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Editors

H. Naritomi

D.W. Krieger



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Clinical Recovery from CNS Damage

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Preface

Over the last 3 decades, we have become witnesses of various successful and not so successful attempts to minimize sequelae after brain injuries. All of these strategies had one thing in common, the belief that time is brain and salvage becomes impossible at a point of no return. Advances in supportive care, in particular neurocritical care, enhanced the functional outcome even with severe brain injury. For quite some time, recovery from brain injury has been extremely dynamic and individual. Although our understanding of brain recovery is still in its infancy, many eye-opening discoveries will potentially lead to a sea change of neurorehabilitation.

We have believed for many years that injury to the central nervous system is permanent and does not permit compensatory revival of neuronal systems. Recent breakthroughs in neuroscience, however, suggest that recovery from central nervous system injury arises through neuroregeneration and neuroplasticity. Neurorehabilitation is transforming into a thriving field of preclinical and clinical research focusing on understanding the mechanisms of neurological recovery and enhancing repair. Aided by computer science and biotechnology, brain-machine interfaces are being created that can replace lost function but may also one day allow to communicate with unconscious patients. Neurorehabilitation has become the new arena where neuropharmacology, biotechnology, molecular biology and computer science meet traditional approaches, such as physiotherapy, speech therapy, psychology and social services. Novel therapies will require controlled clinical trials. New agents and procedures, such as stem cells, neurotransplantation, electromagnetic stimulation, brain-computer hybrids and neuropharmaceuticals, are being put to test to transform traditional neurorehabilitation. This book intends to provide a current overview of the most promising areas of research prepared by clinicians and scientists entrenched in the field of neurorehabilitation. Each chapter intends to give a concise overview of the basic science underpinning and clinical consequences of the particular area in neurorehabilitation. We have selected the areas according to their importance from a clinical perspective. All authors were invited based on their personal experience in the field and were aided by associates where appropriate. The targeted readership includes neuroscientists,

rehabilitation specialists, geriatricians, neuroscience nurses, ergo-, speech and physiotherapists.

We feel very honored by the distinguished contributions of all authors and the fruitful collaboration with the publishers on this endeavor so close to our hearts.

Hiroaki Naritomi, Osaka
Derk W. Krieger, Copenhagen

Mechanisms of Functional Recovery after Stroke

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Abstract

Stroke is a leading cause of disability. After initial stabilization, neurologic recovery takes place even in the acute phase. Well-known recovery mechanisms from stroke deficits are improvement from diaschisis, or functional reorganization of the ipsilesional or contralesional cortex with involvement of uncrossed corticospinal tract fibers. The importance of coactivation of the perilesional or contralateral cortex is unknown; however, neuronal plasticity plays an important role in neurologic recovery. With the recent advancements in knowledge regarding underlying mechanisms of neuronal plasticity, various functional modulating methods have been developed and studied in humans. In this review, basic mechanisms of functional recovery and potential targets for future research will be discussed.

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The Impact of Stroke

Great strides have been made in clinical stroke research over the last decade. The therapeutic time window of intravenous recombinant tissue-type plasminogen activator has been extended to 4.5 h, and the new Solitaire flow restoration device achieves better recanalization in patients with large vessel intracranial occlusion [1, 2]. However, the majority of patients with ischemic stroke still do not benefit from these advancements because of the narrow therapeutic indications. In patients with intracerebral hemorrhage, treatment with aggressive blood pressure control and hemostatic agents using activated factor VII has failed to translate into improvement in functional outcome [3, 4]. Meanwhile, stroke still is the leading cause of disability worldwide [5].

The functional status of stroke patients spontaneously improves over 6 months after onset. More specifically, rapid recovery is achieved during the first month [6]. From the patient's perspective, rehabilitation is a process of regaining and relearning lost functions. Therefore, functional improvement, augmented by active reha-

bilitation, overlaps with motor learning in terms of underlying mechanisms [7]. Motor learning is associated with structural changes, such as axonal or dendritic growth along with new synapse formation and functional modulation including long-term potentiation or long-term depression, which may enhance or suppress synaptic activities. Together with the mechanisms above, cortical reorganization develops in the damaged brain, which plays an important role in recovery from acute stroke. Herein, we will elaborate on the functional recovery mechanisms after stroke.

Structural Bases of Functional Recovery

Neuroblast Migration

A myriad of evidence from animal experiments suggests that neurogenesis does occur after stroke. Neuroblasts usually originate from their source location in the brain, such as the subgranular zone in the dentate gyrus of the hippocampus and the subventricular zone. In rodent stroke models, neuroblasts divert from the rostral migratory system and move to the ischemic penumbra. These migrated neuroblasts may replace injured neurons or glial cells, and help with remodeling and reorganization processes [8]. This has long been considered a unique process in animals; however, recent evidence shows that neuronal migration occurs in adult human brains as well. Brain biopsy and autopsy studies in humans have shown that neurogenesis occurs after stroke [9]. However, it still remains to be elucidated whether the neurogenesis directly translates into clinical functional benefit in the human brain.

Angiogenesis

Neuronal death after vascular occlusion is a major underlying pathophysiology of ischemic brain injury. Newly formed blood vessels might help with augmenting nutrient supply and repair processes [10]. Simply, proangiogenic balance is associated with mild neurologic deficit and antiangiogenesis status predicts a worse long-term functional outcome in humans [11]. However, it is still elusive whether angiogenesis is a *since qua non* for neurologic recovery. Proangiogenic growth factors promote survival of the neuronal, glial and endothelial cells in the peri-infarct tissues, and transient neovascularization in the ischemic brain helps with the clearance of damaged tissues. Moreover, it may create a vascular niche for neuroblast migration [10]. Therefore, angiogenesis has multiple beneficial roles in the ischemic brain tissue rather than simple blood flow augmentation. Decreased angiogenesis is frequently seen in elderly and those with hypertension or diabetes mellitus, which is associated with poor functional recovery after stroke [10]. Taken together, angiogenesis may be necessary, but not sufficient for neurologic recovery. More studies are needed to verify its clinical utility in humans.

Axonal Sprouting and Regeneration

Axonal sprouting and regeneration also play a significant role in neurologic recovery. The major stimuli for this process are thought to be peripheral deafferentation. Axonal sprouting is mainly driven by the balance between a growth-promoting status and reduction of growth-inhibitory environment. Axonal sprouting may alter cortical sensory or motor maps, and robust evidence exists to show that new connections are formed in peri-infarct cortex areas [12]. Nogo-A protein is closely related with this process. It limits plasticity via inhibiting neurite outgrowth. Anti-Nogo-A antibody enhances functional recovery and promotes reorganization of the corticospinal tract with axonal plasticity [13]. Therefore, it is currently a hot topic for modulating regeneration.

