Mechanisms of neural plasticity following brain injury
Tadeusz Wieloch¹ and Karoly Nikolich²

Brain insults cause rapid cell death, and a disruption of functional circuits, in the affected regions. As the injured tissue recovers from events associated with cell death, regenerative processes are activated that over months lead to a certain degree of functional recovery. Factors produced by new neurons and glia, axonal sprouting of surviving neurons, and new synapse formation help to re-establish some of the lost functions. The timing and location of such events is crucial in the success of the regenerative process. Comprehensive gene expression profiling and proteomic analyses have enabled a deeper molecular and cellular mechanistic understanding of post-injury brain regeneration. These new mechanistic insights are aiding the design of novel therapeutic modalities that enhance regeneration.

Addresses
¹ Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, University of Lund, BMCA13, 221 85 Lund, Sweden
² Neuroscience Institute at Stanford, Stanford University Medical School, Stanford, California 94305-5489, USA and AGY Therapeutics, Incorporated, South San Francisco, California 94080, USA

Corresponding author: Wieloch, Tadeusz (tadeusz.wieloch@med.lu.se)

Introduction
Injuries of the central nervous system (CNS), including stroke, traumatic brain injury and spinal cord injury, cause devastating and irreversible losses of function. Stroke affects very large patient populations with major physical and emotional suffering for the patients and their relatives, and at significant cost to society (http://www.americanheart.org). These injuries, beyond causing significant tissue damage, disrupt the internal intricate circuits of the brain and its external neuronal connections that are involved in cognitive and other higher functions, in addition to those involved in crucial sensori-motor functions. There are no currently approved pharmacological agents that would help to restore such lost functions. New therapeutics will emerge from understanding how to overcome inhibitory mechanisms that block regeneration and from understanding mechanisms that enhance neuronal plasticity. The most comprehensive relevant literature today covers post-stroke mechanisms, and this is the primary focus of our review. However, the processes involved are relevant for other types of damage in the CNS, including spinal cord injury and head trauma. For earlier results, several excellent reviews are recommended [1–7].

During and immediately after stroke, neurological functions associated with the infarcted (see glossary) area are lost. During the subsequent months certain neurological functions recover, but approximately 50% of stroke patients have remaining hemiparesis (see glossary), 30% are unable to walk, and 26% remain severely disabled with a need for daily attention. Recovery is most prominent during the first 30 days but continues for at least 6 months [8]. Recovery of function is dependent on the degree of tissue loss and the preservation and/or engagement of neuronal networks that serve as substrates for the restoration of lost brain functions.

Functional recovery following brain damage
Loss of function during stroke is due partly to neuronal death in the infarcted tissue but also to cell dysfunction in the areas surrounding the infarct. These areas encompass the part of the underperfused penumbra (see glossary) that survives the insult, the non-ischemic peri-infarct tissue, and remote (including contralateral) brain areas that are connected to the area of tissue damage. Dysfunction in the remote areas is believed to be due to diaschisis, an equivocal term, which includes tissue hypometabolism, neurovascular uncoupling, and widespread aberrant neurotransmission [2,9].

Recovery of function involves three distinct phases: first, reversal of diaschisis and activation of cell repair, second, functional cell plasticity, that is, changing the properties of existing neuronal pathways, and third, neuroanatomical plasticity leading to the formation of new connections, as summarized in Figure 1. Phases two and three are involved in normal learning, which is also the driving force during functional recovery, and is enhanced by the milieu created following injury. In humans, recovery processes primarily engage ipsilateral brain regions, although if the damage is severe, contralateral brain areas are also involved [7]. These processes can be readily studied in experimental models in rodents and monkeys [10,11]. As in humans, recovery of function following transient middle cerebral artery occlusion (MCAO) in the rat shows a complex pattern of ipsilateral and bilateral hemispheric activation [9], with clear involvement of the contralateral hemisphere [12**].
Early events in the surviving penumbra

Recovery of neurological function is dynamic and multifactorial, and dependent on the severity of ischemia (see glossary). Hence the time of onset and location of various recovery processes will vary accordingly. Recovery of the penumbral tissue depends on the extent of the cellular stress imposed by the repetitive depolarizations occurring for several hours after onset of ischemia, as well as by edema and inflammation. Surviving neurons that are damaged by catabolic processes are rapidly repaired [13] and resume metabolic function, but might still exhibit aberrant neurotransmission, partly because of the dysfunction of their spines. Ischemia causes spine collapse, forming dendritic beads with actin aggregates [14,15]. Collapsed spines recover, although many spines re-appear at different locations on the dendrites and with different morphological features, which could contribute to post-ischemic dysfunction.

