Brain Plasticity and Stroke Rehabilitation
The Willis Lecture
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Abstract—Neuronal connections and cortical maps are continuously remodeled by our experience. Knowledge of the potential capability of the brain to compensate for lesions is a prerequisite for optimal stroke rehabilitation strategies. Experimental focal cortical lesions induce changes in adjacent cortex and in the contralateral hemisphere. Neuroimaging studies in stroke patients indicate altered poststroke activation patterns, which suggest some functional reorganization. To what extent functional imaging data correspond to outcome data needs to be evaluated. Reorganization may be the principle process responsible for recovery of function after stroke, but what are the limits, and to what extent can postischemic intervention facilitate such changes?

Postoperative housing of animals in an enriched environment can significantly enhance functional outcome and can also interact with other interventions, including neocortical grafting. What role will neuronal progenitor cells play in future rehabilitation—stimulated in situ or as neural replacement? And what is the future for blocking neural growth inhibitory factors? Better knowledge of postischemic molecular and neurophysiological events, and close interaction between basic and applied research, will hopefully enable us to design rehabilitation strategies based on neurobiological principles in a not-too-distant future. (Stroke. 2000;31:223-230.)

Key Words: neuronal plasticity ■ recovery ■ rehabilitation ■ stroke

Since regeneration of transected central axons has never been convincingly demonstrated in higher mammals, it seems in most instances that one must resort to the assumption that intact fibers take over for the damaged ones.1

The above words were written in 1973 by Alf Brodal, a Norwegian neuroanatomist, based on his own experience after a stroke. To what extent has his assumption been shown to be correct? In this review I will present current concepts on brain plasticity in intact and lesioned brain, and evidence that postischemic interventions can alter molecular events and influence functional recovery after brain lesions.

Current Concepts on Brain Plasticity
That neuronal cortical connections can be remodeled by our experience was suggested by Hebb half a century ago.2,3 Since then, many studies have demonstrated chemical and anatomic plasticity in the cerebral cortex of adult animals.4–16 Animals reared or housed as adults in complex environments with access to various toys and activities develop more dendritic branching and more synapses per neuron and have higher gene expression for trophic factors than animals housed individually or in small groups in standard cages.4–12 Similar changes can be induced during learning.13–16

Another aspect of brain plasticity, first and most extensively demonstrated by Merzenich and coworkers, is that cortical representation areas, cortical maps, can be modified by sensory input, experience, and learning (Figure 1), as well as in response to brain lesions.17–30 The potential relevance for stroke rehabilitation of those data was proposed more than a decade ago.19 Transient alterations of cortical representation areas may be common in everyday life, as indicated by transcranial magnetic stimulation studies during learning tasks in human volunteers.31 If we regularly have to perform a very skilled motor task, the cortical representation for the muscles involved will remain enlarged, as seen for the fingers of the left but not the right hand in string players.32 Similarly, the sensorimotor cortical representation of the reading finger is expanded in blind Braille readers33 and, furthermore, fluctuates with the reading activity pattern.34

Possible Mechanisms Behind Brain Plasticity
Several mechanisms are likely to be involved in brain plasticity.35 Activity-dependent modification of synaptic connections and reorganization of adult cortical areas are thought to involve long-term potentiation (LTP) and long-term depression (LTD), mechanisms by which information is stored in the mammalian central nervous system.36–37 Synaptic plasticity in cortical horizontal connections has been proposed to underlie cortical map reorganization.38–40 Glutamate, the main excitatory neurotransmitter, plays a crucial role in these processes.
role. Cortical map reorganization in the primary somatosensory cortex can be prevented by blockade of N-methyl-D-aspartate (NMDA) receptors. \cite{41-43} \gamma-Aminobutyric acid (GABA)-A receptor antagonists can facilitate LTP induction in neocortical synaptic systems, and the induction can be blocked by GABA-A receptor agonists.\cite{40} Transmitters released by the diffuse neuromodulatory systems originating in locus coeruleus (noradrenalin), nucleus basalis (acetylcholine), lateral tegmentum (dopamine), and raphe nuclei (serotonin) may modify the process.\cite{44,45} Nitric oxide is another candidate for dynamic modulation of cerebral cortex synaptic function.\cite{46} There is evidence that mechanisms involved in synaptic plasticity vary between cortical regions.\cite{35,47} Local neurotrophin actions, transmitter release, and synaptic protein synthesis are thought to promote synaptic remodeling and changes in receptor expression or activation.\cite{12} As illustrated in Figure 2, dendritic spines, which receive the vast majority of excitatory synaptic contacts in the mammalian brain, are continuously being formed and modified.\cite{48}

