Synaptic Plasticity: Neuronal Sprouting

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Introduction

In neuroscience the term 'sprouting' refers to any phenomena invoking neurite growth; sprouting occurs through the life span in the mammalian central nervous system (CNS) and in nonmammalian brains and the peripheral nervous system (PNS). These changes in neurite growth are usually represented by modifications to synapses, evidenced as either alterations in synapse number or junctional area. Such alterations can occur in response to normal and pathological stimuli as part of the overall plasticity of nervous system circuitry, believed to underlie changes in behavioral activity. The structural synaptic changes are referred to as 'synaptic plasticity,' as a reference to the term 'behavioral plasticity.' The latter term was first defined by William James in 1890 as any meaningful change in behavior of an organism.

Synaptic plasticity was first recognized during observations of reactive synaptogenesis and sprouting that occurred in response to extension lesions. It was demonstrated that in response to neuritic loss the nervous system was able to form new synapses, and undamaged neurons were able to form new branches (sprouts) with the capability of making new synaptic connections. This led directly to the examination of synaptic plasticity in neurodegenerative diseases and injury. Although the field started with the examination of plastic responses to neuronal injury, it is clear that plasticity occurs throughout life as a normal response and it is recognized that synaptic plasticity is more pervasive than just a compensatory mechanism. It is a subtle, ongoing process in the normal organism, responding to the environment and endogenous rhythms.

Normal Adult Plasticity

The nervous system is very dynamic, even down to the level of its synaptic connections. Proper mental function is based upon a dynamic organization of synaptic structure. The lability of these neuronal networks is highest in the phylogenetically young brain structures involved in realization of perception and self-awareness, which rely upon continual readjustment and refinement. This lifelong self-optimization process underlying neuronal complexity is remodeled to meet environmental demands. Experience-dependent learning combines both additive and subtractive processes. In response to an organism's attempt to control aspects of a new, complex environment there is the creation of new synaptic connections. Then, through the process of pruning, the brain selects the strongest neural circuits, fine-tuning the connections in a subtractive process. Thus experience-dependent learning couples sprouting with selective pruning, with a net gain of synapses. This constant, dynamic synaptic turnover makes it possible to adapt to complex, changing environments. Thus these synaptic connections are in a constant state of flux, responding to hormonal and environmental alterations with adaptive changes in neural circuitry and resulting behavioral alterations. However, the selective dynamic stabilization and destabilization of synaptic connections are accompanied by increasing inherent potential of failure and neuronal vulnerability during the aging process.

Neuronal plasticity mediated by sprouting can involve alteration in dendritic spine number and/or size or alterations in neuritic complexity (length and branching). Dendritic spines are micrometer-sized protrusions of the dendritic membrane that serve as the postsynaptic component for the vast majority of central nervous system excitatory synapses. Spine synapses, in contrast to shaft synapses, act to create spatially isolated compartments, confining biochemical signals and membrane trafficking to localized regions, allowing for fine-tuning of individual synaptic responses. Spine turnover and morphological changes in existing spines are important for the modulation of neuronal circuits during development and plasticity. Dendritic spines are highly dynamic in size, shape, and number, with highest density occurring during late development and decreasing to a relatively stable level throughout adulthood. Changes in spine number and shape are observed in response to high-frequency synaptic activity, behavioral stimuli, or endogenous hormonal cycles.

Further refinement of the neuronal circuitry can be imposed by the function of neighboring glial cells. Soluble glia-derived factors are also important in synaptogenesis, synapse maturation, and plasticity. Neuronal sprouting is dependent upon astrocyte-derived apolipoprotein E (apoE) and cholesterol, which complex into apoE-containing lipoproteins involved in membrane remodeling, repair, and lipid redistribution. Further, neuritic sprouting is impaired by activated glial cells and can be enhanced by reduction in the expression of glial fibrillary acidic protein (GFAP). Increased astrocyte contact with neurons and coverage

of neuronal surface area can modify synaptic activity by controlling local neurotransmitter concentrations and by spatially restricting neuron–neuron contact.