A creative milieu

The non-ischemic peri-infarct area and the repaired penumbra area are prime sites for processes that compensate for lost functions. In this tissue that survived the primary insult, growth-promoting factors that stimulate anabolic processes and growth-inhibitory proteins that hamper axonal outgrowth are released. Survival, repair and plasticity genes (arc [activity regulated cytoskeletal-associated protein], NGFI-A [nerve growth factor induced gene A], homer, BDNF [brain derived neurotrophic factor]) are activated rapidly after ischemia in response to damage, and later in a second phase at 9–24 hours, which could initiate secondary gene programs [16,17]. Indeed, the expression of growth-stimulating genes (GAP-43 [growth-associated protein-43]) is seen during the first week after ischemia, and following that, growth-inhibitory genes (aggrecan, versican, brevican) are expressed [18**], which might determine the efficiency of tissue plasticity.

Figure 1

An overview of the activated parallel processes and therapeutic approaches following brain injury, specifically stroke. The temporal sequence of events is shown along a semi-logarithmic schematic timeline of 180 days after injury. Darker shading highlights the maximum intensity of the specific mechanism. Processes that are detrimental towards recovery are shown in pink. Processes of cell genesis are shown in brown, whereas those that underlie adaptive plasticity are shown in green. Prospective therapies that focus on neuroprotection and enhancement of regeneration and functional recovery are shown in gray.

Glossary

Brain ischemia: Decrease in blood flow to the brain that leads to brain dysfunction or cell death.

Hemiparesis: Loss of muscle function on one side of the body.

Infarct: Degenerated tissue due to ischemia induced by stroke.

Penumbra: Ischemic brain tissue in which damage develops slowly, and that can potentially be salvaged by neuroprotective therapies. The penumbra surrounds a core of irreversibly damaged ischemic tissue.
Synchronous electrical hyperactivity, similar to that observed during development, develops in the peri-infarct region and in corresponding contralateral sensori-motor areas. Within the same tissue that becomes hyperexcitable, LTP is enhanced; GABA_δ receptor subunits are downregulated while the number of NMDA receptor binding sites increases. Such an environment is expected to promote axonal sprouting [2,19] in much the same way as normal use-dependent cortical plasticity. Indeed, post-injury axonal outgrowth appears to be dependent on neuronal activity. Using biotinylated dextran amine (BDA), fibers could be traced from the contralateral cortex to the peri-infarct area, in addition to those to the ipsilateral dorso-lateral striatum. This fibre outgrowth was inhibited by tetrodotoxin (TTX) [3]. Yet, the same area is highly hypometabolic for up to 8 days of recovery after the insult, possibly because of defective glial–neuronal interactions [20].

**Axonal sprouting**

Recovery is often limited partly because of the restricted capacity of the brain and spinal cord for anatomical reorganization following lesions. However, recovery of function following cortical injury is correlated with enhanced axonal growth in the vicinity of the lesion. For example, small ischemic lesions induce horizontal axonal sprouting between areas that are normally not connected [21**], whereas larger lesions cause long distance cortico–spinal axonal sprouting [22]. At the cellular level, adaptive changes in neuronal morphology (axonal growth, dendritic arborization and spine remodeling) are seen particularly in the contralateral cortex [1,4]. During the first 2–4 weeks following stroke, motor function recovers substantially. Certain motor skills recover within a few days of the lesion, suggesting that for such rapid recovery of neuronal function, the theory that silent pathways or synapses are unmasked or activated must be considered [23].

**Cell genesis**

In addition to repair and growth of surviving neurons, cell genesis is greatly stimulated following stroke in neurogenic areas, including the subventricular zone (SVZ). It has been proposed that newborn neurons recruited from neurogenic regions contribute to functional recovery following stroke and that measures promoting proliferation and maturation of new neurons are beneficial [24,25]. These neurons are continuously generated up to 4 months after injury, providing a pool of cells that could potentially develop into mature neurons [26*]. Whether neurogenesis contributes to early phases of functional recovery is uncertain, because the speed with which early recovery occurs is too fast for newly generated neurons to be integrated and correctly connected. Also, it is difficult to envisage how a small number of functional new neurons in the striatum could dramatically enhance functional recovery of cortico–spinal pathways. It appears more likely that neuronal, glial and endothelial progenitor cells supply factors that support adaptive remodeling of surviving neurons and neural networks [27,28]. Angiogenesis is prominent and enhances vascularization of the surviving penumbral area [29], in addition to stimulating neurogenesis. Gliosis is rapidly activated following stroke, and glial fibrillary acidic protein (GFAP) and vimentin expression expands over time over the entire area from the SVZ to the peri-infarct area [16]. However, reactive gliosis could have opposing effects on functional recovery following stroke. In the peri-infarct area beyond the infarct scar, glial cells participate in neuronal remodeling processes by releasing growth factors, and therefore provide lipids for myelination and glial–neuronal interactions [13,16]. At the same time, glial cells hamper axonal growth by secreting growth inhibitors [22,30].