Specific Issues in Intracerebral Hemorrhage

In intracerebral hemorrhage, extravasated blood forms a clot and generates thrombin which is a potent source for post-hemorrhage inflammation. However, recent animal research shows that thrombin might be important in the functional recovery process by stimulating neuroblasts, enhancing neurogenesis, promoting secretion of nerve growth factors, and affecting neurite outgrowth [8]. Thrombin also enhances angiogenesis and synaptic remodeling, and has a strong effect on brain plasticity. By contrast, Hirudin, a specific inhibitor of thrombin, decreases neurogenesis in a rat intracerebral hemorrhage model, suggesting the importance of thrombin in neurogenesis. Moreover, statin has a pleiotropic effect, and has strong beneficial effects on angiogenesis, neurogenesis and synaptogenesis in animal models. However, this should be re-evaluated in prospective clinical trials.

Functional Cortical Reorganization

Advanced functional imaging helps us understand the underlying mechanisms of functional recovery from a neurologic deficit. The suggested mechanisms of cortical functional reorganization are peri-infarct reorganization, recruitment of ipsilesional or contralesional cortex, changes in interhemispheric interactions, or bihemispheric connectivity [14]. Active rehabilitation treatment might improve the neurologic deficit mediated by one of the above mechanisms.

Diaschisis

Several functional imaging studies using SPECT or PET have demonstrated that functionally connected but structurally distant brain regions acted suboptimally after primary brain injury, which is called diaschisis [15]. After the acute phase, spontaneous neurologic recovery happens with the reversal of this type of functional impairment. Therefore, reversal of diaschisis is one of the mechanisms of spontaneous functional improvement. The most common form is crossed cerebellar diaschisis which occurs

in the contralateral cerebellum after hemispheric stroke, mediated by the descending glutamatergic crossed corticopontocerebellar pathway. In middle cerebral artery infarction, the degree of crossed cerebellar diaschisis is well correlated with the neurologic deficit early after stroke [16]. Moreover, functional inhibition may occur ipsilaterally to the subcortical lesion (thalamocortical diaschisis), which is regarded as an underlying mechanism of subcortical aphasia or neglect [17].

Cortical Reorganization

Perilesional Cortex

Experimental studies in nonhuman primates showed that the representative hand areas in the motor cortex started to shrink after lesioning, and the cortical areas representing elbow or shoulder expanded [18]. Even in humans, ipsilateral perilesional cortical activation including premotor or supplementary motor area is a common finding after primary motor cortex injury. The descending fibers from the premotor area are less dense and less excitatory, and project to the proximal part of the arm [5]. Therefore, there is a possibility that chronic ipsilateral premotor area activation sometimes competitively inhibits distal hand motor recovery. Studies from well-recovered stroke patients suggest that ipsilateral perilesional cortical activation is associated with functional recovery, at least in the acute period. Inhibition of those recruited areas using transcranial magnetic stimulation resulted in reappearance of previous neurologic deficit. Even in cases of aphasia, the major component of recovery is associated with perilesional tissue activation, which underscores the importance of the integrity of perilesional brain issues [9, 19].

Contralesional Cortex

In the recovery phase, the corresponding area in the contralateral cortex frequently shows coactivation. However, it is still debatable whether contralateral cortical activation is beneficial. In patients with aphasia, the contralateral nondominant hemisphere helps with neurologic recovery [20]. Studies from aphasic patients showed that cerebral blood flow was increased in the right inferior frontal lobe along with recovery. Other studies showed bihemispheric temporal and frontal engagement in auditory verbal processing during the recovery process. Meanwhile, a new balance in the cortical activation is needed in the chronic stage. Therefore, a decrease in the activation in the contralateral cortex is observed in patients with better functional recovery. Continuous coactivation of the mirror cortex represents maladaptive cortical mapping, which is related with nonoptimal functional recovery. The underlying mechanisms of change in contralateral cortical activation share similar physiologic changes such as unmasking of latent synapse, facilitation of alternating network, synaptic remodeling, and axonal sprouting [21].

Uncrossed Fibers from the Contralesional Hemisphere. A growing number of evidence supports that the contralesional (ipsilateral) motor cortex was activated after stroke [22]. Although the exact mechanism of coactivation of the contralesional mo-

tor cortex is still elusive, the disinhibition hypothesis is the most widely accepted [23]. With the development of hemispheric stroke, interhemispheric transcallosal inhibition is decreased from the affected side, which is translated into more activation of the contralesional motor cortex. The potential descending motor pathway from the contralesional hemisphere to the ipsilateral arm is via uncrossed ipsilateral descending corticospinal fibers, or noncorticospinal fibers, which is the corticoreticular projection, fibers passing through the red nucleus and pontine and olivary nucleus [24].

Generally, the neurologic outcome of the patients who recovered with ipsilateral (contralesional) motor cortex activation is worse than of those who recovered with perilesional reorganization [25]. Moreover, those patients experience mirror movements with recovery, which is attributed to the ipsilateral motor pathway [26]. The severity of mirror movements showed a reverse correlation with hand motor function. Therefore, abnormal involuntary mirror movement, or proximal-distal inter-joint coupling may have a detrimental effect on functional recovery. Even with these conflicting results, the ipsilateral descending pyramidal tract helps trunk muscle recovery, and is an important factor in motor recovery in children.

Recovery from Miscellaneous Stroke

In patients who recovered from unilateral cerebellar infarction, it seems that the cerebello-cortical loop on the opposite side might be important [27]. When recovering from thalamic infarction, a somatosensory gaiting process plays a significant role in sensory improvement [28].

Pharmacologic Options Targeting Functional Improvement

With the help of a sound understanding of the underlying mechanisms of the neurologic recovery and neural plasticity, pharmacological and nonpharmacological approaches to augment neurologic recovery were attempted.

Central Noradrenergic Stimulation

Amphetamine is a monoamine agonist which increases norepinephrine, dopamine, and serotonin levels in the brain. Animal experimental studies using rats and cats showed that administration of amphetamine concomitantly with motor practice accelerated recovery from cortical injuries. Although amphetamine is a potent psychomotor stimulator, this effect is thought to be independent of its psychostimulatory effect, which is mediated by dopamine. Several human randomized clinical trials were performed to identify the beneficial effect of amphetamine on neurologic recovery. Although several anecdotal reports support that it may help a 'speedy recovery' in small numbers of patients, it is still inconclusive whether amphetamines are beneficial for the quality of stroke recovery [29].