There is increasing evidence that astrocytes take an active part in synaptic plasticity.\cite{49,50} Rapid astrocytic changes in cortex and ultrastructural evidence for increased contact between astrocytes and synapses in rats reared in a complex environment suggest a close relationship between astrocytic plasticity and experience-induced synaptic plasticity.\cite{51,52} Nonsynaptic transmission may also play a role in plasticity processes.\cite{53} Plastic changes occur not only in the cortex but have also been demonstrated in subcortical regions, including thalamus and brain stem.\cite{54,55}

**Spontaneous Events and Training Effects**

After a brain lesion, changes in other brain regions have been documented at different postlesion times, from minutes to months.\cite{54,55} Postlesion events may be due to deafferentation, removal of inhibition, activity-dependent synaptic changes, changes in membrane excitability, growth of new connections, or unmasking of preexisting connections.\cite{56} Unmasking has generally been proposed to be responsible for rapid changes in cortical maps,\cite{56} and there is evidence that synaptic plasticity can be very rapid.\cite{48}

Cortical mapping by intracellular recordings in primates has demonstrated that the tissue surrounding a small lesion in the hand-representation area of primary motor cortex in adult monkeys undergoes a further territorial loss in the functional representation of the affected body part, perhaps through nonuse or disruption of local intrinsic cortical circuitry.\cite{28} This further tissue loss could be prevented and functional reorganization in theundamaged surrounding motor cortex stimulated by retraining of hand use, starting 5 days after induction of the lesion.\cite{29} Similarly, reorganization of primary somatosensory cortex occurs in parallel with sensorimotor skill recovery in monkeys after restricted lesions. No significant changes were recorded in the hand representation area in somatosensory cortex in the opposite intact (untrained) hemisphere.\cite{30}
Morphologic studies in the rat indicate that cortical lesions can induce an increase of dendritic branching in the contralateral hemisphere, with a maximum 2 to 3 weeks after the lesion. If the rats were prevented from using the intact forelimb, both the morphologic changes and functional recovery were inhibited. Although some studies with other techniques could not verify those findings, a detailed electromicroscopic longitudinal study has confirmed the development of time-dependent morphologic changes with a significant increase in dendritic volume in cortical layer of the contralateral motor cortex 18 days after the lesion, and in the number of synapses per neuron 30 days after the lesion.

After a focal brain infarct, an increased density and distribution of GAP 43 immunoreactivity has been observed in the ipsilateral cortex 3 to 14 days after the vascular occlusion and of synaptophysin immunoreactivity in the same areas at postoperative days 14 to 60. A larger distribution of synaptophysin immunoreactivity was also noted in the contralateral hemisphere. An increased neuronal labeling of MAP-2, GAP-43, and cyclin D1 immunoreactivity from day 2 and up to 28 days has been found in the penumbra zone.

**Enriched Environment and Neurotrophic Factors**

There is substantial evidence that the postoperative environment can influence the outcome after experimental brain damage, such as traumatic brain lesions, hippocampal sectioning, and cortical ablation. After an experimental brain infarct, rats housed in an enriched environment with the opportunity for various activities and interaction with other rats perform significantly better than rats housed in standard laboratory environment, even when the transfer to an enriched environment was delayed for 15 days. A comparison between enriched environment, social interaction, and physical activity in the form of wheel-running indicated that social interaction was superior to wheel-running and that an enriched environment which allowed free physical activity combined with social interaction resulted in the best performance.

