation of the entorhinal cortex illustrates many of the general principles of lesion-induced synapse turnover. One day after the lesion is made, 90 percent of the synapses in the outer two-thirds of the dendritic tree of the granule cells on the side of the lesion are lost. A massive growth response by all of the residual fiber systems in the dentate gyrus except the raphe begins 3 days after the lesion. New synapses are formed by sprouting fibers originating in the contralateral entorhinal cortex (*5*), the septum (*6*) and in the CA4 area (commissural-associational system) (*7*). Synapse replacement is complete by about 60 days. The newly formed synap-

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ses made by CA4 and contralateral entorhinal neurons are functional (*8*); the homologous entorhinal fibers probably mediate the same function as the entorhinal path they replace (*9*).

Several generalizations can be derived from studies on lesion-evoked synaptic growth (*1, 4, 10*). The most obvious is that in the mature CNS new synapses can grow and replace those lost by injury (*11*). Replacement occurs over several weeks, and its rate parallels the rate of clearing of degenerating synapses, suggesting that one process may regulate the other (*12*). A lag in the initiation of replacement increases with age (*13*). Once synaptic replacement begins, its rate is similar to that in the young adult (Fig. 3). Axonal sprouting is selective in that it is restricted to the fiber populations already present within or adjacent to the denervated zone (Fig. 4). The relative contribution of each system to the total is not random. In general, the input most closely related to the injured one accounts for the majority of the new connections (*5, 14*). The remaining inputs are redistributed hierarchically (*3, 10, 15*), generally in proportion to their relative abundance. No examples of a permanent hyperinnervation have been reported. A key concept in lesion-induced synapse turnover is that the total postsynaptic surface per neuron is conserved (*16*).

Although sprouting can be robust, axonal regrowth in the mature CNS occurs only over very short distances. The greatest expansion of a terminal field so far reported is that for hippocampal CA4 fibers, which sprout 20 to 30 μm in the dentate outer molecular layer (*17*). This contrasts with the potential of many of the same systems to elongate over a distance of several millimeters into peripheral nerve grafts or CNS transplants placed in proximity to regenerating axons.

Lesion-Induced Synapse Turnover in Nondenervated Zones

Readily quantifiable changes in synapse number also occur in areas not denervated by a lesion, but connected to a partially deafferented neuron. This can be illustrated in the hippocampus after a unilateral entorhinal lesion. Such a lesion does not denervate the inner third of the granule cell dendritic tree on either side of the brain. However, in this zone the number of synapses declines by 22 percent 2 to 4 days after the lesion and returns to normal by 10 days. In the same zone on the opposite side of the brain, the number of synapses also decreases, although the loss is slower, reaching 38 percent at 60 days and returning to near control values by 120 days (*18*). Thus, on the side of the lesion synapse turnover is compressed into a 10-day period, whereas on the opposite side the cycle requires several months. Synapse turnover extends transynaptically to the synapses made by the deafferented granule cells. After unilateral entorhinal ablation, the mossy fiber synapses made by the granule cell axons on hippocampal CA4 cells (Fig. 4) undergo one cycle of loss and replacement (*19*). In all of these cases, synapses disconnect without our being able to detect degenerating axon profiles by electron microscopy.

Manipulations of the peripheral nervous system can also evoke transynaptic growth and remodeling in the brain. Detailed studies of this type of turnover in the red nucleus have been carried out by Tsukahara and co-workers (*20*). Neurons of the red nucleus receive two kinds of afferents: those from the cerebellum, which contact red nucleus neurons on their somas; and those from the sensorimotor cortex, which synapse on the distal parts of their dendrites. Efferent axons from the red nucleus project onto spinal cord motor neurons. Cortical afferents form new connections closer to the cell body after (i) destruction of cerebellar input, (ii) cross-union of nerve input to forearm flexor and extensor muscles, and (iii) classical conditioning of forepaw position (*20, 21*). These studies demonstrate that a variety of perturbations other than injury can elicit synaptic plasticity.

Natural Synapse Turnover

Synapse turnover in the mature nervous system is an ongoing process, evoked by nondamaging stimuli and the normal physiological activity of the organism (*1*). Synapse remodeling observed in the absence of tissue damage has been called "natural" or "spontaneous" to distinguish it from that elicited by lesions. Examples of its occurrence, inferred from the observation of synapse loss (degenerating endings and vacated postsynaptic sites) and new synapse formation (axonal sprouting, growth cones, and dendritic growth), are found in both the peripheral nervous system and the CNS.

