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Review

The changeable nervous system: Studies on neuroplasticity in cerebellar cultures

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ABSTRACT

Circuit reorganization after injury was studied in a cerebellar culture model. When cerebellar cultures derived from newborn mice were exposed at explantation to a preparation of cytosine arabinoside that destroyed granule cells and oligodendrocytes and compromised astrocytes, Purkinje cells surviving in greater than usual numbers were unensheathed by astrocytic processes and received twice the control number of inhibitory axosomatic synapses. Purkinje cell axon collaterals sprouted and many of their terminals formed heterotypical synapses with other Purkinje cell dendritic spines. The resulting circuit reorganization preserved inhibition in the cerebellar cortex. Following this reorganization, replacement of the missing granule cells and glia was followed by a restitution of the normal circuitry. Most of these developmental and reconstructive changes were not dependent on neuronal activity, the major exception being inhibitory synaptogenesis. The full complement of inhibitory synapses did not develop in the absence of neuronal activity, which could be mitigated by application of exogenous TrkB receptor ligands. Inhibitory synaptogenesis could also be promoted by activity-induced release of endogenous TrkB receptor ligands or by antibody activation of the TrkB receptor.

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1. The issues

The nervous systems of subhuman mammals and man have a remarkable capacity to change and reorganize after various insults resulting from disease or injury. The purpose of these changes is to preserve some functional capacity. The degree to which function can be retained or restored depends on many factors, including the stage of maturity of the affected individual, the critical location and/or magnitude of the area affected, and whether the involved nervous system cells are completely destroyed or only partially damaged. Changes can occur at the level of single cells, such as altering the type of neurotransmitter expressed by a nerve cell, to reorganization of a significant portion of the circuitry of the nervous system. Our interest was in injury-induced reorganizational changes in the central nervous system (CNS). Given the complexity of the CNS, the changes that take place to preserve function cannot be random, but must follow some rules or patterns, as had been indicated by experimental animal studies from a number of laboratories (Cotman et al., 1981; Lynch et al., 1976; Raisman and Field, 1973; Tsukahara et al., 1975). In these studies, axon collateral sprouting by neurons whose projections overlapped those of lesioned neurons were identified as a key element in circuit reorganization after injury in septal nucleus (Raisman and Field, 1973), red nucleus (Tsukahara et al., 1975) and dentate gyrus of the hippocampal formation (Cotman et al., 1981; Lynch et al., 1976) in adult animals. Synapses formed with different presynaptic elements from those originally present, but the newly formed synapses were functional. In order to obtain further definition of some of these rules, my colleagues and I undertook a series of experiments with a simplified CNS in which the injury to the system could be controlled and the subsequent reorganizational changes could be documented.

Why use a simplified CNS? The brain contains a very large number of neurons, each of which is a compartmentalized unit consisting of a cell body (soma) with multiple processes, one of which, the axon, projects electrical impulses away from the soma and the remainder, the dendrites, project electrical impulses toward the soma. Most neurons are either excitatory or inhibitory, their axon terminals, or endings, releasing chemical neurotransmitters in response to electrical impulses at specialized junctions (synapses) with a dendrite or cell body of a target cell. The released neurotransmitter either promotes or inhibits discharge of electrical impulses in the target neuron. The soma acts as an integrator of excitatory and inhibitory signals impinging on its dendrites and somatic membrane, the sum of which determines whether or not an electrical impulse is discharged down its axon. The magnitude of the complexity of the system is in the realization that a neuron may have thousands of synapses, and the number of neurons in a human brain may be on the order of 86 billion (Azevedo et al., 2009).