Serotoninergrics

Antidepressants may promote neuroplastic changes mediated by surges of the amount of synaptic monoamines. Based on this, a pivotal randomized controlled clinical trial was performed and the results were recently published [30]. Patients treated with fluoxetine and physiotherapy showed better distal motor power improvement and less dependency at 3 months, compared with those with physiotherapy alone. Although the precise underlying mechanisms are unknown, fluoxetine seems to be effective via modulating brain plasticity. With the positive results, it is still unclear whether other selective serotonin reuptake inhibitors have a similar effect on neurologic recovery, or whether the routine use of fluoxetine is justifiable in patients without post-stroke depression. More studies are needed.

Dopaminergics

A randomized single-blind crossover trial was done before using levodopa administration in the chronic stage of stroke patients. Although the treated dose was low (100 mg per day), the treatment group showed better motor performance at 5 weeks after treatment, and better cortical excitability measured by repetitive transcranial magnetic stimulation [31]. This study was based on a small number of patients; therefore, it needs to be verified in a larger study.

Nonpharmacologic Therapeutic Options

Noninvasive Cortical Stimulation

Repetitive transcranial magnetic stimulation or transcranial direct current stimulation are noninvasive cortical stimulation methods to modulate cortical excitability in humans [32]. These noninvasive cortical stimulation techniques administered alone or in combination with various methods of neurorehabilitation were reported to be safe in the short term. However, more studies are needed to verify their long-term effect on motor recovery.

Constraint-Induced Movement Therapy

After severe motor stroke, patients may preferentially use the nonaffected limbs. This pattern of movement activates the contralesional hemisphere which may inhibit the damaged hemisphere via interhemispheric transcallosal inhibition. Constraint-induced movement therapy consists of forced use of the paretic arm aiming to decrease transcallosal inhibition in the affected hemisphere. Reduced unwanted inhibition improves the latent pathway and helps motor recovery via unmasking of the latent pathway. With constraint-induced movement therapy, expansion of ipsilesional motor maps with concomitant decreases in contralesional motor cortex activation was observed, strongly correlating with motor gains [33].

Here, we briefly reviewed the basic neurologic recovery mechanisms after stroke. Modern functional imaging helped with the understanding of basic mechanisms underlying functional improvement; however, more studies are needed to better understand the optimal mechanism in individual patients.

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Diagnostic Approach to Functional Recovery: Functional Magnetic Resonance Imaging after Stroke

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Abstract

Stroke remains the most frequent cause of handicap in adult life and according to the WHO the second cause of death in the Western world. In the peracute phase, intravenous thrombolysis and in some cases endovascular therapy may induce early revascularization and hereby improve prognosis. However, only up to 20–25% of patients are eligible to causal treatment. Further, care in a specialized stroke unit improves prognosis in all patients independent of age and stroke severity. Even when it is not possible to prevent tissue loss, the surviving brain areas of functional brain networks have a substantial capacity to reorganize after a focal ischemic (or hemorrhagic) brain lesion. This functional reorganization contributes to functional recovery after stroke. Functional magnetic resonance imaging (fMRI) provides a valuable tool to capture the spatial and temporal activity changes in response to an acute ischemic lesion. Task-related as well as resting-state fMRI have been successfully applied to elucidate post-stroke remodeling of functional brain networks. This includes regional changes in neuronal activation as well as distributed changes in functional brain connectivity. Since fMRI is readily available and does not pose any adverse effects, repeated fMRI measurements provide unprecedented possibilities to prospectively assess the time course of reorganization in functional neural networks after stroke and relate the temporospatial dynamics of reorganization at the systems level to functional recovery. Here we review the current status and future perspectives of fMRI as a means of studying functional brain reorganization after stroke. We summarize (a) how fMRI has advanced our knowledge regarding the recovery mechanisms after stroke, and (b) how fMRI has been applied to document the effects of therapeutical interventions on post-stroke functional reorganization.

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Background

Stroke and other cerebrovascular diseases remain the world's second leading cause of death [1] and stroke is the leading cause for acquired disability in adults, including hemiparesis, dysphasia, neglect or other focal neurological deficits. Recent advances

in neuroimaging enable rapid and precise diagnosis and new treatment options have become available for patients with acute ischemic stroke, if diagnosis is made within the first hours after the onset of ischemia [2, 3]. However, the majority of patients have either only limited effect or are uneligible for revascularization therapy and long-term rehabilitation remains the most important treatment option. In patients with acute stroke, it is difficult to predict functional recovery and the long-term functional outcome varies from patient to patient [4]. A detailed assessment of lesion location and size with structural magnetic resonance imaging (MRI) is often of limited value in terms of explaining or predicting interindividual differences in long-term recovery because structural MRI provides only little information regarding the potential of the nondamaged brain regions to promote recovery of function [5–7].

Here functional MRI (fMRI) comes into the picture because the distributed neural activity of functional brain networks can be readily studied with fMRI at rest and while patients perform a specific task [8]. In healthy individuals, fMRI has proven to be a valuable tool to study functional brain reorganization due to learning and long-term practice [9, 10] or associated with brain maturation during childhood and adolescence [11] or healthy aging [12]. In a wide range of diseases, fMRI has been extensively used to study how a given brain disease changes the functional neuro-architecture at the systems level [13–15]. In the last 10 years, cross-sectional as well as longitudinal fMRI studies after stroke have provided important insights into changes of the brain in recovery after stroke.

In this chapter, we review the application of fMRI to study the reorganization of functional brain networks after stroke.

What Is Functional Magnetic Resonance Imaging?

When stroke patients undergo fMRI, we measure local changes in regional neural activity using the blood-oxygenation-level-dependent (BOLD) signal [16]. A regional increase in neural activity triggers an increase in local blood perfusion. Under normal physiological conditions, regional oxygen supply increases as a consequence of increasing perfusion, exceeding the local activity-dependent increase in oxygen consumption. Accordingly, an increase in regional neural activity leads to a rise in the local oxyhemoglobin concentration and a decrease in the local concentration of deoxyhemoglobin. The activity-driven reduction of paramagnetic deoxyhemoglobin causes the regional increase in the BOLD signal. Hence, the BOLD signal provides an endogenous contrast which is sensitive to regional changes in neural activity, yet it needs to be borne in mind that the BOLD signal is an indirect (vascular) measure of neural activity which relies on neurovascular coupling [16, 17]. This explains why fMRI can identify functional brain networks, which show a temporally correlated BOLD signal increase in response to a stimulus or in relation to an experimental task [18, 19].