The best documented example of synapse renewal in the peripheral nervous system occurs in the parasympathetic innervation of the ciliary muscle of monkeys. Townes-Anderson and Raviola (*22*) showed that 2 percent of the axonal profiles observed by electron microscopy were degenerating and as many were regenerating. From these data we estimated that the half-life of the synapses on the ciliary muscle is about 18 days (*1*). Such active turnover is probably necessary because of extensive wear and tear associated with eye movement. Similar examples have also been described in other systems subjected to continual use, such as skeletal muscle (*23*) and sensory endings (*24*).

In the CNS, natural synapse turnover was first reported in 1912 by Tello (*25*) for neurosecretory axons. This system still offers one of the most interesting and best documented examples of natural axon renewal and reversible synapse formation. The axons in the neurohypophysis originate in the magnocellular neurons of the hypothalamus and terminate in the spaces around the fenestrated capillaries, where they deliver small peptide hormones. Two of these, oxytocin and vasopressin, have well-characterized actions on water retention and smooth muscle contraction. Hypothalamic neurons in the supraoptic nucleus of a well-hydrated, nonpregnant and nonlactating rat are separated from each other by astroglial processes. Their axon endings are similarly isolated from the capillaries by specialized astroglial cells (pituicytes) that surround and engulf them (*26*). Water deprivation for as little as 4 hours, as well as lactation or late pregnancy and parturition, initiate the following cascade of concomitant events: (i) the withdrawal of glial processes and, therefore, the appearance of contact between the cell bodies and dendrites of neighboring neurons (*27*), thus making electrotonic coupling between neurons possible (*28*); (ii) the appearance of synaptic contact between adjacent magnocellular neurons (*29*); (iii) the retraction of pituicytes, allowing access of the axon endings to the perivascular space (*30*); (iv) the substitution of the slow unpatterned activity of supraoptic neurons by fast continuous firing with occasional high-frequency bursts (*31*); (v) the onset of protein synthesis, particularly that of peptide hormones and their precursors (*32*). All of these changes occur in phase with the appropriate peripheral response—that is, enhanced water retention in the kidneys or a rise in the mammary pressure—and are totally reversible (*27*). In addition, peptide-releasing hypothalamic axons take part in a slower cycle of turnover,

the intermediates of which (Herring bodies) can be easily observed microscopically (*33*).

Natural synapse turnover has also been described in the adrenergic innervation of the uterus during pregnancy (*34*), in the olfactory system (*35*), hypothalamus (*36*), vestibular nucleus (*37*), cerebral cortex, and cerebellum. Turnover in the last two systems has generally been detected by light microscopy as changes in number of dendritic spines, in the size of the dendritic tree, or both.

A noninvasive stimulus such as spontaneous activity can cause synapse renewal in the appropriate area of the brain. Thus, in the cerebellum of adult mice, animals maintained for 17 days where they could exercise as much spontaneous activity as desired ("enriched" environment) showed an increase of 23 percent in the number of spines in the dendrites of Purkinje cells beyond that of mice housed in cages with only enough space to allow them access to food and water (*38*). Similar results were obtained in young monkeys (*39*).

Because the neocortex is believed to be one of the sites of learning and memory, most of the studies of the synaptic effect of natural stimuli have concentrated on this area. Enrichment of the environment increases dendritic branching up to 10 percent in the rat occipital cortex (*40*). Similar increases are caused by age (experience) in both rodents (*40*) and humans (*41*). Smaller but reproducible increases are observed after learning of particular tasks if the relevant cortical region is examined (*42*). Thus, Larson and Greenough (*43*) taught rats to reach for food with the nonpreferred paw. In untrained animals, apical dendritic branching in layer V pyramidal cells of the motor cortex, the primary efferent from this region to the corticospinal motor system, was greater in the side opposite the preferred paw. After 16 days of training to reverse their preference, branching was greater on the side opposite the trained paw (*43*).

Synaptic Growth in the Adult Human Brain

The dendritic arbor of some cortical neurons appears to become elaborate with age, probably reflecting growth throughout life (*41*). Mossy fiber terminals in the hippocampal dentate gyrus appear more abundant in the inner molecular layer of the aged than the young (*44*). This proliferation may be natural growth, or it may be induced by loss of intrinsic pyramidal neurons as a result of aging. In rodents, destruction of the mossy fiber targets causes the same type of growth (*45*), thus validating the use of animal models to predict synaptic plasticity in the human brain. Extensive and abnormal dendritic growth has also been described in the brains of patients with presenile dementia of the Alzheimer's type (*46*). Both dendrites and axons apparently grow into neuritic plaques in Alzheimer's disease (*47*), whereas dendritic atrophy has been shown in other areas of these patients (*41*). This suggests that the capacity for growth in the brain of Alzheimers' patients is expressed in a pathological manner but can be expressed nonetheless.