**Enhancement of post-injury plasticity**

Evidently, spontaneous functional recovery after stroke develops through the following partially overlapping sequence of events that can be enhanced by external interventions: first, a phase during which tissue is cleaned of debris, cells are repaired and metabolism and neuronal function recovers; second, a phase of axonal growth, spine remodeling and spine activation; and third, a phase of establishing and consolidating new neural networks. The spontaneous process can be enhanced by various means, as discussed below.

**Enriched environment**

Functional recovery after experimental stroke is enhanced by experience driven re-learning and, to a limited extent, by physical therapy in humans. Giving injured animals an enriched environment (EE), for example, housing them in large cages, housing them with several other individuals together with toys and tools, or by training them on particular skills 2–5 days after stroke, is an extremely efficient way to stimulate functional recovery [4,31–33]. This effect of experience-driven functional recovery declines with time and is not effective if started 30 days after the stroke, and if initiated earlier than 2 days post-stroke it might even be detrimental. An EE stimulates physical activity and sensory experience, but most importantly it provides a social component [34]. An EE induces multiple biological effects in the brain that could account for the positive effect on recovery. It enhances dendritic arborization and spine density on the contralateral pyramidal neurons, supporting the notion that the contralateral hemisphere is particularly involved in the recovery process when stimulated by an enriched environment [4]. This enhanced plasticity is reflected in a recent complementary DNA (cDNA) array analysis of mRNA transcript levels in the contralateral cortex [35**]. Here, plasticity genes, such as synapsin II, metabolic genes and growth factor genes, in particular insulin-like growth factor-1 (IGF-1), were elevated. However, in the ipsilateral
peri-infarct area, more than half of the differentially regulated genes were downregulated. These included metabolic enzymes, and genes related to cell signaling and cell structure, which is indicative of a hypometabolic state. Although levels of neurotrophins are elevated by an EE, the importance of BDNF as a plasticity-inducing factor is equivocal [4,36].

An EE increases the number of neuronal stem cells and precursor cells in the subventricular zone and striatum, but not the number of mature neurons [37,38]. By contrast, astrocytogenesis is enhanced in the ipsilateral peri-infarct area, and the number of perineuronal NG2 positive polydendrocytes increases in both the ipsilateral and the contralateral hemispheres. Current data, therefore, suggest that the recovery-enhancing effect on sensorimotor function after stroke by an EE is not due to generation and recruitment of new neurons to the peri-infarct or remote cortical areas. Rather, the increased proliferation of glial cells and neuroblasts might enhance recovery by releasing regenerative factors [39] or by providing cellular constituents, in particular lipids, for axonal growth [16].

Brain activation
Earlier findings showed that D-amphetamine and L-DOPA enhanced recovery of function in experimental stroke models, whereas scopolamine had an ameliorative effect. These results implicated subcortical adrenergic and cholinergic neurotransmitter systems in the recovery process, and led to clinical trials that demonstrated limited efficacy [7,40]. Recently, lesions to the cholinergic system of the basal forebrain have been demonstrated to dramatically depress functional recovery after cortical injury in rats [41], suggesting that cortical map remodeling following stroke is indeed dependent on cholinergic input. Amphetamine is thought to stimulate those mechanisms that enhance brain activity and LTP, thereby stimulating axonal sprouting, possibly by decreasing the signal-to-noise ratio of relevant input in the affected areas. Stimulation of glutamatergic neurotransmission by modulators of the NMDA receptor might appear risky, but applied later than 1 day, this stimulation enhances functional recovery after brain trauma [42**]. Brain tissue can also be activated by physical means, thereby ‘conditioning’ the neurons for plasticity. For example, forced training of the paretic limb, in addition to direct or indirect electrical and transcranial magnetic stimulation, improves motor function when applied in the chronic phase of stroke [40].