We hypothesized that the beneficial effect of enriched environment might be caused by increased synthesis of neurotrophic factors. Neurotrophic factors are polypeptides capable of promoting neuronal survival. Local neurotrophin action may promote synaptic remodeling and changes in receptor expression. Ischemia is a strong inducer of gene expression in the brain. More than 90 different genes have been shown to be acutely induced, generally with an early peak within minutes or hours of onset of ischemia and a rapid return to normal or subnormal levels. Whether the early transient increase in gene expression during the initial postischemic hours is related to outcome is not known. There is some evidence that trophic factors can rescue neurons in the acute stage. In addition to reducing infarct size even when given hours after the ischemic insult, basic fibroblast growth factor (bFGF) may attenuate the thalamic degeneration following cortical infarction. Nerve growth factor (NGF) has been reported to improve memory and motor functions and reduce dendritic atrophy in the remaining pyramidal neurons. As reviewed elsewhere, several other growth factors, including brain-derived growth factor (BDNF), insulin growth factor-1, transforming growth factor β1, and glial cell line–derived neurotrophic factor, have been reported to be beneficial in the early ischemic period. Whether some of these trophic factors can be beneficial in the rehabilitation phase has not yet been evaluated. Because of the poor penetration of many growth factors into the brain, an interesting approach is to use substances that induce endogenous growth factor synthesis in the brain after peripheral administration. A β2-adrenoceptor agonist as been shown to reduce infarct volume and induce an earlier and more pronounced increase in mRNA of nerve growth factor (NGF), bFGF, and transforming growth factor-β1 (TGF-β1) than was seen in untreated rats after permanent focal ischemia. Cholecystokinin-8 increases both NGF protein and NGF mRNA in mouse cortex and hippocampus when injected intraperitoneally at physiological doses and may thus represent a potential experimental model for investigating the effects of endogenous NGF upregulation after brain lesions.

Based on data on the role of BDNF for plasticity in the intact brain, we have studied the late posts ischemic gene expression for this protein in rats killed 2 to 30 days after the ischemic event. Moreover, intraventricular BDNF has been shown to rescue neurons in acute ischemia. Unexpectedly, a secondary increase in BDNF gene expression observed in control rats did not occur in rats housed in an enriched environment, who had significantly lower BDNF expression in ipsilateral and contralateral cortex than rats in a standard environment 2 to 12 days after the insult. Similar results were obtained for expression of NGFI-A mRNA, a gene that earlier had been shown to be activated in an enriched environment in intact rats. However, a late increase of NGFI-A mRNA expression was observed at 30 days after the lesion, which suggested that it might be important for later posts ischemic events.

The reason for the early dampening of the posts ischemic increase seen in control animals for BDNF is not clear. BDNF is related to synaptic activity. Cortical networks adjacent to a focal brain ischemia are hyperexcitable because of an imbalance between excitatory and inhibitory synaptic function. Hyperexcitability has been recorded not only around the infarct but also in the contralateral hemisphere measured 1 week after the ischemic event. Both a detrimental role (impaired processing of incoming information) and a beneficial role (adaptation and favorable recovery) of this hyperexcitability have been proposed. Could it be that rats in an enriched environment have a better balance between inhibitory and excitatory transmission and that depression of hyperexcitability might be beneficial in the early stage? Clearly, more studies are needed before any conclusions can be drawn. The relationship between growth inhibitory and growth promoter factors are likely to be important, and it should be noted also that growth inhibitory factor mRNA is increased during the posts ischemic period after focal ischemia in the rat. The recent reports on increased corticofugal plasticity after neutralization of a myelin-associated neurite growth inhibitor is a reminder that inhibitors of brain plasticity may be just as important as stimulating factors for posts ischemic functional outcome.
Pharmacological Interventions

The possible role of pharmacological interventions in the postischemic rehabilitation phase has been extensively reviewed by other authors.\textsuperscript{93–98} I will here restrict myself to a few comments. Considering the many complex events that occur in postischemic brain, it is likely that the efficacy of a drug can vary with the postischemic time, size and type of lesion, and interactions with other therapeutic interventions. In a recent review, Schallert and Hernandez\textsuperscript{27} write: “depending on the site, extent and nature of the injury and secondary degenerative events, and the timing of drug administration, GABA agonists may have either negative or positive effects.

In many research reports, rather bold suggestions have been offered about the potential clinical efficacy of GABAergic drugs without regards to these important variables”. Interactions between drugs and environmental factors is another aspect. If combined with test-specific training, norepinephrine, amphetamine, and other α-adrenergic stimulating drugs can enhance motor performance after unilateral ablation of sensorimotor cortex\textsuperscript{95,99} and have also been shown to enhance the immunoreactivity to synaptic proteins after focal brain ischemia.\textsuperscript{100} However, amphetamine had no additional effect on outcome in rats housed in an enriched environment after focal brain ischemia,\textsuperscript{101} perhaps due to enhanced noradrenergic release under such housing conditions.

What Is the Possible Role of Neurogenesis?