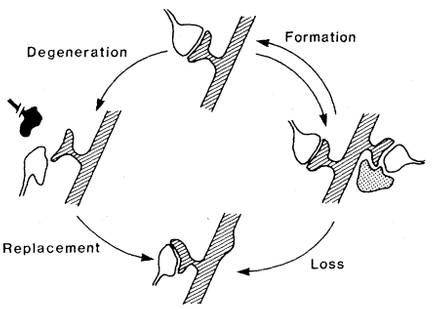

Fig. 1. Synapse turnover. Natural stimuli or injury can give rise either to synapse loss, synapse formation, or synapse replacement.

Time Course of Synaptogenesis in the Mature Brain

Synapse replacement after injury in the adult is a slow process. Its maximum rate is controlled by the initiation of sprouting, which requires 1 to 5 days in adults and is even slower in aged subjects (*1*), seems to depend on the rate of clearing of degeneration products (*12*). This situation contrasts with that in vitro, where an axotomized neuron can sprout a growth cone in 15 minutes (*48*) and synapses can form in less than 1 day (*49*).

A similar situation in this respect occurs in "natural" synapse turnover, in which synapse formation and disconnection take place with the intervention of glial cells and occur in hours (*50*). Since the distances over which growth occurs in the brain are very short, the rate of new synapse formation is never limited by the rate of axonal growth (2 to 4 millimeters per day). Injury-induced synapse growth in the rat brain, judged by the increase in the area of synaptic contact, occurs within 24 hours of its initiation (*51*). In summary, synapse turnover can be fast, provided that extensive new growth is not required or, if required, provided that the rate of sprouting is not hindered by the need to clear degeneration products.

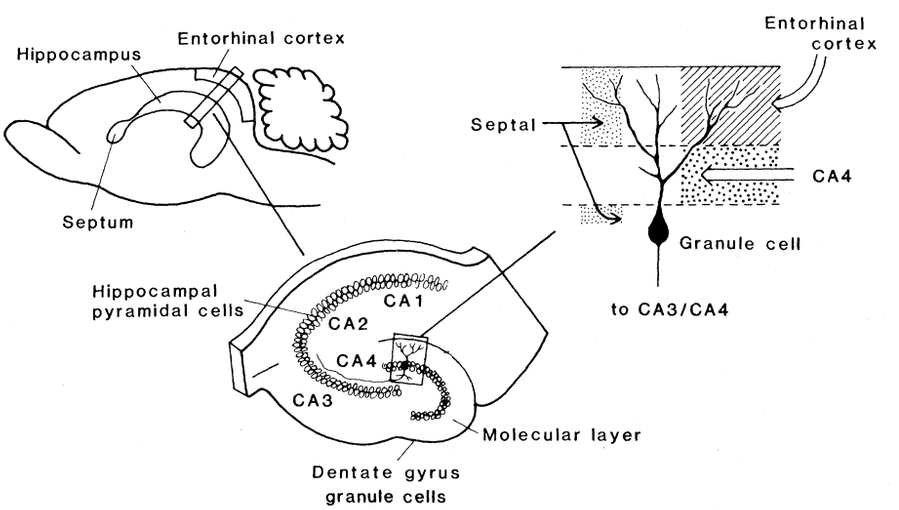

Fig. 2. Two distinct neuronal populations in the hippocampal formation. Pyramidal cells are the major cell type in subfields CA1 to CA4 in the hippocampus proper. Granule cells are predominant in the dentate gyrus. The inputs to the dentate gyrus granule cells are strictly laminated along their dendritic tree. The entorhinal cortex collects input from about eight cortical and subcortical areas and projects to the outer two-thirds of the dentate gyrus molecular layer. This projection is excitatory and provides about 60 percent of the total input to the granule cells. The other prominent extrinsic afferent originates in the septum is cholinergic and projects to both the dentate gyrus molecular layer and the hippocampus proper. Minor inputs arrive from locus coeruleus, raphe, hypothalamus, and nucleus reuniens. The remaining innervation originates in the hippocampal formation itself. The dentate granule cells send their axons to hippocampal subfields CA3 and CA4. Area CA4 cells, in their turn, project bilaterally back to the granule cells, innervating the inner third of the dendritic tree of the ipsilateral (associational fibers) or the contralateral (commissural fibers) dentate gyrus.