The structural plasticity described by synaptic plasticity is beginning to be linked to functional plasticity. Such functional plasticity can be seen as alterations of synaptic transmission that are strengthened or weakened in response to previous activity. These alterations in synaptic transmission can be of short duration, as in paired-pulse depression and posttetanic potentiation, or long-lasting, as in long-term depression and long-term potentiation (LTP). LTP, generated in the hippocampus, is thought to be a synaptic component of learning and memory, one form of behavioral plasticity. LTP is expressed as an increase in synaptic strength in response to a stimulus train, with increased amplitude being observed after 10–20 s and for many hours, or even up to days or weeks. Accompanying the change in synaptic transmission there is an expansion of the area of the synaptic junction. Although the number of synapses and the shape of dendritic spines do not change after the induction of LTP, stimuli that alter spine number or shape have been shown to also regulate LTP generation. Thus, the functional plasticity represented by LTP is tightly linked to structural changes associated with synaptic plasticity.

Synapse turnover is a constant process of loss and replacement of synapses, with total synapse number determined by the relative rates of synaptogenesis and synaptic regression. One set of stimuli may result in a net increase in synapse number by increasing synaptogenesis. Likewise, competing stimuli may induce synaptic regression, resulting in a net decline in synapse number. The subtle balance between stimuli may represent a mechanism underlying the synaptic plasticity observed as part of the normal life cycle, dependent upon developmental stage, experience, and age.

Neurons have the capacity to respond to perturbations to maintain their function within normal physiological range, such that the neuron can regulate the function of each impinging synaptic terminal, decreasing or increasing the strength of each synapse, resulting in a normal sum total excitation despite inappropriate innervation. Such homeostatic regulation of neuronal activity would maintain the robust function of the nervous system during the restructuring and refinement of neural circuits during the postembryonic developmental period. Such homeostatic regulation would establish limits beyond which activity-dependant changes in synaptic number would not reasonably alter cellular activity. This allows for maintenance of cellular physiological responses during the tumultuous periods of synaptic pruning required for establishment of synaptic structure and function. As the dendrites of the central nervous system, preexisting synaptic connections grow physically and chemically more removed from the cell soma, and more surface area is exposed for the inclusion of new synaptic contacts, leading to new functionality imposed by the changing response properties of the neuron.

The continual turnover of synapses, coupling sprouting with selective pruning underlying experience-dependent learning, allows for efficient memory storage in the brain, balancing the energy and metabolic demands of synapse maintenance with the extra energy cost of synaptic overgrowth required to obtain selective removal. Such a scheme allows for the judicious refinement of neural circuits required for proper memory storage within a highly adaptable context that is modifiable by endogenous and extrinsic environmental cues. However, the reliance of this system on exquisite balance between continual synaptogenesis and synaptic regression is vulnerable to maladaptive alterations in either side of the equation, leading to declines in cognitive function. The importance of correct pruning of the neural circuitry is observed in seizure patients. In the dentate gyrus, aberrant development of recurrent collaterals branching from granule cell axons (the mossy fibers) may contribute the scrambling of neural circuitry and to the epileptogenic activity in patients with temporal lobe epilepsy.

Puberty, Reproduction, and Sex Steroids in Synaptic Sprouting

Synaptic density peaks in the early postnatal period; this is followed by an extended time period of continuous synaptic turnover balanced toward synaptic removal or pruning, leading to a gradual decline to adult levels of synapses by puberty. Puberty is the point of transition, marking the metamorphosis of the child into the adult, with accompanying changes in reproductive maturity and alterations in body growth and composition, as well as profound changes in brain function. Not only does puberty mark the end of the extensive period of synaptic pruning that occurs during development, but it is also represented by the initiation of adult patterns of synaptic plasticity and sprouting. Neuronal connectivity is not fixed once development is complete but instead continues to change throughout life. However, there is a reduction in synaptic elimination, shifting the balance of the continuous synaptic turnover from a state of pruning to a state of maintenance.