Growth factors, axonal sprouting and cell genesis
When administered several days after the insult, growth factors promote functional recovery by stimulating neuronal plasticity or cell genesis. Some of these factors have even reached the stage at which they are being tested in clinical trials. Erythropoetin (EPO), which is neuroprotective when given hours after MCAO, has a recovery-enhancing effect when administered for 7 days starting 24 h after stroke [43]. EPO stimulates angiogenesis, neurogenesis and increases the levels of other growth factors (BDNF and vascular endothelial growth factor [VEGF]). Gene expression profiling after experimental stroke led to the identification of granulocyte-colony stimulating factor (G-CSF) as being an inducible gene in the brain [44**]. Recombinant G-CSF administration enhanced functional recovery, possibly by through stimulation of neural progenitor cells [44**,45]. Similar effects were observed following treatment with heparin-binding epidermal growth factor-like growth factor delivered by an adenoviral vector [46]. Furthermore, statins (HMGCoA reductase inhibitors) and phosphodiesterase-5 inhibitors, when administered one day after injury, enhance functional recovery, in addition to stimulating angiogenesis and synaptogenesis [47,48]. Likewise, a sigma-1 receptor agonist given to rats 2 days after stroke or brain trauma enhances functional recovery and increases axonal outgrowth [49]. Intrathecal administration of inosine enhances functional recovery after stroke, long distance sprouting of fibers and synaptogenesis [50].

Blocking growth inhibitors
Following injury, the growth cone extension is effectively inhibited by myelin-associated proteins, such as Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp), which activate the growth inhibitory Nogo-66 receptor, and therefore slow and prevent axonal sprouting [22]. This inhibition is overcome by antibodies directed towards the Nogo protein [51**], by peptides blocking the Nogo receptor or by genetic depletion of the Nogo or Nogo66 receptor [52**]. Intrathecal delivery of Nogo antibody for 1 month, starting as late as 7 days after ischemia, dramatically enhances functional recovery after stroke. This is associated with increased sprouting of cortico–rubral projections with a significant crossover of fibers [51**]. Likewise, fiber growth is stimulated after stroke in mutant mice lacking the Nogo-66 receptor and Nogo A/B, or in rats with the Nogo receptor blocked [52**].

Stem cells
To reconstitute a loss of human neocortex following stroke is a substantial challenge, taking into account the layered structure, the vast number of cell types and the complexity of the synaptic connectivity. Still, various stem cells or progenitor cells have been either transplanted directly into the damaged brain or introduced systemically. Some of these cells differentiate into brain cells of different phenotypes, and in some cases enhance functional recovery. Although the data are encouraging from a therapeutic perspective, there are still several unresolved issues [53,54]. Only a limited number of transplanted cells differentiate into neuronal phenotypes, and whether the new neurons contribute to any
significant degree to the restoration of function through their integration into networks is unclear. Also, the transdifferentiation of pluripotent precursors to brain cells is debatable, as transplanted cells appear to fuse with host cells [55]. Transplanted cells might instead release factors (trophic factors, extracellular matrix molecules and cytokines) [28,56,57] that enhance survival and plasticity of host cells. Optimizing the methods of cell growth [58] and survival [59] might promote the efficiency of the transplantation procedure. Indeed, human fetal neural stem cells and non-human primate embryonic stem cells integrate and differentiate in the peri-infarct area and the damaged striatum, respectively [58,60]. There are several caveats associated with cell transplantation, in particular the risk of tumorigenesis of homologous transplants [61]. Importantly however, stem cells can be engineered to become vehicles for the delivery of various recovery-promoting proteins to the injured brain [62].

The two faces of Janus
The location and sequence of post-stroke mechanisms are crucial in the recovery process and can offer different types of treatment opportunities. Cell death and recovery of function involve distinct mechanisms in different time windows that can serve as a basis for different types of treatment strategies after stroke. Yet, whereas some therapeutic agents are complementary in being both protective and pro-regenerative (FGF, EPO, G-CSF, sigma-1 receptor), others might have opposite effects in neuroprotection and regeneration (glutamate receptor blockade, GABA mimetics, anti-inflammatory agents). For example, glutamate receptor activation is detrimental during the early post-injury phase, but is crucial for functional recovery. Also, inhibitors of metalloproteinases are neuroprotective when given in the acute recovery phase after tMCAO, but prevent recovery of function and tissue remodeling when treatment is initiated several days after the insult [63].