How Can Functional Magnetic Resonance Imaging Be Used to Assess Brain Function after Stroke?

A given brain function is maintained by the functional integration of neural processing among specialized brain regions. Stroke causes a focal brain lesion, which involves one or more specialized brain regions and their interaction with the remaining nodes of the functional network. In other words, the post-stroke brain is characterized by an altered functional network architecture, one which is less effective as opposed to the intact brain, but which will use its remaining processing capacities to maintain as much as possible functional integrity. The altered neural processing within post-stroke brain networks can be studied with BOLD fMRI which can reveal altered levels of regional brain activation within the network as well as changes in the functional interactions between the remaining network nodes.

In stroke patients, fMRI can either be performed while patients are 'at rest' (i.e., resting-state fMRI) or while patients are exposed to sensory stimuli (i.e., stimulus-related fMRI) or perform a well-defined task in response to a sensory stimulus (i.e., task-related fMRI). These fMRI techniques have been successfully applied in post-stroke patients to assess functional remodeling of brain networks as reflected by regional changes in neuronal activation and distributed changes in functional brain connectivity. Stimulus-related, task-related and resting-state fMRI capture different aspects of functional reorganization and should be considered as complementary techniques with specific strengths and weaknesses. For resting-state and stimulus-related fMRI, it is not necessary that patients can perform a specific task. This has the advantage that these fMRI examinations are feasible even in severely affected stroke patients and can be used to study spontaneous fluctuations in regional BOLD levels (i.e., resting-state fMRI) or changes in regional BOLD signal driven by 'passive' sensory stimulation (i.e., stimulus-related fMRI).

Resting-state fMRI can be used to study alterations in functional brain connectivity after stroke because the low-frequency (<0.1 Hz) BOLD signal fluctuations at rest are temporally correlated in functional brain networks [8, 20]. The resting-state BOLD signal correlations are sensitive to head movements [21]. Moreover, comprehensive filtering should be applied because physiological noise from cardiac and respiratory cycles causes BOLD signal changes resembling those observed in resting-state fMRI [22]. A resting-state fMRI time series can reveal functional connectivity of several functional brain networks, including the so-called default mode network and the motor network [8]. Studies on healthy resting subjects have shown that brain networks which display correlated resting-state activity strongly overlap with the topography of brain networks as identified by task-related fMRI [8].

In contrast, task-related fMRI offers the possibility to identify changes in the task-specific activation pattern after stroke and to examine how task-specific activation patterns dynamically change during the course of recovery. Task-related fMRI studies offer better possibilities to directly relate specific activity or connec-

tivity changes in the relevant brain networks to the degree of functional impairment and to recovery of a specific brain function such as hand paresis, aphasia or neglect. In summary, resting-state, stimulus-related and task-related fMRI measure different aspects of functional integration and therefore, should be used as complementary approaches when assessing functional brain reorganization after stroke.

The above-mentioned fMRI approaches can be combined with an intervention. For instance, focal transcranial brain stimulation might be combined with fMRI to experimentally manipulate the function of one or more of the nonaffected cortical areas [23, 24]. This combined brain stimulation-fMRI approach is particularly interesting if one wishes to test the functional relevance of a specific cortical area for recovery of a specific brain function. Another interventional approach is to map distributed changes in the BOLD signal in response to an acute pharmacological intervention compared to placebo. Pharmacological fMRI might be useful to examine how the pharmacological manipulation of a specific neurotransmitter or ion channel alters the functional integration within brain networks and hereby promotes recovery of function [25].

Feasibility of Functional Magnetic Resonance Imaging in a Clinical Post-Stroke Setting

Stroke patients frequently undergo MRI as part of their diagnostic workup. fMRI carries the same contraindications as conventional MRI scans, that is metal implants, claustrophobia etc. Artifacts induced by head movements remain a limiting problem in the acute phase [26]. Here prospective motion correction of head movements using data from optical tracking systems might significantly help to reduce motion artifacts in future studies [27].

As pointed out above, resting-state fMRI is suited for patients with any neurological deficit of any severity as there is no task to perform. The only practical limitation might be related to spontaneous body movements during the resting-state fMRI session. The estimation of resting-state functional connectivity becomes more reliable the more time points (i.e., brain volumes) are acquired during a single resting state, because resting-state connectivity describes the temporal correlation of spontaneous BOLD signal fluctuations within functional brain networks. Van Dijk et al. [28] reported that a scanning session of 5 min is sufficient to acquire reliable resting-state fMRI data with a TR of 2.5 s and a spatial resolution of 2–3 mm. Usually, a resting-state fMRI session lasts between 5 and 10 min which allows resting-state fMRI to be incorporated into existing clinical MRI protocols for stroke.

Stimulus-related fMRI is also relatively easy to establish in a clinical setting and might be used even in patients with a severe deficit. For instance, an auditory language comprehension paradigm with alternating periods during which speech or

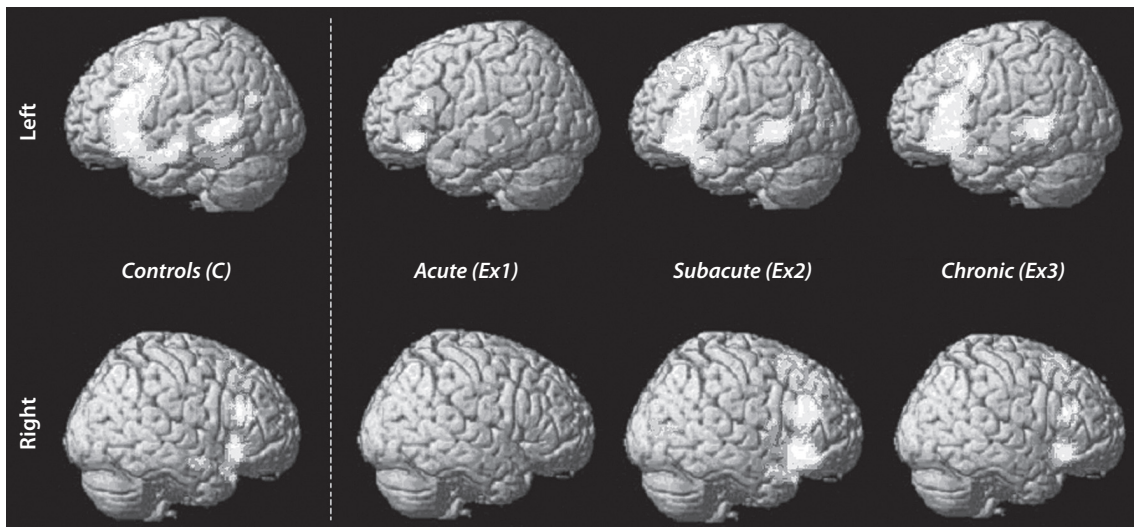


Fig. 1. Temporofrontal language network activation measured in a task-based language paradigm in healthy controls and patients with aphasia after stroke in acute, subacute and chronic phases after stroke. Group analyses of 14 controls and 14 patients; marked areas are voxels significant at $p < 0.05$ corrected for multiple comparisons. Results are surface-rendered onto a canonical brain with the left side in the upper row and the right side in the lower row. From Saur et al. [29], with permission.