Most dramatic are the alterations in synaptic connectivity in the hypothalamic centers governing sexual physiology and behavior. Here, steroid hormones both remodel and activate neuronal circuits during

adolescent brain development, establishing recurrent interactions between steroid hormones and the central nervous system. The release of gonadotropin-releasing hormone (GnRH) from GnRH neurons, a process that couples brain activity and gonadal function, is accomplished by these recurrent interactions, establishing a controlled cyclic gametogenesis and gonadal hormone production. In females, the midovulatory gonadotropin surge that stimulates ovulation is preceded by an estrogen-inducible retraction of axosomatic synapses in the arcuate nucleus. Following ovulation there is a recovery to baseline levels of synaptic connections, representing defined cyclical synaptic plasticity under control of sex hormones governing reproductive physiology. Due to the lack of alterations in spine or neurite morphology, these events are not sprouting *per se*; however, they remain reminiscent of the sex hormone-induced sprouting involved in the development of these same neural circuits.

Although the ovarian cycle-related synaptic changes in the hypothalamus do not involve neuritic outgrowth, in the hippocampus and prefrontal cortex there is an accompanying alteration in dendritic spine density. Specifically, there is significantly lower spine density during estrus than during proestrus. In addition, removal of circulating gonadal steroids via ovariectomy results in a significant decrease in spine density of the hippocampus in adult female rats, and spine density increases upon replacement of estrogen. This effect is reminiscent of the estrogen-induced sprouting during development of hypothalamic circuits underlying reproductive physiology and behavior.

Further supporting a role of sex hormones as environmental regulators of synaptic sprouting in the adult animal, gonadal steroids have been shown to be involved in regulating lesion-induced axonal sprouting. Although lesion-induced sprouting is more akin to pathological alterations in neuronal function, it is a useful paradigm to study the interaction of aging and environmental factors on normal aging-related synaptic plasticity and sprouting. These effects are often too subtle to isolate the mechanisms underlying normal synaptic turnover that are revealed by magnifying the effect via reactive regenerative processes. Sprouting is increased in the presence of sex hormones and in response to environmental enrichment or exercise *in vivo* following perforant path lesions of the hippocampus, and *in vitro* in the wounding-in-a dish model. The role of glial cells in the sprouting response is supported by the inverse relationship between astrocytic glial fibrillary acidic protein expression and neuritic sprouting. However, the influence of sex steroids on synaptic sprouting invites another point of vulnerability to the proper maintenance of the synaptic circuitry with the decline in hormonal control in aged animals, emphasized by the loss of sex hormone production in reproductive senescence, as discussed later.

In addition to the intrinsic regulation of synaptic density by gonadal hormones, extrinsic environmental cues can support sprouting as well. Environmental enrichment and exercise have been well established as one means of promoting neuritic sprouting and the incorporation of newly generated neurons into existing neuronal circuits. Exposing rodents to an enriched environment, consisting of large cages with toys ladders, mazes, and social interactions, results in increased performance on spatial learning tasks, compared with rodents housed in impoverished conditions. Similar results are obtained with increased physical exercise. These behavioral modifications are complemented by physical brain changes consisting of increased synaptic density and increased complexity of dendritic branching. While these adaptive responses are most pronounced in the young brain, environmental stimulation produces effects in the aging brain as well.

Neuronal Sprouting and Aging

Aging is associated with a decline in cognitive function that can be explained in part by alterations in cellular response directly affecting plasticity, in which the ability to learn new tasks decreases with age. On the cellular level, synaptic contacts, synaptic strength, and plasticity are reduced with age. These changes are much more subtle than are the dramatic alterations in neuronal morphology and survival that occur in age-associated neural disorders such as Alzheimer's disease and Parkinson's disease. In contrast to these pathological states, neuron loss does not appear to be an important contribution to age-related functional decline. Rather, subtle shifts in dendritic branching and spine density occur in region-specific patterns.

Although there is little evidence of significant neuronal loss during normal aging, alterations in dendritic extension of the neuronal soma occur in a large number of cells during aging. Many neurons show progressive restriction and atrophy of their more peripheral dendrite branches and, especially in cortical pyramidal cells, among the basilar shafts. The remaining dendritic branches often demonstrate beaded swellings, in correlation with the irregular dendritic spine loss. However, during these aging-related losses to dendritic systems, the potential for neuronal growth is not lost. Possibly as a compensatory response to increase available synaptic area, other neurons grow further dendritic extensions.