In addition to neurons, the central nervous system is composed of an even greater number of glia, or supporting cells. Aside from ependymal cells, which line the fluid filled cavities of the brain, there are two major glial types, oligodendroglia and astrocytes. Oligodendroglia form the myelinated sheaths that facilitate conduction of electrical impulses in axons of nerve cells. Myelin is formed by the wrapping of axons in "jelly roll" fashion by processes of oligodendroglia, followed by the extrusion of cytoplasm from the oligodendroglial processes so that the membranes of the processes become closely compacted, providing a multilayered sheath with insulation-like properties along the lengths of the axons. Astrocytes have multiple functions, including structural support for neurons, secretion of a variety of factors that promote neuron survival and growth of neuronal processes, taking up neurotransmitters and ions released into the extracellular space after neuronal discharge, serving as guides for neuronal migration and axonal pathfinding during development, and taking up debris and forming glial scars after injury. Astrocytes also have some function in compartmentalizing the nervous system and in some cases isolating neuronal membranes by ensheathing neuronal somata and dendrites, even covering the somatic and dendritic synapses. Still other functions have been attributed to astrocytes.

There is another category of glia whose origin and function differ from the previously described categories, namely the resident microglia. These cells are derived from the primitive mesodermal layer, as opposed to the ectodermal origin of other glia and neurons, and they enter into the nervous system and become widely distributed early in development. They function as macrophages, cells that become active during pathological conditions, such as after trauma, infection or loss of blood supply. Their role is to attack foreign elements in the CNS, like invasive bacteria, and to scavenge and digest neural cell debris (phagocytosis). They work in conjunction with the immune system to monitor and respond to adverse conditions in the nervous system and activate immune responses by presenting antigens (molecules that trigger immune or inflammatory reactions) to lymphocytes, immunoreactive cells of the immune system. They are an important part of the nervous system's defense mechanism, but their role in the experiments to be described is minor, and thus they will not have a prominent place in the following discussion.

In selecting a simplified central nervous system to use as a model for studies of circuit reorganization, a desirable feature was a system with a limited number of major neuronal types whose interconnections and functions were known. Thanks to the efforts of Santiago Ramón y Cajal (1960), John Eccles, Masao Ito and Janos Szentágothai (1967), and Sanford Palay and Victoria Chan-Palay (1974), as well as other notable neuroanatomists and neurophysiologists, the structural and functional relationships of the rodent cerebellum have been well characterized. The cerebellar cortex contains five major neuronal types, only one of which, the Purkinje cells, projects axons to other parts of the nervous system, and this projection is primarily to the deep cerebellar nuclei, which underlie the cortex. Purkinje cell axons also emit collateral axonal branches that project to all other cortical neurons, including other Purkinje cells. Purkinje cells are inhibitory, and their neurotransmitter is gamma-aminobutyric acid (GABA) (Ito, 1984). Granule cells are the only excitatory neurons in the cerebellar cortex, their neurotransmitter being glutamic acid (glutamate). Most of the excitatory inputs to the cerebellum from other areas of the nervous system (extracerebellar afferents), which are excluded in standard cerebellar cultures (see below), enter as axons called "mossy fibers." Mossy fibers are cholinergic and synapse with the dendrites of granule cells. The granule cells relay excitatory impulses from the mossy fibers to the dendrites of all other cortical neurons *via* bundles of parallel axons known as "parallel fibers," as well as to Purkinje cell dendrites and dendritic spines *via* their ascending fibers. The remaining three neuronal groups, the basket, stellate and Golgi cells, all inhibitory, are interneurons, with their afferents and efferents confined to the cerebellar cortex. Their presumptive neurotransmitter is GABA. Basket cells project to Purkinje cell somata and proximal dendrites, while stellate cells project their axons to more distal portions of Purkinje cell dendrites. Golgi cells give rise to complex axons that project to dendrites of granule cells. The relationships of the cerebellar cortical neurons, minus the mossy fibers, are summarized in the simplified circuit diagram in Fig. 1.

There are other extracerebellar inputs in the intact animal. Another excitatory input is *via* the climbing fibers, which originate in the inferior olivary nuclei in the brain stem and project directly to Purkinje cell dendrites, where they form numerous synapses while branching to conform to the branching of the Purkinje cell dendrites. Still other inputs include catecholaminergic fibers (both norepinephrine and dopamine) from the locus