Conclusions
The recovery of neurological function in patients who have suffered a stroke can be mimicked in experimental animal models, in which a repertoire of overlapping recovery-promoting processes have been identified (Figure 1). Concomitant with the resolution of tissue edema and cessation of inflammation during the first days of recovery, injured neurons are repaired. The synapses of surviving neurons in addition to silent synapses and pathways of areas remote from the infarct are activated. Early during recovery, new glial cells and neuroblasts are generated that migrate into peri-infarct areas or contralateral remote regions. During the following 2–3 weeks, neuroanatomical plasticity (axonal sprouting, dendritic and spine growth) is enhanced by the milieu created by surviving neurons and proliferating parenchymal cells. Also, some neuroblasts resume a neuronal phenotype. In addition, angiogenesis is stimulated, and contributes to the formation of new vessels in the peri-infarct areas. Finally, once novel neural networks are established, they are stabilized by an experience driven learning process.

Importantly, current research using sophisticated molecular tools has revealed new insights into the mechanisms of pro-regenerative and inhibitory processes after injury. This has provided novel prototype therapies that enhance recovery of function during the first month after stroke. Results suggest that activators of the noradrenergic, dopaminergic and cholinergic systems promote functional plasticity, that growth factors and attenuators of axonal growth inhibition boost neuroanatomical plasticity, and that cortical stimulation and physical therapy appear to enhance recovery during the chronic phase, and could be enhanced by the two earlier treatments as adjuvants. The near future will be an interesting arena for promising new therapies for such devastating conditions.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- - of outstanding interest


6 Signalling mechanisms


The authors found that applying lidocaine to the contralateral hemisphere 4 weeks after stroke (induced by endothelin-1 injection) abolished motor recovery in animals with large infarcts. In animals with small infarcts, a similar treatment only abolished recovery of motor function to a limited degree, which demonstrates the bicortical dependence of recovery of function. This demonstrates that following large infarcts recovery is dependent on the contralateral hemisphere, because the tissue that could participate in functional recovery on the ipsilateral side is damaged. When smaller infarcts are induced the ipsilateral residual tissue will function. This demonstrates the bicortical dependence of recovery of function. This demonstrates following large infarcts recovery is limited and migrate into the striatum for up to 4 months following the insult.


In a model of small permanent cortical infarct in the rat, the authors analyzed the expression patterns of 12 growth promoting genes and 10 growth-inhibitory genes using quantitative PCR in peri-infarct tissue at weeks 1, 4 and 8 days of recovery. During the first two weeks, growth-promoting genes are overexpressed, whereas later, chondroitin sulphate proteoglycans, which potentially inhibit axonal growth, are upregulated.


The M1 (primary motor cortex) and PMv (ventral premotor cortex) cortical motor areas in the monkey have reciprocal connections that regulate different aspects of motor movement. The authors show that when the PMv-M1 neuronal connections are destroyed, axonal growth is stimulated from PMv to region 1/2 of primary somatosensory cortex, a brain area normally devoid of innervation from PMv.


The authors demonstrate that following TMCAC0 neuroblasts are generated and migrate into the striatum for up to 4 months following the insult.


The authors study the effect of enriched environments on gene expression in the peri-infarct cortex and in the homotopic contralateral cortex at 2 weeks of recovery after a phototothrombotic ischemic lesion. In the peri-infarct cortex 28 genes were downregulated and 13 genes were upregulated, whereas in the contralateral cortex 43 were upregulated and 15 downregulated.


41. Conner JM, Chiba AA, Tuszynski MH: The basal forebrain cholinergic system is essential for cortical plasticity and...


The authors demonstrate that activating NMDA receptors at 24 h after brain injury enhances recovery of function, whereas NMDA receptor blockade is detrimental. This contrasts with the brain protective effect of NMDA receptor blockade when instituted immediately after brain injury.


The authors discovered that G-CSF was induced and promoted functional recovery following experimental stroke in the rat. They demonstrated a marked recovery effect when the growth factor was administered during the first three post-injury days.


This group earlier demonstrated that a Nogo A/B antibody promotes functional recovery following experimental stroke in the rat. Here, they demonstrate a marked recovery effect when the antibody was administered 7 days post-injury.


The authors demonstrate the involvement of the Nogo receptor in the inhibition of functional recovery after experimental stroke in mice and rats. Treatment with a Nogo receptor blocking peptide or knocking out the Nogo receptor enhances motor recovery in two stroke models.