Neural Plasticity Mechanisms and Transplant-Host Integration

In 1917, Dunn (*52*) showed conclusively that fragments of immature cerebral cortex could be transplanted successfully into the cortex of a developing host. The transplants survived and appeared to project into the host brain. Few studies appeared subsequently until Bjorklund and Stenevi revived this approach in the mid-1970's (*53*). Many types of fetal neurons have now been successfully transplanted; however, neurons transplanted from mature animals do not survive.

Many issues in reactive synaptogenesis in the CNS are most conveniently tackled by means of transplants. Transplantation per se poses perhaps one of the most severe tests of CNS plasticity. Is the mature CNS capable of integrating new groups of cells and forming with them functional connections? To date, transplants have been used to evaluate the survival requirements of CNS neurons in situ, long distance growth of central axons, and the guidance of fibers to their targets to form specific synapses. CNS transplants have also been tested for their clinical potential—their capacity to restore function when cells are lost as a result of trauma, disease, or genetic insufficiency.

In vivo survival requirements of CNS neurons. The effective use of transplants for studies of neuronal plasticity requires the optimal survival of the implanted neurons when they are placed in any location in the CNS. Fetal neurons have been transplanted as tissue pieces or cell suspensions in the injured or "uninjured" CNS. When transplants are placed in the host parenchyma, usually in a created wound cavity, the survival of different neuron types is variable. Cortical neurons survive very well, septal and raphe neurons less well, and striatal and peripheral sympathetic neurons survive poorly or not at all. Survival and growth could be enhanced by placing the transplant in the host cavity several days after the cavity was made (*54*). Survival of a transplant requires an adequate oxygen and nutrient supply and is hindered by toxic substances. To a large extent, this means survival requires adequate vascularization. It was initially assumed that a delay in transplantation improved survival by allowing the growth of a vascularized bed for the transplant. However, vascularization alone does not account for either the great variability in survival or the existence of an optimum delay in transplantation, beyond which survival again decreases (*55*). A feature well documented

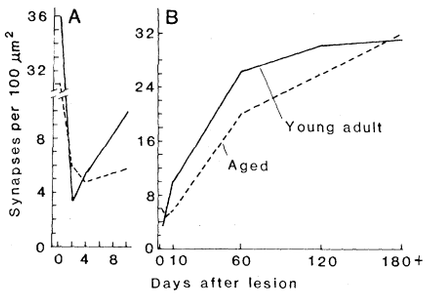

Fig. 3. Loss and replacement of synapses after an entorhinal lesion. Most of the synapses in the outer two-thirds of the dentate gyrus molecular layer disappear within 2 days and are replaced within several months after the lesion. The rate of sprouting and synapse replacement depends on the age of the animal.

for neurons in culture is that specific neurons differ from each other in the specific trophic factors that they need for survival (*56*). We hypothesized that injury to the host brain (the host cavity) caused an increase in central neurotrophic factors. Extracts were prepared from the area of injury and assayed for neurotrophic factors at various times postlesion. Neurotrophic activity was low in uninjured brain tissue but increased after a lesion, reaching a maximum in the adult by 8 to 10 days after the lesion. This maximum was 5- to 50-fold the basal level in the normal brain, depending on the cell type used as the test cell in the assay. The time course of increase in neurotrophic activity correlated closely with that for enhanced survival of striatal transplants placed in the wound cavity at various times after injury (*55*). Exogenous administration of wound fluid to a transplant made its survival possible without a delay period (*57*). Thus, the response of the CNS to injury can be used to enhance transplant survival. Using the delay-transplant procedure, most any embryonic brain area can survive when transplanted into a wound cavity in the host brain. In addition, adult neurons will also survive transplantation under these conditions, although the overall survival is still poor (*58*).

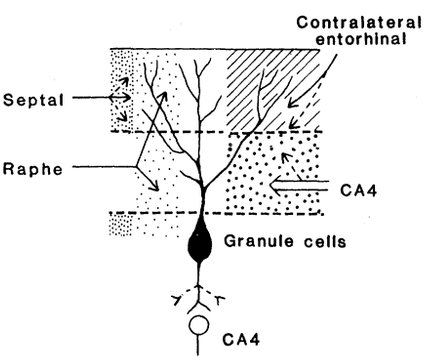

Fig. 4. After an entorhinal lesion, synapse replacement is achieved by selective growth of residual inputs. Dashed lines refer to sprouting of undamaged inputs.