reversed speech is presented via headphones can be applied in patients with acute aphasia [29]. For task-related fMRI studies, selection of the experimental task is constrained to tasks the patient is able to perform [30, 31]. Usually, the experimental task should be as simple as possible, but still specifically activate the neural networks of interest (e.g. the language system in dysphasia or the motor system in motor stroke). If a task is used that is too difficult for the patients, task-related fMRI will inevitably reveal an alteration of task-related brain activity in stroke patients relative to healthy controls, but it will be impossible to interpret the functional significance of the change in brain activity. It is advisable to match task performance in terms of effort. For instance, in patients with hand paresis caused by motor stroke, one might use a grip force task in which patients have to produce a force level relative to their individual maximum grip force rather than a fixed grip force level that is identical for all subjects [32]. Task-based fMRI paradigms may consist of interchanging periods of task performance and rest (i.e., blocked design) or intermingled trials (i.e., event-related design). Blocked fMRI designs are usually preferred as they reveal more robust task-related activations. Regardless of which task patients perform during fMRI, task performance should be monitored as closely as possible. Measures of task performance should be obtained and used as external variables to inform the fMRI data analysis.

Depending on the complexity of the fMRI design, stimulus- and task-related fMRI sessions usually last between 5 and 15 min. Once the equipment for stimulus presentation and performance monitoring is established in the fMRI environment, stimulus- and task-related fMRI studies can be performed in a clinical setting, even shortly after stroke onset [29, 32]. However, task-related fMRI is logistically demanding, especially in the acute stage after stroke, because patients need to be familiarized with the task before scanning and task-related fMRI requires a nonroutine setup (i.e., stimulus presentation and synchronization with fMRI data acquisition as well as task performance monitoring). Figure 1 illustrates changes in activation pattern in a longitudinal task-based fMRI study of patients with aphasia after stroke compared with healthy controls.

Network Reorganization after Stroke

Early fMRI studies in stroke have focused on changes in regional brain activation rather than assessing the functional interaction between the activated brain regions. In recent years, fMRI has been successfully applied in analyses of functional and effective connectivity on fMRI data acquired in post-stroke patients to investigate how the focal brain lesion caused by stroke alters the interaction between the nonaffected areas of a functional network and how changes in connectivity relate to functional impairment and recovery [33].

Interactions between functionally specialized areas can be described in terms of functional or effective connectivity [34, 35]. Studies of patterns of ‘functional connectivity’ are based on coherence or correlation of signal changes among cortical regions and thus, merely reflect statistical dependencies among brain regions. It should be noted that functional connectivity neither makes any explicit reference to specific directional effects or causal interactions between brain areas nor refers to an underlying structural network model. Functional brain connectivity can be estimated in a variety of ways, for example through computing cross-correlations in the time domain or mutual information [35].

‘Effective connectivity’ describes causal interactions among distinct neural nodes. In contrast to functional connectivity, effective connectivity specifies directional effects of one neural element of a brain network over another. Functional connectivity patterns are often extracted from fMRI time series that have been acquired in a (task-free) resting state, whereas effective connectivity is usually inferred from task-based fMRI time series [36]. It should be mentioned that BOLD fMRI is not the only method with which one can study functional and effective connectivity. Other neuroimaging modalities such as electroencephalography and magneto-encephalography can also be used to analyze functional or effective connectivity patterns in the human brain. The techniques used for extracting effective connectivity patterns from a BOLD fMRI time series are either based on a prespecified anatomical model

(e.g. dynamic causal modeling) or largely model free (e.g. Granger causality [37]). Especially dynamic causal modeling has been successfully applied to fMRI data in subcortical motor stroke to reveal impaired integration within the motor network [38].

Assessment of Brain Reorganization after Stroke

As pointed out above, fMRI studies in stroke patients can broadly be divided into task-related and resting-state fMRI studies and the focus of interest can be on the distribution of activation within a network ('activation pattern') or on changes in functional integration ('connectivity pattern'). Due to space restrictions, we only review key fMRI studies of stroke-induced reorganization in the motor system. However, we wish to stress that fMRI has been successfully used to study functional reorganization in post-stroke patients presenting with other neurological deficits such as spatial neglect or dysphasia [29, 39–41].

Cross-Sectional Functional Magnetic Resonance Imaging Studies in Motor Stroke

fMRI studies on motor stroke have focused on recovery of motor hand function by mapping task-related brain activation during whole hand grasp or finger movements. Since most studies were restricted to patients with subcortical lesions, little is known about motor reorganization following cortical or corticosubcortical stroke. This complicates the comparison with animal studies, which have almost exclusively examined motor reorganization triggered by cortical stroke lesions [42, 43].

After subcortical motor stroke, patients commonly show overactivations in secondary motor areas, including the dorsal premotor cortex (PMd), ventral premotor cortex, supplementary motor area (SMA) and cingulate motor area in the affected and unaffected hemisphere as well as the contralesional primary motor cortex. As a rule of thumb, activation of secondary cortical motor areas is more pronounced in patients with poorer outcome, suggesting stronger recruitment in those patients 'with greatest need' [44]. Several studies associate persistent overactivation negatively with function and recovery, while refocusing and normalization of activation patterns point towards new network organization akin to the network before stroke and correlate with better outcome [23, 45–47]. Yet other studies showed a persistence of movement related overactivity, even after nearly full functional recovery [48–50]. This divergence may be explained through differences in the degree of impairment, time after stroke and the imaging task.

Using the coordinate-based activation likelihood estimation (ALE) method, a recent meta-analysis of 36 task-related neuroimaging studies specifically addressed the question which motor activation patterns are consistent across studies [51]. Increased activation in contralesional M1 and bilateral premotor areas was a highly consistent finding after motor stroke despite considerable differences among studies in terms of

fMRI tasks and motor impairment levels. With respect to motor outcome, the recruitment of the original functional network rather than on contralesional activity was associated with good motor recovery. However, Ward et al. [52] showed that the ipsilateral PMd showed a linear increase in task-related activation as a function of hand-grip force in chronic stroke patients with significant impairment, but not in chronic stroke patients with good outcome or in healthy controls. This observation suggests that activity in ipsilateral motor cortical areas such as PMd might also be functionally relevant in patients with poor outcome.

