Themes and Strategies for Studying the Biology of Stroke Recovery in the Poststroke Epoch

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Background and Purpose—This review will focus on the emerging principles of neural repair after stroke, and on the overlap between cellular mechanisms of neural repair in stroke and clinical principles of recovery and rehabilitation.

Summary of Review—Stroke induces axonal sprouting and neurogenesis. Axonal sprouting occurs in tissue adjacent to the stroke and its connected cortical areas, and from sites that are contralateral to the infarct. Neurogenesis produces newly born immature neurons in peri-infarct striatum and cortex. Stimulation of both axonal sprouting and neurogenesis is associated with improved recovery in animal models of stroke. A unique cellular environment in the poststroke brain supports neural repair: an association of angiogenic and remodeling blood vessels with newly born immature neurons in a neurovascclar niche. Controversies in the field of neural repair after stroke persist, and relate to the locations of axonal sprouting in animal models of stroke and how these correlate to patterns of human remapping and recovery, and to the different models of stroke used in studies of neurogenesis.

Conclusions—On a cellular level, the phenomenology of neural repair after stroke has been defined and unique regenerative environments in the poststroke brain identified. As the field moves toward specific studies of causal mechanisms in poststroke repair, it will need to maintain a perspective of the animal models suited to the study of neural repair after stroke as they relate to the patterns of recovery in humans in this disease. (Stroke. 2008;39:1380-1388.)

Key Words: axonal sprouting | regeneration | neurogenesis | neurorehabilitation | angiogenesis

Stroke is the leading cause of adult disability. An estimated 55% of the total yearly costs of stroke occur in the chronic setting, which means that the long-term management of disabled stroke patients costs upwards of $34.5 billion per year. From 1994 to 2004 the death rate from stroke declined 20.4%. However, stroke incidence is expected to increase to an estimated 1.14 million per year in 2025. This translates to a disease with an ever-larger number of disabled survivors. These statistics have led to a research focus on mechanisms of neural repair as they relate to the patterns of human recovery after stroke.

The patterns of recovery in the human brain after stroke have been determined with functional imaging and transcranial magnetic (TMS) and direct current (TDC) stimulation. In the early stages after stroke, brain activation in sensorimotor tasks of the affected limb occurs in a wide-ranging network of cortex in primary motor, premotor and supplementary motor areas in both hemispheres. In patients that experience a good recovery over time, a more focused set of cortical areas are involved in sensorimotor tasks, and these often relate to peri-infarct and connected cortical areas. Patients with poorer recovery often retain a more diffuse, or contralesional, activation of cortical areas to sensorimotor tasks. Similarly, deactivation with TMS or TDC of perilesional areas disrupts recovered functions after stroke. Deactivation of contralesional hemispheric areas may also disrupt motor performance in recovered stroke patients, but these are often patients with larger stroke and/or poorer overall recovery. Areas of cortical remapping in the stroke hemisphere undergo an expansion in cortical thickness that is reminiscent of the dendritic sprouting and increase in cortical volume in areas that mediate recovery of function in ischemic lesions in animals. There is variability in this pattern of diffuse to focused, and bilateral to ipsilesional activation with stroke recovery in humans. At least some of this variability relates to lesion location and size, with larger lesions producing functional recruitment of contralesional cortical areas into a recovery network. This review will focus on one major set of findings in these human studies of recovery after stroke: imaging, stimulation and recent structural studies indicate that a major pattern of successful recovery after stroke in humans establishes a target for the study of cellular
and molecular mechanisms of neural repair in the peri-infarct and connected cortical areas ipsilateral to the stroke. Within these regions of peri-infarct and connected cortical areas two main cellular processes of neural repair have been extensively described: poststroke axonal sprouting and poststroke neurogenesis. This review will focus on the cellular and molecular events in peri-infarct and connected cortical areas that are associated with neurological recovery after stroke.

**Poststroke Axonal Sprouting**

Axonal sprouting occurs after injury in the peripheral nervous system, and with specific therapies, in the spinal cord and optic nerve. In these sites, sprouting neurons activate specific molecular elements of a growth program to elaborate a growth cone, extend an axon and form new synapses. Nervous system injury also induces glial and meningeal growth-inhibitory proteins that block axonal sprouting.