Long-distance axon growth and guidance to the target. Inherent in the formation of new synapses in the CNS is the necessity of axonal growth, be it limited, as in reactive synaptogenesis, or extensive as in axonal regeneration. Unaided regeneration of long tract fibers does not occur in the CNS, probably because the extracellular territory becomes inappropriate for axons to navigate after developing connections are established. If so, transplants placed far from their targets should not be able to reach them. The most detailed examination of this hypothesis has been carried out in the hippocampal formation after removal of the native afferents. Unmyelinated septal fibers can relocate their proper termination fields in the hippocampus when placed back in their natural position (rostral to their target) or forced to grow in the opposite direction from an entorhinal or parietal position (caudal to their target) (*59*). Axons from normally myelinated embryonic entorhinal cortex also reach their correct targets in the hippocampal formation. Therefore, the normal CNS terrain is adequate for the progress of growth cones of both normally myelinated and unmyelinated fibers from transplanted embryonic neurons, but inadequate for the progress of regenerating growth cones from adult long-tract neurons. This is not due to lack of capacity for growth in the central axons, however, as shown by the work of Aguayo and co-workers (*60*).

Fibers of the CNS can be induced to grow many times their normal length (up to several centimeters) by placing them in contact with an implanted segment of myelinated peripheral nerve. Such implants have been used to join two stumps of transected spinal cord, to bridge two widely separated levels of the brain and spinal cord, and to guide the growth of CNS axons into other tissues (*60*). Neurons whose axons grow into peripheral nervous system grafts can be excited or inhibited by natural or electrical stimulation of their natural afferent connections, and the spontaneous and induced activity recorded resembles that for the same region of the intact brain. However, the axons that emerge from peripheral nerve grow into the CNS for a distance of only about 2 millimeters (*60*). It is unknown whether the central fibers make functional connections with CNS cells after leaving the peripheral nervous system implant.

Thus, the success or failure of axonal regeneration seems to depend on signals

that neurons exchange with their immediate environment. Axons from the embryonic neurons can navigate in the adult CNS. Adult axons, on the other hand, need the environment provided by either peripheral nerves or embryonic CNS transplants (*61, 62*).

Specificity of synapse formation. Can the axons of transplanted neurons project to their correct target and recreate the proper terminal fields? In general, they are remarkably successful. Thus, septal fibers form the same trilaminar synaptic pattern on granule cell dendrites as that of the normal septal projection (Fig. 5). In growing to their proper zones they must pass denervated fields and, in the developing brain, will bypass apparently available synaptic sites. Septal fibers from a transplant respond to an entorhinal lesion as native axons do, creating a pattern typical of the sprouted septal system. Striatal cholinergic neurons placed into an entorhinal cavity can also form a projection pattern similar to that of septal fibers even though normally they do not project to the hippocampus (*63*). Transplants of monoaminergic cells also establish their own distinctive nerve ending fields (*64*). Therefore, the specificity of synapse formation resides in both the transplant and the host target cells. Neurotransmitter identity seems to be one of the main specificity determinants for the afferent fibers. Nearly all studies to date have been carried out after removing the native connections in order to readily distinguish fibers originating in the transplant from the normal fiber system. If native septal fibers, for example, are left intact, they compete with septal transplant fibers and are more effective than other host nonseptal systems (*65*), indicating that a strict hierarchy exists.

Little is known about the inputs from the host into the transplanted neurons. Transplants probably receive synaptic input from local neurons, including those in the transplant itself. We have recently investigated the connectivity of embryonic entorhinal cortex implanted into adult hosts because it contains complex mixtures of neuronal types that both form and receive myelinated and unmyelinated axons (*61*). The major output of the entorhinal cortex is to the hippocampal formation (perforant path); most perforant path fibers are myelinated. The entorhinal cortex receives inputs primarily from eight other brain areas several of which are myelinated. Entorhinal transplants form their correct output but apparently receive only three of their normal eight inputs, none of which are myelinated. These are generally unmyelinated fiber systems (for example, septal or raphe inputs). Fibers from mature myelinated neurons rarely, if ever, regenerate into transplants, just as they fail to do so in the host brain itself. In this sense then, transplants are incompletely integrated relay stations in injured networks. The terrain that axons have to cross on their way to the target presents a problem primarily for mature long-tract myelinated fibers.