Even though there are these dendritic alterations in many neurons, they do not represent a gross regression of dendrites and remain region specific. There is

a reduction in dendritic branching with age in the prefrontal cortex in humans and rodents. In contrast, the hippocampus of humans and rodents does not experience significant changes in dendritic length with age, with net stabilization of dendritic extent in CA1, CA2, CA3, and the subiculum. In fact, in many brain regions, including the parahippocampal gyrus and the dentate gyrus, the aging brain is associated with increased dendritic branching, until very old age, when there is a consistent regression of the dendritic tree back to that of mature young adults. The region specificity of the dendritic losses indicates that the declines in neuronal plasticity are not merely consequences of wear and tear or global aging. This variability in aging that occurs within subpopulations of neurons reflects the different demands throughout the life span.

The observed region-specific increases in dendritic plasticity may be a compensatory response to partial cell loss that occurs during normal aging. New connections would be formed by healthy neurons within the population, assuming parallel functions. Such an extension of existing inputs would maintain circuit function, but would limit the amount of redundancy in the system, making it more vulnerable to further alterations in synaptic connectivity. Alternatively, the new connections could be formed by fibers from converging pathways that can act to boost weakened signals and maintain functional stability. This response may be a recapitulation of the developmental environment in which many neurons are programmed to form a certain number of terminals or to synapse, and if they cannot do so in one region, they will tend to increase terminal growth elsewhere. The increase in available synaptic area in response to the progressive regression of neighboring dendritic systems works to maintain the total postsynaptic surface per neuron. This leads to a concept of two neuronal responses to aging, one involving dendritic retraction and one involving reactive dendritic expansion. This two-stage response of dendritic spines (loss of total number but enhanced sprouting of remaining spines) represents a neuronal response to the decreased allostatic load, defined as a decrease in the capacity of neurons to oppose the damaging effects of strong, excessive stressors, compatible with full function at normal levels of load. The decreased allostatic load, or homeostatic reserve, is a characteristic of normal aging, resulting from continual or repeated stress.

Even though the structural and behavioral plasticities associated with sprouting are present in the normally aged brain, functional decline and its associated synapse loss are still observed. The aged rodent brain has a remarkable capacity for sprouting and synaptogenesis. However, aged synapses respond to plasticity-inducing stimuli differently than do young synapses, and the degree of sprouting is often blunted with respect to young animals. Further, even though in many brain regions the absolute number of spines is often not altered, there are significant changes in the shape and distribution of synaptic contacts. With increasing age there is a shift from L-type (lollipop shape) to N-type (nubby-like shape) synapses. The spine head becoming smaller, the spine shaft thickening, and a decrease in spine length characterize this shift. Cognitive-sparing diet alterations have been demonstrated to prevent the loss in L-type synapses. The aging-related shift in spine makeup and response is demonstrated by the interaction between vulnerability to aging and sex steroids in female rats. The estrogen-induced spine increase seen in CA1 of young rats does not occur in response to estrogen in aged rats. However, in the aged rat, estrogen increases the number of *N*-methyl-D-aspartate (NMDA) receptor per synapse, restoring the more youthful receptor profile that is lost in normal aging. Thus estrogen may help preserve hippocampal function in the context of a decreased synaptic density. This is another case in which the aged response to environmental stimuli maintains the circuit function, but with a decrease in the system's redundancy. This alteration in estrogen responsiveness is species specific. In contrast to the rodent, the CA1 region of the aged monkey hippocampus is still as responsive to estrogen-induced spine density and synapse number increases as is the young monkey.

Many of the other age-related changes in neuronal plasticity are generally subtle and may only be observationally manifested under conditions of perturbation. This was first observed in 1978 by Scheff and colleagues, who demonstrated that there is a clear reduction in collateral sprouting and regeneration in the aged nervous system. This limited response of reactive sprouting in aged animals has been well characterized by many groups since. Following entorhinal lesion, both young and aged rats can replace synapses lost in the dentate molecular layer of the hippocampus. However, this reinnervation, which begins very rapidly in young animals, is delayed in aged animals, with impairment in the initial phases of synaptic sprouting. Thus the rate of reactive sprouting is decreased as a function of age.