The unequivocal demonstration of poststroke axonal sprouting has required direct axonal quantification. This is because the proteins associated with the growth cone, such as GAP43, which have been traditionally used to “map” sprouting axons are in fact not neuron- or sprouting-specific: GAP43 is found in astrocytes, oligodendrocytes and is induced in neurons with LTP. With this requirement for a direct demonstration of poststroke axonal sprouting, axonal sprouting after stroke has been shown in peri-infarct cortex, in the cervical spinal cord and brain stem, and between parietal and frontal lobes.

Long distance axonal sprouting after stroke has been shown in the brain stem and cervical spinal cord and in cortico-striatal projections. Axons sprout from the intact projections of the sensorimotor cortex contralateral to the stroke, into the deafferented regions of cervical spinal cord and midbrain that previously received a projection from the now infarcted sensorimotor cortex. This sprouting can be unequivocally demonstrated as it develops a novel contralateral projection. A similar process occurs in the motor cortex contralateral to ischemic sensorimotor cortical lesions, where projections from the contralateral cortex sprout into the region of striatum on the other side of the brain that normally received input from the now infarcted sensorimotor cortex.

In these cases of long-distance axonal sprouting in the rat, the sprouting axons appear to arise from intact, contralateral projections that were not injured by the stroke. It is not clear at present if this sprouting involves in situ axonal branch formation and growth, but it would be unlikely for axonal projections to grow de novo across the long distances from cortex to cervical spinal cord or brain stem after stroke. Axonal branch formation involves specific molecular events, and these appear to be at least partially distinct from those that regulate axonal growth cone behavior. For example, the guidance cues netrin-1 and semaphorin 3a (sema3a) and the growth factor fibroblast growth factor 2 (FGF2) can directly regulate the formation of axon branches independently of an effect on the growth cone. The molecular control of cytoskeletal dynamics also involves different stathmin family proteins in growth cone versus axon branching. Thus, if poststroke axonal sprouting from cortex contralateral to stroke into brain stem and cervical spinal cord is mediated by axonal branch formation, it may differ molecularly from that seen in axonal sprouting in peri-infarct cortex (see below). In terms of functional assessment, pharmacological stimulation of axonal sprouting from cortex contralateral to stroke into cervical spinal cord and brain stem is correlated with functional recovery, suggesting that this axonal sprouting from intact, contralateral projections may mediate recovery in rats. However, generalizing this process from the rat to the human is problematic (see last section).

Stroke also induces axonal sprouting in the cortex adjacent to or connected with the stroke site. Small strokes in the rodent somatosensory cortex induce axonal sprouting in the cortex within 1 to 4 mm from the infarct. This sprouting causes a topographical remapping of the normal somatosensory connections. In the more complex brain of the New World Monkey, stroke in the motor cortex induces axonal sprouting from premotor cortex in the frontal lobe into the somatosensory cortex of the parietal lobe. This axonal sprouting develops a novel projection from premotor cortex to somatosensory area 1/2. There is precedence for such a long distance axonal sprouting in the primate brain, in that deafferentation of somatosensory cortex also leads to long-distance axonal sprouting in cortex. In these examples of axonal sprouting in regions adjacent or connected to stroke, the axonal sprouting occurs in the same areas that are associated with functional recovery in humans. However, there has been no direct demonstration that axonal sprouting in peri-infarct or connected cortical areas promotes functional recovery.