Neural stem cells, multipotent cells that are precursors to neurons and glia, have been identified in the adult vertebrate central nervous system. Although first reported to be present in brains from rodents in the sixties,\textsuperscript{102,103} it is only during the last years that they have been extensively studied.\textsuperscript{104,105} They are predominantly found in the periventricular ependymal or subependymal zone and in the dentate gyrus but may also be present in small numbers in other regions.\textsuperscript{106} Stem cells from the adult brain proliferate and differentiate into neurons and glia in tissue culture with the same efficiency for neuronal differentiation as found in fetal stem cells. That stem cells are present also in human brains was first shown in tissue culture from the subependymal zone and periventricular white matter.\textsuperscript{107} Recent studies have shown that they can differentiate to neurons in the adult human dentate gyrus in vivo.\textsuperscript{108} With the observation that such cells in experimental studies can be manipulated in vivo by growth factors and by environmental enrichment,\textsuperscript{109–111} the clinical potential of in vivo manipulation of stem cells in humans is currently subject to much speculation.\textsuperscript{104–106}

The aspects of neurogenesis that are relevant for this review are the following: (1) Does neurogenesis increase in response to brain lesions? (2) If so, is neurogenesis related to outcome? (3) Can those events be influenced by postlesion interventions? and (4) Can stem cells or progenitor cells be used for transplantation after stroke? The first question can be answered affirmatively. It has been shown to occur in excitotoxic and mechanical lesions in the dentate gyrus (hippocampus) in the adult rat\textsuperscript{112} and after transient global ischemia in gerbils.\textsuperscript{113} The second and third questions remain to be answered, and the fourth question will be discussed in the transplantation section below.

Transplantation

As reviewed elsewhere, implantation of fetal neocortical cells after cortical lesions has been performed successfully in several laboratories.\textsuperscript{76} Transplanted cells can interact with the host tissue by forming connections but also by being a source of trophic factors that can influence the surrounding tissue. Although both anatomic and functional integration with the host brain have been observed,\textsuperscript{114} improvement in behavioral tests have been noticed only when transplantation is combined with posttransplantation housing in an enriched environment.\textsuperscript{115,116} If performed 1 week after ligation of the middle cerebral artery, but not if delayed for 3 weeks, the combined procedure can improve functional outcome more than an enriched environment alone, and it can also reduce the secondary thalamic atrophy that occurs after cortical lesions. Although grafting can successfully be performed at later postischemic times, there is so far no evidence for functional improvement at later times.

Immortalized embryonic neuronal cells lines or cultured neural multipotent progenitor cells (the transition from stem cell to progenitor cell is not sharp) implanted into a lesioned brain have shown promising results in other experimental models. When implanted into the neocortex of adult mice undergoing targeted apoptosis of neocortical pyramidal neurons, they migrate long distances into the regions of cell death, where they differentiate and make appropriate long-distance projections.\textsuperscript{117–119} The results indicate the presence of environmental signals that promote differentiation of the implanted cells. Furthermore, adult mature astrocytes in the host brain have been shown to retain the capacity to transform into developmental radial glia that may help the active migration of transplanted neural precursors.\textsuperscript{120} Whether transplantation of neuronal precursor cells to neocortical infarct cavities would be a good substitute for the currently used fetal neocortical tissue block techniques remains to be demonstrated.

Clinical Evidence for Reorganization of Cortical Networks

Studies using PET, functional MRI (fMRI), transcranial stimulation, and magnetoencephalography (MEG) support the concept of functional reorganization after stroke.\textsuperscript{121–130} PET studies on blood flow distribution during finger movements in a previously paretic hand have demonstrated complex patterns of activation, with increased activity with large individual variations.\textsuperscript{121,122} Until now, studies comparing the degree and pattern of activation in patients with good and less-than-good recovery and with specific therapeutic interventions are few, and the published data are sometimes contradictory. Because of large individual variations, careful longitudinal studies of individual patients with specific deficits and well-defined lesions are needed. Individuals may use different compensation strategies before and after training,\textsuperscript{128} and the activation pattern can change with time. It has been reported that changes in activation pattern can be induced by forced training of the paretic hand even 4 to 15 years after stroke onset.\textsuperscript{131} The knowledge that the degree of cortical lateralization and interhemispheric interaction varies for specific language components in the normal human brain may be
Stroke Units and Early Training