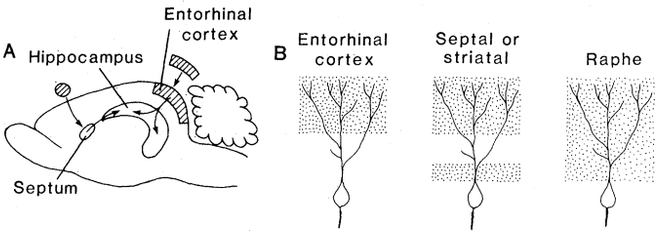

Fig. 5. Transplants of embryonic cortex, septum, corpus striatum, or raphe, when placed in their normal position or in the parietal cortex project to the dentate gyrus and form the correct synaptic fields.

Restoration of function. Transplants have proven effective at restoring some types of lost function. Thus, secretory CNS neurons can be replaced by similar kinds of transplanted cells with recovery from the specific functional deficits (*66*). Cognitive deficits caused by cortical or fimbrial lesions (*67*) or associated with old age (*68*) can be restored by transplants of embryonic cells. Transplants of dopamine neurons (or even adrenal medullary tissues) are also capable of reducing motor deficits caused either by lesions that destroy dopamine substantia nigra (*69*) or by natural dopamine cell loss with aging (*70*). In this respect, transplants parallel the known actions of exogenously supplied dopamine or dopamine agonists. In fact, in all these cases the transplants appear to act as an endogenous source of additional transmitter (and perhaps trophic factors), delivered in sufficient quantity to appropriate receptors. Transplants into the mature CNS do not fully reform the native circuitry and seem to act primarily by delivering chemicals to the proper area much as an endogenous drug delivery system does. They serve primarily in a modulatory rather than connectivity function. Transplants are capable of profound behavioral restoration, nonetheless, thus providing valuable information on the minimal requirements for recovery of function.

Molecular Basis of Neuronal Plasticity

Many cellular and molecular mechanisms similar to those that operate during development continue to be available to the adult and are actualized when necessary (*1, 71*). Perhaps the main difference between developmental and adult synaptogenesis is that during development there is a net gain of synapses, whereas in the adult the process is predominantly one of turnover.

During synapse turnover, existing synapses must be disconnected (Fig. 1). This step of the turnover cycle is formally similar to the elimination of synapses observed during development (*72*). At least two processes of synapse disconnection have been observed in adults. In one of them, synaptic endings degenerate over comparatively long periods of time and intermediates can be observed microscopically (*22, 34, 37*). The second process takes place within a few hours, is not characterized by degeneration, is fully reversible, and seems to require the participation of glial cells that interpose themselves between pre- and postsynaptic elements. This process has been observed in the supraoptic nucleus (*50*) and during the loss of input to axotomized neurons (*73*). Although a plausible mechanism of nerve ending degeneration has been proposed (*1*), we do not know the detailed mechanism of either process of synapse disconnection.

The second part of synapse turnover, synapse formation, involves axonal or dendritic growth and subsequent differentiation of mature synapses. Two essential requirements for growth seem to be the presence of growth factors and an appropriate substrate for axonal extension.

General working hypotheses are that the source of growth factors is the target of the growing inputs and that their production is restricted by innervation. The concentration of growth factor falls in the fully innervated state and rises after partial denervation. The hypothesis that the production of growth factors is regulated by the extent of innervation sheds some light on why fibers grow only over short distances in the CNS or, when induced to grow over long distances, why they fail to penetrate extensively into the target area. After injury, local sprouting reactions occur very rapidly, reinnervating the denervated target cells and arresting the production of growth and guidance factors. The hypothesis predicts that partial denervation at the time of arrival of regenerating fibers

should result in further regenerative growth, but the prediction has not been tested yet.

Molecules capable of inducing neurite outgrowth in vitro have been detected in both the peripheral nervous system (*74*) and the CNS (*75*). The production of trophic factors in the brain probably increases dramatically after partial denervation (*55, 76*). Denervation also initiates sprouting in vivo and increases the production of neurite-promoting factors in both muscle (*77*) and brain (*78*). Polyribosomes in dendritic spines in the dentate gyrus increase after deafferentation (*79*) and so do astrocyte mitogenic factors (*80*). The time dependence of those processes parallels the increase in trophic activity after brain injury (*55, 76*), suggesting potential cellular sources of the growth factors.

There are no reports of sprouting of CNS fibers induced or accelerated by administration of a growth factor. However, nerve growth factor (NGF) does increase septal choline acetyltransferase activity in both the developing and the mature brain (*81*). In the peripheral nervous system, denervated rat muscle secretes a protein of molecular weight 56,000, antibodies to which prevent the terminal sprouting caused by botulinum toxin muscle paralysis. Antibodies to this unidentified protein are found in the serum of patients suffering from amyotrophic lateral sclerosis (*82*).