**Growth Promotion**

Axonal sprouting in stroke, in neural development and in the regenerating peripheral nervous system occurs through the elaboration of a molecular growth program in temporal phases after stroke. Within days after injury, abnormal patterns of neuronal activity are generated in injured neurons or brain regions that will undergo sprouting. Within the first 3 days after ischemic cortical lesions, postlesion neuronal activity takes the form of rhythmic neuronal discharges that develop in peri-infarct and then contralateral cortex and are tightly linked to axonal sprouting in this model. At this early phase after stroke, molecular events associated with axonal sprouting are initiated. This molecular growth program includes molecules that may form a link between membrane signaling events, intracellular signaling cascades, transcriptional activation and cytoskeletal reorganization. Gene expression profiles of peri-infarct cortex within the first day after stroke demonstrate induction of neuronal growth factors (IGF-1) and transcription factors, such as members of the ANIA class and ARC, that are associated with neuronal plasticity. Within the specific region of poststroke axonal sprouting in peri-infarct cortex over the 1-month period of axonal sprouting, specific patterns of growth-promoting genes are present. The AP-1 transcription factor c-jun, the lipid raft growth cone proteins CAP32 and GAP43, and SPRR1 are activated within the first week after stroke, with CA23, GAP43 and c-jun mRNA induction increased for
1-month after stroke. At later time points, between 1 and 3 weeks after stroke, genes involved in axonal outgrowth or cytoskeletal reorganization, such as L1-NCAM, p21/Waf1, MARKS and Tad tubulin are induced. At 28 days after stroke in the rat, new patterns of cortical connections can be detected. During this later stage, genes involved in microtubule reorganization are still being activated, such as the stathmin family members, SCLIP and SCG10.

This pattern of growth-promoting gene activation differs from that seen in peripheral nerve sprouting. Genes associated with cytoskeletal reorganization, including the cytoskeletal reorganizing proteins SCLIP and SCG10, are induced late in peri-infarct cortex after stroke and RB3 and stathmin gene expression decline in the region of axonal sprouting in peri-infarct cortex after stroke. However, RB3 is upregulated in peripheral nerve regeneration and SCG10 and SCLIP are induced throughout the sprouting response in peripheral nerve. Similarly, gene expression for p21 and SPRR1 are induced throughout the duration of the peripheral nerve sprouting response unlike their transient induction in the region of poststroke axonal sprouting in peri-infarct cortex. These differences between PNS and CNS postinjury axonal sprouting may reflect true differences in the molecular response of these two systems, and may account for the limited ability of the CNS to sprout. However, the present gene expression profiling of axonal sprouting after stroke has involved whole tissue analysis—isoation of the region of axonal sprouting and its constituent glia, blood vessels, inflammatory cells, many types of neurons and relatively small number of sprouting neurons. A definitive study of the molecular growth program of the sprouting neurons after stroke will require selective isolation of this cell population.

Growth Inhibition

There are three main classes of axonal growth-inhibitory molecules: myelin-associated proteins include NogoA, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin associated glycoprotein (OMgp). Extracellular matrix proteins include the chondroitin and heparin sulfate proteoglycans and tenascin. These organize with other extracellular proteins in a complex three-dimensional matrix that may inhibit axonal growth, mediate axon fasciculation or even promote growth through the binding of growth factors (such as FGF2). The developmentally associated axonal guidance molecules are loosely grouped in a category based on their initial description in neurodevelopment. These include members of ephrin A and B and EphA/B tyrosine kinase signaling systems and sema 3a/neurpilin 1. Stroke alters the expression of all three classes of growth-inhibitory molecules both locally near the infarct and in distant areas in a temporal and spatial pattern that places several of these molecules in a direct position to block poststroke axonal sprouting.

Stroke massively induces the expression of chondroitin sulfate proteoglycans and other growth inhibitory molecules in the immediate vicinity of the infarct, within the glial scar. This is a region in which axonal growth promoting proteins are also induced, such as GAP43, CAP23 and SPRR1. Thus glial scar can be identified as an area of massive simultaneous upregulation of both growth inhibitory and growth promoting genes. However, this region of glial scar is not the major area of poststroke axonal sprouting. As demonstrated with tract tracing experiments, this region includes more distant areas of peri-infarct cortex, away from the glial scar. In this region, only a small subset of growth-inhibitory molecules are induced by stroke during the initial periods of axonal sprouting: neurocan, NG2, EphB1, ephrin A5 and MAG. These molecules are induced directly in regions in which growth cone proteins, such as GAP43, CAP23, MARCKS and SPRR1 are upregulated, and in a pattern of overlap with these molecules in peri-infarct cortex. EphrinA5 and MAG are of further interest in the biology of poststroke axonal sprouting because they are not only induced by stroke in the region of axonal sprouting in peri-infarct cortex in young adult animals, but also are induced to even higher levels by stroke in this region in the aged brain. Because stroke largely occurs in aged individuals, EphrinA5 an MAG may provide targets to manipulate axonal sprouting in a clinically important manner.