Another important question which can be addressed with fMRI is how a focal stroke lesion affects the functional integration among brain regions forming a functional network. In patients with subcortical motor stroke, a task-related fMRI study employed dynamic causal modeling to demonstrate a reduction in intrinsic connectivity between ipsilesional SMA and ipsilesional primary motor hand area very early after stroke (less than 72 h after symptom onset) [53]. The reduction in positive coupling between ipsilesional SMA and primary motor hand area was found during finger movements with the affected and unaffected hand and correlated with individual motor impairment [53]. While these data point to impaired information flow between ipsilesional brain regions, other fMRI studies suggest that changes in interhemispheric connectivity between homologous motor regions might be associated with poor recovery. In patients with subcortical motor stroke, the primary motor cortices express an abnormal pattern of interhemispheric effective connectivity with the contralesional motor cortex exerting an abnormal inhibitory drive towards the ipsilesional M1 during movements with the affected hand [53]. Likewise, a recent resting-state fMRI study showed that a loss in homologous interhemispheric functional connectivity in the somatomotor network predicted individual impairment of upper extremity function in 23 patients with acute stroke [54]. Finally, it should be noted that interleaving transcranial magnetic stimulation (TMS) with fMRI could be used to test changes in effective connectivity in specific cortical connections by targeting a specific cortical area with focal TMS [55]. This possibility was exploited in a recent concurrent TMS-fMRI study [23], in which short bursts of TMS were applied to the contralesional PMd during fMRI. Interleaved TMS-fMRI revealed that the contralesional PMd had a stronger influence on the ipsilesional sensorimotor cortex when patients moved the affected hand in patients with greater clinical impairment [23].

It needs to be borne in mind that the results obtained with fMRI are correlative in nature. While fMRI can be used to demonstrate a task-related overactivation of a cortical area or a change in corticocortical connectivity, this does not imply that these changes are functionally relevant. In contrast to fMRI, focal TMS is an interventional method that can transiently interfere with ongoing neuronal activity in the stimulated brain area. The 'interventional nature' of TMS opens up unique possibilities to probe the functional relevance of a change in regional activity as revealed by fMRI [55]. For instance, the observation that the PMd is overactive during motor

tasks in patients with motor stroke does not prove that this constitutes a functionally mechanism for recovery. However, this was demonstrated by disrupting neural processing in the PMd with focal TMS: focal TMS given to ipsilesional [56] or contralesional PMd [57] affected the performance of a simple motor task in patients with chronic stroke but not in healthy controls. The disruptive effect of TMS to contralesional PMd was found to be stronger in patients who showed greater motor impairment [57].

Longitudinal Functional Magnetic Resonance Imaging Studies in Motor Stroke

Cross-sectional fMRI studies provide a snapshot of the change in functional neuroarchitecture at a given time point after stroke, but they are not suited to clarify how functional reorganization dynamically evolves after stroke. Using a grip force task, a longitudinal fMRI study of motor recovery after subcortical stroke found an initial overactivation in many primary and secondary motor regions when patients performed manual motor tasks with their affected hand [32]. This was followed by a gradual focusing of movement-related activation, in a way that is typical for motor skill learning in healthy individuals. The degree to which the activity pattern shrunk towards a normal (minimized) activity pattern correlated with long-term motor recovery. Poor recovery was associated with a persistence of task-related overactivation, whereas the activation pattern normalized in patients with good recovery. Another longitudinal fMRI study also found dynamic changes in task-related activation during the first 2 weeks after subcortical motor stroke, which depended upon the degree of initial motor impairment. In that study, bilateral increases of activity in the primary motor cortex, lateral premotor cortex, and SMA correlated with short-term motor recovery [47].

With respect to impaired connectivity, a longitudinal fMRI study used dynamic causal modeling to show reduced positive coupling of ipsilesional SMA and lateral premotor cortex with the ipsilesional primary motor hand area in the acute stage (≤ 72 h [58]). This ipsilesional premotor-to-motor coupling increased over time and the increase was associated with better recovery. The same study also found dynamic changes in interhemispheric effective connectivity. In the acute stage, the negative coupling strength from ipsilesional motor areas to the contralesional primary motor hand region was attenuated. The subacute stage was characterized by a positive influence of the contralesional primary motor cortex on ipsilesional primary motor cortex. The negative coupling between ipsilesional areas and the contralesional primary motor hand area M1 normalized over time. Interestingly, poor recovery in the chronic stage was associated with enhanced negative coupling from the contralesional to ipsilesional primary motor cortex.

Repeated resting-state fMRI measurements also revealed dynamic changes in functional connectivity in the motor network after stroke [59]. Patients with subcortical motor stroke underwent 5 resting-state fMRI measurements in the first year after stroke. A functional connectivity matrix among 21 motor brain regions was

constructed and analyzed using graph-theoretical approaches. Overall, the topology of the motor execution network gradually became more random over time, indicating a less efficient network topology. The ipsilesional primary motor area and contralateral cerebellum showed increased regional centralities within the network, whereas the ipsilesional cerebellum showed decreased regional centrality over time. These topological connectivity measures correlated with different clinical outcome measures.

Together, the fMRI studies on patients with motor stroke consistently show that a focal stroke lesion typically affects neural integration of the entire motor system, and clearly emphasize the relevance of a network-based neuroimaging approach to understand functional brain reorganization after stroke [60]. Yet the reported findings are partially conflicting and the functional relevance of specific connectivity changes for motor recovery, for instance the relevance of interhemispheric versus (ipsilesional) intrahemispheric connectivity changes, remains to be clarified.

Predicting Recovery Based on Early Functional Magnetic Resonance Imaging

In recent years, several groups have started to address the question whether fMRI can contribute to predict recovery of a focal neurological impairment in a single patient. In patients with acute ischemic stroke, there are well-established clinical variables such as age and NIHSS score within 6 h of symptom onset that help to predict 3 months' survival and independence (Barthel index ≥ 95) [61]. However, these clinical variables are too nonspecific to enable prediction of recovery with respect to specific deficits such as upper limb or language improvement. Accurate prediction of upper limb or language recovery might inform rehabilitation planning and assist clinicians and patients in realistic goal setting. This has prompted some researchers to measure brain activation with fMRI in the first few days after stroke in order to test whether the fMRI data contain some information that predicts subsequent improvement in manual motor function [62, 63] or language abilities [64].