It is well established that early mobilization can reduce secondary thromboembolic events, pneumonia, and mortality in acute stroke. It is now recommended that stroke patients be admitted to specialized stroke units with specially trained medical and nursing staff, coordinated multidisciplinary rehabilitation, and education programs for patients and their families. Stroke unit care has been shown to be associated with a long-term reduction of death and of the combined poor outcomes of death and dependency, effects that were independent of patient age, sex, or variations in stroke unit organization. No study has shown to what extent the beneficial effect is due to specific rehabilitation strategies, to the daily time spent in physiotherapy and occupational therapy, or is a nonspecific effect of a more stimulating environment with competent staff that can encourage and support the patients and family members. Scientific evidence demonstrating the values of specific rehabilitation interventions after stroke is limited. Comparisons between different methods in current use have so far failed to show that any particular physiotherapy or stroke rehabilitation strategy is superior to another. There is some evidence that forced use of a paretic arm may improve function in the chronic stage.

Clinical data are thus strongly in favor of early mobilization and training. On the other hand, there are some disturbing animal data indicating that overtraining of the lesioned forelimb induced by immobilization of the intact forelimb can expand cortical lesions. Referring to those studies, Nudo et al. started training monkeys 5 days after the lesion. Housing animals in an enriched environment with the opportunity to perform various activities but no specific training significantly improves functional outcome without increasing tissue loss. However, if combined with more specific training from 24 hours after the insult, an increased tissue loss was observed. Despite the larger tissue loss in the early training group, the rats improved more than standard rats, confirming earlier data of poor correlation between infarct volume and functional outcome in rats housed in an enriched environment. The better outcome in the early training group than in standard rats may be related to compensatory adaptation in the contralateral hemisphere, subcortical region, or cerebellum.

Even if the increased tissue loss did not correspond to unfavorable outcome, it is clearly an unwanted effect, one which might make the brain more vulnerable to additional insults or aging. What could be the course of this vulnerable early postischemic period? Motor activity stimulates the release of glutamate and catecholamines. One possible explanation for the increased tissue loss might be that hyperexcitability of the surrounding tissue in the early postischemic period makes the surrounding neurons vulnerable to excitation. As discussed above in the section on enriched environment and neurotrophic factors, cortical networks adjacent to a focal brain ischemia are hyperexcitable because of an imbalance between excitatory and inhibitory synaptic function with increased NMDA receptor-mediated excitation and decreased efficacy of GABAergic inhibition. In the presence of excitatory and toxic substances from the ischemic tissue, an additional release of excitatory substances induced by motor activity may be harmful in the early postischemic stage. Consistent with this hypothesis is the observation that the NMDA receptor antagonist MK-801 can prevent secondary cortical damage in rats forced to use their impaired limb after a lesion in the sensorimotor cortex.

It is important to define the window for a possible increased vulnerability and additional peri-infarct neuronal loss. However, I do not think these animal data should make us change the policy of early mobilization of stroke patients. Housing animals in an enriched environment, which may correspond to early mobilization, has no aggravating effects. The most important tasks in the early stage are to prevent complications and train the patient to regain balance and body symmetry. In addition, cortical and lacunar infarcts may differ.

Age and Plasticity

Age influences the impact of vascular occlusion in rodents but does it also influence functional recovery? In a comparison between 3- and 20-month-old rats, upregulation of MAP1B and MAP2 was diminished but not abolished in the aged compared with the young animals. It might not be fair to compare humans with rats living their entire lives in a laboratory with little stimulation. The decrease in synaptic density in aged laboratory rats can be prevented by rearing in an enriched environment. The loss of neurons in aging humans is to some extent compensated for by selective dendritic growth. A postmortem comparison of dendrites of layer II pyramidal neurons in the parahippocampal gyrus of adult (aged 51 years) and neurologically healthy aged (80 years) individuals showed that the dendritic trees were longer and more branched in the healthy 80-year-olds. In a population of persons aged 65 years and older, the level of cognitive function is positively related to the frequency and intensity of cognitive activity. However, it has been shown that a head injury in young adulthood, although well compensated for at the time, exacerbates cognitive decline in later years. This might be relevant for individuals with repeated stroke.

Concluding Remarks

The current trend to equate tissue loss with outcome is not relevant when it comes to postischemic interventions, which can improve outcome without a change in infarct volume. That environmental stimulation improves recovery is not a new observation but it is worthwhile to point out that it can enhance the effect of other therapies, as shown with neocortical grafting. It is not only the number of neurons left, but how they function and what connections they can make that will decide functional outcome.

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