Stroke causes a reduction in specific chondroitin sulfate proteoglycans in the region of poststroke axonal sprouting. The chondroitin sulfate proteoglycans aggrecan, versican and phosphacan are organized as peri-neuronal nets around both inhibitory and pyramidal neurons in cortex. After stroke, the mRNA for these genes is at steady state until a late induction, but immunohistochemical staining for these proteins is lost within the first week. This early loss of protein levels suggests that stroke causes enzymatic cleavage of chondroitin sulfate proteoglycans in peri-infarct cortex. Peri-neuronal nets modulate cortical plasticity, as they appear in cortex during the closure of the critical period, the time period in the developing brain in which environmental alterations can produce large scale changes in physiology and structure in neurons. Enzymatic removal of peri-neuronal nets prolongs the critical period, and behavioral maneuvers that prolong the critical period delay deposition of chondroitin sulfate proteoglycans into peri-neuronal nets. Thus, stroke may promote axonal sprouting in a region of peri-infarct cortex through enzymatic removal of chondroitin sulfate proteoglycans in manner analogous to the initial period of connection formation in the developing cortex.

Getting in the Mood for Growth

Neuronal connections form during development when immature neurons that are in a growth state project through a series of inhibitory and facilitating guidance cues to a target. Axonal sprouting in the developing brain thus has two sides to the process: a growth program that is active in the embryonic/early postnatal sprouting neurons, and a favorable environment or set of cues through which to grow. As neurons mature this growth state is lost, and in parallel myelin and chondroitin sulfate proteoglycans are deposited and form a more inhibitory growth environment. If the process of initial axonal sprouting in the developing brain is a guide for axonal sprouting during neural regeneration, to stimulate axonal sprouting after stroke or other CNS injuries in the adult will take both blockade of axonal growth inhibitors, and
stimulation of neurons into a growth state. The idea that both stimulation of a growth program, and blockade of growth inhibitors, is necessary for CNS regeneration is supported by recent in vivo experiments. MAG or NogoA knockout animals do not show enhanced neuronal sprouting in spinal cord injury. Blockade of either NogoA signaling or of RhoA, a common intracellular effector for MAG, NogoA, chondroitin sulfate proteoglycans and ephrins, alone in optic nerve injury does not promote robust regeneration across the lesion site. However, blockade of NogoA or RhoA signaling and induction of a neuronal growth state do promote substantial axonal sprouting across the lesion site. Similarly, in peripheral nerve sprouting into the spinal cord, degradation of chondroitin sulfate proteoglycans and stimulation of a neuronal growth state are both required for robust regeneration. Activation of a neuronal growth state after stroke may be achieved with pharmacological treatments, such as inosine or amphetamine. However, it is possible that a neuronal growth state may be activated through a behavioral paradigm, such as a neurorehabilitation protocol. Exposure to an enriched environment or overuse of the affect limb after stroke promotes neuronal sprouting and synaptogenesis in cortex. Motor learning is associated with cortical remapping and increases in synaptic number in corresponding motor cortex. In humans, overuse of the affected limb after stroke promotes cortical remapping within the peri-infarct and connected cortical areas. Thus, it is possible that neurons in peri-infarct and connected cortical areas after stroke may be activated for neuronal growth—dendritic and/or axonal sprouting—through behavioral measures to overuse the affected region of cortex, and that this can be coupled with blockade of the key growth-inhibitory molecule(s) to promote functionally important axonal sprouting after stroke.

Poststroke Neurogenesis

Stroke induces cell proliferation within the subventricular zone, migration of newly born immature neurons into peri-infarct tissues and long-term survival and maturation into a small number of cells with a mature neuronal phenotype and ultrastructural evidence for synapses. Poststroke neurogenesis appears to divert migratory immature neurons from their normal path in the rostral migratory stream (RMS). Immature neurons in peri-infarct cortex appear at odds with the reported association of immature neurons and astrocytes in the ischemic striatum. As reviewed below, the differences in association of newly born immature neurons in stroke predominantly with astrocytes in ischemic striatum and with angiogenic blood vessels in peri-infarct cortex likely relates to important, and often overlooked, differences in the stroke models used in neural repair studies.