Marshall et al. [62] studied 23 patients with fMRI within the first days after acute stroke. During fMRI, patients performed a simple repetitive hand closure task in synchrony with a 1-Hz metronome click alternating with rest. A multivariate analysis yielded a correlation between brain activation and change in Fugl-Meyer score over the next 3 months as indicator of motor recovery. Additionally, voxel-based univariate statistical analysis revealed 2 small clusters in the ipsilesional postcentral gyrus and cingulate cortex where initial task-related activation correlated with subsequent recovery. Using the same motor task, the same group subsequently reported that the distributed fMRI activation pattern in combination with the initial Fugl-Meyer score improved the prediction of upper limb recovery in patients with severe initial upper limb paresis (predictive explanation: 47%) as opposed to the Fugl-Meyer score alone (predictive explanation: 16%) [63]. However, this improvement in outcome predic-

tion did not reach significance. In patients with mild initial paresis, the clinical predictive variable (i.e., initial Fugl-Meyer score) already predicted motor recovery with a very high accuracy (predictive explanation: 96%) [63]. It should be mentioned that other mapping techniques such as TMS and diffusion tensor imaging might also help prediction of upper limb recovery [65]. Therefore, the additional value of task-related fMRI needs to be determined.

Saur et al. [64] applied a multivariate machine learning approach (i.e., support vector machine) in 21 stroke patients with moderate or severe aphasia to show that language fMRI data obtained early after stroke contain substantial predictive information about subsequent recovery of language function. In that study, fMRI was acquired during an auditory comprehension paradigm 2 weeks after stroke. Outcome after 6 months was either classified as good or bad. In addition to the fMRI activation pattern, age and initial language deficit were included in the predictive model. Of note, the classification algorithm allowed for the possibility that within a given voxel, the same outcome could be coded by either an increase or decrease in activity during the language comprehension task. A bad outcome could be coded by both high and low activation, while medium activation would then predict a good outcome or vice versa. The multivariate machine learning approach correctly separated patients with good and bad language performance 6 months after stroke in 3/4 of patients when classification was only based on the fMRI activation pattern. Classification accuracy further improved to 86% when age and the initial language impairment were included for classification. A comparable accuracy was reached for the relative language improvement when fMRI data were restricted to a region of interest in the right frontal gyrus.

Together, these initial studies support the idea that the task-related fMRI activation pattern performed early after stroke might be used to predict the recovery of specific brain functions. The same may hold true for fMRI-based connectivity patterns, both at rest or during a specific task. For instance, interhemispheric functional connectivity as revealed by resting-state MRI in combination with clinical variables may constitute a useful predictive marker for recovery [60].

Mapping Treatment-Induced Functional Reorganization

Many prospective fMRI studies have shown that therapeutic interventions can induce significant changes in task-related activation and connectivity [33, 66]. Even short-term interventions such as a single session of TMS [24, 67] or a single pharmacological challenge [25] can cause consistent shifts in brain activation and connectivity. Other fMRI studies have reported changes in task-related activation or connectivity after long-term training of motor or language functions that were correlated with training-induced improvement [57, 66, 68]. For instance, James et al. [68] used resting-state fMRI to investigate the impact of 3 weeks of intensive upper limb rehabilita-

tion therapy on interhemispheric connectivity between the ipsi- and contralesional PMd. Structural equation modeling of the fMRI data yielded a stronger influence of ipsilesional PMd on its contralesional homologue after therapy.

In summary, the majority of studies showed that remodeling of cortical functions is possible even years after stroke, including homologous ipsilesional and contralesional regions. Usually, therapy-induced reorganization occurred within the pre-existing functional brain network rather than recruiting new brain regions that belong to other functional brain networks.

Methodological Considerations

It is recommended to screen for macrovascular abnormalities in the arteries supplying the brain. The presence of uni- or bilateral stenosis or occlusion in the major intra- or extracranial arteries supplying the brain may hamper downstream perfusion in specific vascular territories and alter the temporal dynamics of the BOLD response in specific vascular territories. This might be of relevance when assessing stimulus-induced or task-related changes in regional brain activity or connectivity.

Another phenomenon which needs to be considered when performing fMRI in the acute phase of stroke is diaschisis. Diaschisis is defined as a dysfunction of preserved cortical brain regions that are remote but functionally connected to an acutely damaged brain area. This dysfunction leads to a depression of regional neuronal metabolism and cerebral blood flow and thus, will affect the regional activation and interregional connectivity patterns as revealed by fMRI in acute stroke patients. It is therefore difficult to determine how much the patient's initial deficit and subsequent recovery can be attributed to the focal brain damage or secondary diaschisis-related phenomena [41].

Another intrinsic problem relates to the heterogeneity of patient populations. Patients usually present with a combination of neurological deficits, which vary in magnitude from patient to patient. Deficits such as aphasia, hemianopsia or neglect might affect task performance in other tasks because patients might not understand the instruction or fail to appropriately perceive the stimulus that instructs the task. The same applies to the interindividual variability of the localization and extent of brain lesions. Here voxelwise lesion-behavior mapping might help to figure out which areas of the brain need to be damaged by stroke to result in a specific neurological deficit [69]. To this end, the infarcted brain area is delineated manually and a binary brain map is generated containing either affected or preserved voxels for each patient. Patients are further classified according to the presence or absence of a specific neurological deficit. By pooling the data of a large group of stroke patients, a statistical brain map can be generated which identifies those clusters of voxels where local brain damage is most consistently associated with the symptom of interest. Another strategy to cope with the large interpatient variation in terms of ana-

tomical lesion and clinical deficits is to apply more stringent inclusion criteria by including only patients with a prespecified neurological deficit or stroke location. While this increases the comparability among patients, the results of such fMRI studies cannot easily be generalized to the general stroke population. As pointed out above, the majority of fMRI studies on motor stroke have only included patients with subcortical stroke. Therefore, the fMRI results obtained in this subgroup of patients might tell little about motor reorganization that occurs in stroke patients with cortical involvement.

Finally, the potential for functional reorganization critically depends on the nondamaged brain regions and connections that offer the anatomical substrate supporting functional recovery. Therefore, thorough structural mapping of the nondamaged brain will greatly facilitate the interpretation of the fMRI data. In this context, diffusion-sensitive MRI techniques are of great value as they not only allow to define the infarcted area, but also to test whether and how much the major fiber tracts in the cerebral white matter are still intact after stroke. Here diffusion MRI-based tractography can be used to find out which corticocortical or corticosubcortical routes are still available for compensation after stroke-induced focal brain damage [70].