Recent reports suggest that gangliosides, particularly G_{M1}, have effects similar to those predicted for growth factors in that they promote neurite outgrowth in vitro (*83*) and enhance axonal sprouting in vivo (*84*). Antibodies to gangliosides prevent neurite outgrowth from regenerating retinal explants (*85*) and dorsal root ganglia (*86*). The clinical potential of these small molecules is suggested by the fact that chronic parenteral administration of gangliosides prevented loss of dopaminergic innervation after unilateral hemisection in the rat (that is, prevented the death of the axotomized nigral neurons) and reversed diabetic peripheral neuropathy (*87*). Gangliosides may promote growth by acting on neuronal membrane receptors for growth factors, enhancing their uptake or exerting equivalent intracellular actions to prevent cellular degeneration.

The extracellular matrix, particularly the proteoglycan constituents of the synaptic basal lamina of the muscle, have recently received much attention as promoters of neurite formation (*88*). Its two major noncollagenous constituents, fibronectin and laminin, mediate neuronal adhesion to the growth substratum. In addition, fibronectin promotes neurite extension from peripheral but not central neurons (*89*), whereas laminin also causes neurite formation in central neurons (*89, 90*). The CNS lacks a basal lamina. However, early astrocytes secrete laminin (*91*) suggesting that this molecule has a role during CNS development and perhaps a similar one in adult sprouting. The factors described above may act simultaneously and synergistically, as reported for NGF and gangliosides (*92*).

Growth of sprouts over significantly long distances requires an adequate substrate pathway. The extracellular matrix, by regulating the relative adhesivity of the growth cone, seems important in regulating the direction and rate of growth of the new axons (*93*). Specific membrane glycoproteins (cellular adhesion molecules) that mediate neuron-neuron and neuron-glia adhesion that have been demonstrated in developing brain (*94*) probably contribute to the guidance of newly growing axons to their targets. Local electric fields have also been shown to enhance neurite outgrowth and accelerate neurite extension towards the cathode (*95*). Other variables, however, must also play a role to explain the observed precision of growth orientation and target innervation. Growth cone chemotaxis has been demonstrated both in vitro and in vivo for NGF (*96*) and non-NGF (*97*) but, except for NGF, no neuronal chemotactic factor has been characterized. In the adult CNS an adequate substrate pathway does not seem to exist for long distance growth of most mature neurons. Peripheral nerve transplants in the CNS probably facilitate long distance growth (*69*) by providing such a substrate.

Turnover mechanisms must also account for the specificity of synapse formation in the mature CNS. For example, transplant fibers not only locate precise target cells but also occupy precise positions on their dendritic tree. Similarly, new synapses formed in response to partial denervation also occupy specific laminar locations. An indication of the processes responsible has been obtained in the hippocampus, where the cellular mechanisms that control the development of the septal cholinergic input seem to be the same that control its reorganization after lesions. In this system another afferent (originating from CA4 pyramidal cells) directs the growth of the septal system by excluding septal fibers from its territory. The position of CA4 fibers on granule cell dendrites excludes the growth of septal fibers from the CA4 domain (*15, 98*).

The control of innervation territories and the absence of hyperinnervation (*1, 99*) may require inhibitory factors carried by afferent fibers or target cells. Hyperinnervation can be caused in the CNS or PNS by blocking axonal transport (*99*). Inhibitors of neuronal survival and sprouting have recently been described (*55, 100*). The growth status in the mature CNS is probably the result of a dynamic balance between the actions of growth-promoting and growth-inhibitory factors.

Little information exists on the molecular processes leading to synapse maturation in the CNS, the final step of synapse turnover. The brain contains soluble molecules capable of inducing synaptic characteristics in muscle basal lamina (*101*). Laminin, acting together with a neuronal factor, can promote neurotransmitter receptor clustering in the postsynaptic site (*102*). In turn, the molecular components of the synaptic basal lamina can, in the absence of target cell, direct the differentiation of regenerating neuromuscular junctions (*103*). Although the brain lacks a structured basal lamina, the extracellular proteoglycans present could play an analogous role, becoming organized under the influence of soluble factors.