Cellular Environments for Neural Repair After Stroke

Angiogenesis, neurogenesis and axonal sprouting occur in common areas of peri-infarct tissue after stroke and may form a unique regenerative triad that supports neural repair in this disease. Studies in stroke have defined specific receptor-ligand signaling systems that link angiogenesis and neurogenesis. As noted above, blocking angiogenesis severely reduces poststroke neurogenesis. These angiogenic blood vessels in peri-infarct cortex secrete SDF-1 and angiopeitoin-1 in the first week after stroke. Administration of SDF-1 or ang-1 stimulates neuroblast migration into peri-infarct cortex, and blockade of their receptors, CXCR4 and Tie2, blocks or disperses the migration of immature neurons after stroke. This work identifies two intercellular signaling systems that mediate poststroke neurogenesis within a neurovascular niche in peri-infarct cortex. Erythropoietin (EPO) and VEGF are also in a position to mediate a neurovascular coupling of angiogenic blood vessels and migrating neuroblasts. EPO is induced in blood vessels and astrocytes in peri-infarct tissue after stroke. This endogenous increase in poststroke EPO production promotes poststroke neurogenesis. Pharmacological doses of EPO also promote angiogenesis and neurogenesis after stroke. VEGF is induced in peri-infarct tissue after stroke and may be
secreted by angiogenic blood vessels. VEGF receptor block-
ade downregulates poststroke neurogenesis and exogenous
VEGF promotes poststroke neurogenesis. VEGF is also
produced by neurons and astrocytes in peri-infarct cortex and
is strongly bound to the extracellular matrix, so the exact
cellular communication pattern within the VEGF system in
stroke remains to be determined.

Angiogenesis, neurogenesis and axonal sprouting are more
broadly linked in neurodevelopment. This linkage has been
particularly supported for members of the ephrin B/EphB
signaling system. Members of the ephrin B tyrosine kinase
signaling system serve as important axonal guidance cues in
many areas of the nervous system. Ephrin B molecules also
play key roles in vascular sprouting, vasculogenesis and
angiogenesis. Ephrin B2 guides vascular sprouting, in parallel
to its mechanism for axonal sprouting. Ephrin B2 is
upregulated during angiogenesis and guides mural cell re-
cruitment to angiogenic blood vessels. Ephrin B class
members also mediate neuroblast migration from the normal
SVZ and control an aspect of neural crest progenitor migra-
tion. Thus ephrin B/EphB proteins on immature neurons
or neural progenitors and vascular endothelium are known to
mediate sprouting, cell migration and recruitment. EphB
receptors are upregulated in peri-infarct cortex in the region
of axonal sprouting, angiogenesis and neurogenesis during
the time course for these processes. A similar overlap
between angiogenesis, neurogenesis and axonal sprouting,
and an induction in expression level peri-infarct cortex in
stroke, is present for the ephrin A system and sema 3a/neuropilin 1/VEGF. In the neuropilin 1 system, sema 3a or VEGF competitively bind the neuropilin 1 receptor to mediate their distinct cellular effects, such as axonal repulsion or neurite outgrowth and neurogenesis. Because astrocytes, meningeal fibroblasts and blood vessels will be sources for sema 3a and/or VEGF, this suggests that neuropilin 1 signaling in the neurovascular niche after stroke will be a dynamic representation of local ligand concentration as the processes of angiogenesis and astrocytosis develop along their different time courses. A common theme within ephrin and semaphorin systems is that these operate within growing axons, blood vessels and immature neurons, are induced within the region of the neurovascular niche in peri-infarct cortex and may provide a molecular network for coordinated regulation of these three processes as the brain repairs after ischemic injury.