The age-related alterations in neuronal plasticity are emphasized again with the interplay between sex steroids and reactive sprouting. As discussed earlier, estrogen greatly enhances neurite outgrowth in various models of sprouting. However, in the aged female rat the induction of neuronal sprouting is impaired and, unlike in young adults, the sprouting is not sensitive to estradiol. This effect may be due to the increased glial activation associated with aging,

as evidenced by an increase in the activation marker GFAP. GFAP expression is usually regarded as secondary to neurodegenerative processes. However, glia become activated early in aging without concurrent clinical manifestations of pathology. Since neuritic sprouting is inversely related to GFAP expression, normal aging-related glial activation may have a profound impact on synaptic functions. Increased glial activation and GFAP expression may shift the balance of sprouting and retraction unfavorably during the ongoing synaptic turnover, leading to age-related declines in cognitive function.

Age-related alterations in synaptic plasticity may be due to changes in the properties of individual neurons or due to changes in the nervous system itself, as the individual neurons exist within a highly organized system. The decrements in neuronal plasticity could be due to inherent changes in the neuron's ability to extend neurites or form new spines. Alternatively, the decrement could be due to alterations in the complex system of extrinsic signals that trigger the requisite outgrowth. However, there has not been a consistent observation of age-related changes in neurotrophic factors to support the latter scenario. Another alternative to intrinsic changes to the aged neuron diminishing its capacity for growth involves alterations in the target neural circuitry in a way that lessens the accommodation or stimulation of sprouting. This is a model supported by the fact that the aged hippocampus supports less neuron ingrowth than does young hippocampus, whereas aged transplants show robust innervation in young host hippocampal tissue. This is an effect that does not appear to be due to reductions in baseline substrate properties that promote neurite outgrowth. Further support is provided by the observation that sprouting in the wound-in-a-dish model is diminished when neurons are co-cultured with astrocytes from aged animals, as compared to astrocytes from young animals. Thus, in the target region preventing efficient sprouting, there are likely changes that are marked by increased glial activation. This increased glial activation could result from the increase in allostatic load imposed by the results of cumulative reactive oxygen species (ROS) production or excessive stress. Both increased oxidative load and stress (or its hormonal effector, glucocorticoid) have been shown to reduce neuronal plasticity, LTP, and behavioral learning. Further implicating oxidative load in aging-associated cognitive decline, spine losses can be attenuated by superoxide dismutase overexpression (antioxidant defense).

In addition to reduced oxidative load and hormonal modification, synaptic sprouting can be altered favorably by other means. Calorically restricted diets can retard synapse loss, mostly through maintenance of L-type synapses, rather than preventing a decrease in total spine number. The processes of synaptic plasticity appear to be strengthened by increased neuronal activity, driven by enriched environment or behaviors such as exercise. Such age-associated dendritic morphologies are also reflected in the region-specific alterations in spine density. This continued structural plasticity of the aged brain is reflected behaviorally, in that age-related loss in speed of mental processing can be offset by continual environmental enrichment and challenge so as to promote synaptic plasticity. The fact that aging-vulnerable circuits are still responsive to environmental stimuli indicates that the aged synapse remains plastic. This is in contrast to cognitive deficits due to neuronal loss, in which case little can be done to restore circuit function. If the cognitive deficit of normal aging is due to the frailty of the synapse, the mechanisms of synaptic plasticity might be harnessed to for therapeutic intervention.

See also: Adult Cortical Plasticity; Aging of the Brain; Axonal and Dendritic Identity and Structure: Control of; Cognition in Aging and Age-Related Disease; Developmental Synaptic Plasticity: LTP, LTD, and Synapse Formation and Elimination; Gene Expression Regulation: Steroid Hormone Effects; Neuronal Plasticity after Cortical Damage; Spine Plasticity; Synaptic Plasticity: Neuronogenesis and Stem Cells in Normal Brain Aging.

Further Reading

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