The most prominent subsynaptic membrane structures, the postsynaptic densities (PSD's) seem to be as dynamic as the synapses to which they belong. PSD's can be lost within one day after deafferentation (*104*). During synapse turnover they themselves seem to be involved in a turnover cycle, of which one of the intermediates is a large PSD with multiple perforations and tears. This intermediate probably breaks down, and each of the resulting PSD fragments may either be totally degraded or serve as the nucleus of a new PSD (*104*) and perhaps generate a new dendritic spine (*105*), thus increasing synaptic surface.

Conclusion

Studies on synaptic plasticity have revealed that the mammalian CNS has a remarkable capacity to renew its circuitry. The systems that respond to denervation are also, in some cases, modifiable by other stimuli, giving credence to the notion that neuronal growth is more pervasive than yet appreciated. Synapse formation in vivo, once viewed as a slow event, can now be demonstrated to occur within hours and can be induced by natural stimuli. The trend of recent research is shifting from a phenomenologi-

cal level to a more molecular level. New exciting discoveries now appearing at the molecular level (growth factors, gangliosides, and so forth) suggest future approaches that will emerge to lead to new therapeutic agents.

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Immunological Approaches to the Nervous System

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In the past decade, immunological approaches have helped to revolutionize neurobiology. Monoclonal antibodies in particular have been versatile reagents and have provided a major impetus to research. In this review, we discuss examples of applications of antibodies to research in neuroanatomy, developmental neurobiology, neuronal cell biology, and protein structure. Antibodies have been used to map the distribution of molecules, both within the cell and throughout the nervous system. Often the use of antibodies has permitted the distribution of cells containing an antigen to be determined. Sometimes, this has led to the discovery of previously unappreciated relationships or differences among neurons. Antibodies have also helped to purify and characterize neuronal antigens, many of which were first identified with a monoclonal antibody. In some cases, related families of molecules have been discovered. Often antibody-blocking experiments have suggested functions for new antigens. Finally, "differentiation antigens," frequently defined by monoclonal antibodies, have been used to study many developmental problems.

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Nervous System Anatomy

Antibodies specific for major cell types. An important objective of neuroimmunology has been to develop reagents able to distinguish different subsets of cells in the nervous system. Antibodies to cell-surface antigens are particularly important for isolation of a cell type by positive or negative selection [reviewed in (*1*)]. Positive selection procedures include adhesion to antibody-conjugated resins or magnetic beads and cell sorting (*2*). Negative selection procedures include antibody-directed complement- or toxin-mediated killing of unwanted cell types (*3*).

The major cell types in the nervous system can now be distinguished with antisera to defined antigens [reviewed in (*4*)]. In the peripheral nervous system, for example, neurons, Schwann cells, and fibroblasts can be recognized by the use of antisera to tetanus toxin, laminin, and fibronectin (*5*). In the central nervous system, antisera to tetanus toxin, galactocerebroside, glial fibrillary acidic protein, and thy-1 serve to distinguish neurons, oligodendrocytes, astrocytes, and ependymal cells (*6*). Monoclonal antibodies specific for many of these cell types have also been isolated [reviewed in (*4*)].

Antibodies specific for neuropeptides and transmitters. The most straightforward way to detect differences between neurons has been to use antibodies specific for different transmitters. During the past decade, immunocytochemical methods have shown that more than 30 peptides, often previously identified elsewhere, are localized in specific neurons, coexisting in many areas with other peptides or classical transmitters (*7*). Techniques in molecular biology have revealed even larger numbers of peptides encoded in the genes for the precursors to these peptides. Tissue-specific RNA splicing and prohormone processing have further expanded the numbers of known peptides (*8*). Since only a few of the genes encoding these peptides have been examined, there is scope for further growth in the number of identified peptides. Antibodies to synthetic peptides containing specific epitopes, especially those that can distinguish between related peptides, should be particularly valuable for future work. In some cases, behavioral responses have been shown to require more than one of the peptides encoded by a single gene (*9*).

The use of antibodies has not been restricted to peptide transmitters. It has been possible to visualize amine transmitters, including serotonin, glutamic acid, and γ-aminobutyric acid (GABA), by the use of antibodies to these amines coupled to protein carriers (*10, 11*). This approach should be particularly useful in identifying neurons utilizing amino acids as transmitters—for example, glutamic acid and aspartic acid. Antibodies to enzymes required for the synthesis of other transmitters, such as GABA, catecholamines, and acetylcholine (ACh) have been used for many years to identify neurons likely to contain these substances. In recent years, monoclonal antibodies specific for several of these enzymes, including glutamic acid decarboxylase, tyrosine hydroxylase, dopa-