Atque inter silvas Academi quaerere verum: And Seek for Truth in the Garden of Academus

With the phenomena of axonal sprouting and neurogenesis now described, the field of neural repair after stroke appears poised to move into a productive series of studies on causal molecular mechanisms. However, at least three lingering problems remain in the studies of neural repair that may impact how these mechanisms are translated into the clinic. First, the exact sites of poststroke neurogenesis remain controversial. Many investigators have not been able to identify the long-distance migration of immature neurons from the SVZ into peri-infarct cortex. Long distance migra-
tion in poststroke neurogenesis will be a key process if this is to have relevance to the human. Several studies document robust local migration of immature neurons from the SVZ into the immediately adjacent ischemic striatum, but not beyond this site. Similarly, one study found that astrocytes in the ischemic striatum produce SDF-1 and that this is a tropic signal for migration of immature neurons, whereas in peri-infarct cortex blood vessels are a source for SDF-1 and for its migratory signal to immature neuro-
blasts. Second, many studies have documented axonal sprouting from cortex contralateral to the stroke site in rats, and established that this contralateral sprouting into deter-
vated brain stem and cervical spinal cord mediates a degree of
functional recovery. However, human studies suggest that cortex contralateral to stroke does not mediate direct control of the affected limb during recovery and that activation of contralateral cortex after stroke negatively correlates with recovery and occurs primarily after larger strokes. Third, as evidence mounts for a cellular link between angiogenesis, neurogenesis and axonal sprouting in peri-infarct tissue, stroke studies employ a variety of models with differing amounts of reperfusion and penumbral area, and in some cases utilize animal models in which there are genetic mutations associated with the vasculature that limit angiogenesis and angiogenic growth factors and affect neuro-
genesis, such as the spontaneously hypertensive rat. The type of stroke model used in a particular study will dramatically affect the type of neural repair observed in that study. The middle cerebral artery occlusion model with permanent, or one to two hours of occlusion, produces infarcts in a substantial portion of the cerebral hemisphere, from 21% to 45%, and usually spares a small region of medial striatum adjacent to the SVZ (Figure). While rodents survive this type of stroke, this amount of ischemic damage in humans leads to death or a devastated neurological out-

come. Similarly, the three-vessel occlusion model with distal middle cerebral artery occlusion, also damages much of the cortex in the affected hemisphere (Figure). Because in these two models there is not much peri-infarct and connected cortex remaining after stroke, it is likely that axonal sprouting and neural recovery will need to occur in contralateral cortex. This has been reported in humans, where patients with the largest infarcts maintain contralateral activation of cortex as compared to the transfer of cortical function to peri-infarct and connected areas that occurs in smaller infarcts. In terms of neurogenesis in the middle cerebral artery occlusion models, it is likely that there is no migration of immature neurons to peri-infarct cortex because this region is either dying or dead (Figure) or that the migratory route is encased in gliotic scar, in which secreted proteins are inhibitory to the migration of immature neurons. Furthermore, the region of long-term tissue survival and immature neuron migration in middle cerebral artery occlusion models contains a significant astrocytic response that in many cases directly abuts the SVZ. In stroke models with restricted cortical infarcts, there is a large distance between the infarct and the SVZ, with several millimeters of normal white matter and striatum between the two. Thus immature neurons that migrate out of

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the SVZ in the middle cerebral artery occlusion models migrate through a very different environment than in stroke models in which the stroke site is well removed from the SVZ, and this may account for the different cell signaling patterns between the two models. The middle cerebral artery and other large stroke models provide consistent measures of cell death for neuroprotection studies. However, this consistency comes in the setting of damage to many of the regions associated with recovery. Thus for neural repair stroke studies, rather than consistency of cell death, there should be a focus on the damage in the regions that survive. To paraphrase Horace, as one seeks for truths in the field of neural repair, it is important to remember the garden in which one is seeking.

Conclusions
Stroke induces not only a region of cell death and scar formation, but regions of neural repair and reorganization. In peri-infarct cortex, this reorganization and repair are seen in the formation of new patterns of connections and in the generation of new neurons. Axonal sprouting and neurogenesis occur in the same cellular environment as angiogenesis after stroke. Angiogenesis, neurogenesis and axonal sprouting share many molecular signaling systems in development, and it appears that stroke induces a microenvironment in peri-infarct brain regions in which these three processes share molecular signals during tissue regeneration. Future studies will better define the molecules that control tissue regeneration in peri-infarct cortex and striatum so as to generate pharmacological targets for neural repair after stroke. Present studies have shown an association of axonal sprouting and neurogenesis with neurological recovery. Future studies will also need to determine if these two processes have a causal role in recovery after stroke.

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None.

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