Summary and Outlook

Within the last decade, the use of fMRI in patients with stroke has substantially advanced our understanding of the mechanisms underlying functional brain reorganization in response to a focal brain lesion. There is also some evidence to suggest that fMRI in the acute phase might have some potential to predict recovery. It also appears possible that the results obtained with fMRI will inspire the development of new rehabilitation strategies and assist the planning of future intervention trials. However, the clinical use of fMRI in post-stroke patients is still in its infancy and the establishment of clinically feasible fMRI applications remains a challenge for translational research.

Previous fMRI work in stroke has been confined to small-scale single-center studies and most studies were designed as proof-of-principle studies. Future studies should aim at investigating larger patient cohorts and should include a broader range of stroke patients in terms of lesion location. This will facilitate the generalization of the results and help to identify subgroups of patients that show distinct patterns of functional reorganization. Further, the isolated use of fMRI to study functional reorganization after strokes has clear limitations. We anticipate that a multimodal assessment of functional reorganization that combines different methods, such as fMRI, diffusion MRI and TMS, but also magnetic resonance spectroscopy or electroencephalography will offer a deeper understanding of the mechanisms underlying post-stroke reorganization and its functional relevance. Longitudinal studies starting al-

ready few days after stroke are preferable to cross-sectional studies in the chronic stage because they can unravel the spatiotemporal dynamics of recovery. Finally, fMRI research of post-stroke recovery is mainly limited to academic neuroscience centers. It remains a challenge to implement the fMRI approach into nonacademic community hospitals where the majority of patients are treated [71]. This requires the establishment of simple fMRI protocols and automated analysis pipelines that can be implemented as clinical routine. All these considerations need to be taken into account if one wants to foster the clinical use of fMRI in post-stroke patients. This requires a close interaction between ‘Imaging Neuroscience’ and ‘Clinical Neurology’ to fully realize the potential of fMRI as a means of monitoring the efficacy of therapeutic interventions and stratifying patients based on the likely response to a therapeutic intervention.

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Diagnostic Approach to Functional Recovery: Diffusion-Weighted Imaging and Tractography

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Abstract

There is evidence showing that white matter changes are clinically relevant and can be associated with cognitive disorders, slower mental processing speed or motor impairment. The complex structural organization of the white matter can be depicted in vivo in great detail with advanced diffusion-weighted imaging (DWI). From the simplest and most commonly used technique (e.g. the mapping of apparent diffusion coefficient values) to more advanced techniques (e.g. diffusion tensor imaging), it is now possible to visualize white matter fibers of the brain in a noninvasive way. This chapter will first provide a basic understanding of the principles of these techniques and describe the current clinical applications of DWI and tractography in two common brain diseases, stroke and traumatic brain injury. We will emphasise on what these techniques may add to our understanding of the natural course of the pathology and especially, how they can help in predicting the outcomes of the rehabilitation phase. We will discuss how DWI and tractography techniques can shed light on possible compensatory disease mechanisms and propose future developments of these techniques in a clinical setting.

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Imaging White Matter Damages

Clinical Relevance of Studying White Matter

The central nervous system is composed of gray matter, containing the cell bodies (dendrites and axon terminals of neurons) and white matter. White matter makes up to 60% of the total brain volume and is composed of bundles of myelinated nerve cell fibers or axons forming up the structural connectivity of the brain. White matter contains major fasciculi, i.e. corticocortical commissural and association fibers as well as cortical projections connecting many regions including the striatum, brain stem and many more. The main feature of white matter in the brain, besides acting as a connection, is to regulate the speed of electrical signals propagating along axons.

The conductance speed regulation is obtained in combination by changing axon diameter and the oligodendrocyte arms wrapping around the axons as tight layers of

myelin. Myelin significantly increases the speed of signal propagation over long distances and therefore helps ensure the necessary conductance speed in the central nervous system to achieve various sensory, motor and cognitive functions. Clinically, white matter damages can result in serious temporal or permanent disabilities, ranging from mild cognitive impairments to gross deficits, motor injuries and altered sensorium.

To appreciate and understand the crucial role of white matter in functional recovery, noninvasive imaging techniques, such as magnetic resonance imaging (MRI), are important clinical tools. They can detect and monitor degenerative disorders and document cerebral mechanisms that underlie brain injuries.

Characterizing White Matter Damages

Very often, the diagnostic workup of a patient is determined by the abnormalities seen on the conventional MR images, such as T2-weighted lesions, but these changes in white matter are nonspecific and they likely have more than one cause. Advanced MR techniques, such as diffusion-weighted imaging (DWI), further enhance the diagnostic sensitivity and specificity of MRI by more accurately identifying and differentiating the above pathological processes [1]. Figure 1 shows examples of T2-weighted lesions with the associated axonal integrity present in various brain diseases. Not only does DWI via for example diffusion tensor imaging (DTI) provide insight into the status of tissue microstructure, but it also allows insight into brain connectivity via tractography. It is essential to understand the basic contrast mechanism of diffusion when applied to biological tissue to understand the possibilities of DWI-based techniques for clinical purposes.

DWI measures the scatter of free water molecules due to random thermal motion, i.e. Brownian motion or diffusion. When observed over a time period of milliseconds (called the diffusion time) molecules in free water can freely displace in any direction i.e. isotropic diffusion. In biological tissue however, obstacles in the cellular spaces having boundaries formed by the cell membranes influences molecular motion by making it less free; Diffusion within the intracellular space is restricted by the boundaries of the cell membrane whereas those outside in the extracellular space is hindered by these. The restricted/hindered molecular motion in different microstructural environments is the unique contrast of DWI. For obtaining the contrast, the diffusion time should be selected long enough to allow molecules to displace over distances longer than the physical size of the cellular space, which can range up to about 10 μm in diameter [2] (we often assume that during the diffusion time molecular exchange across cell membranes is minimal). On the MR scanner, by changing the b-value parameter, one also indirectly controls the diffusion time and a parameter called the q-value that basically acts as a filter controlling the maximal molecular displacement range. So the higher b-value selected (typically $> 1500\text{-}2000 \text{ s/mm}^2$) the more sensitive the diffusion weighting (DW) images get to restricted diffusion and hence to the information of the intracellular space. The reason for the increasing intra cellular contrast with b-value is that, in the extracellular space molecules are not restricted and therefore have displaced over longer distances than the q-value allows.








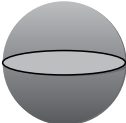


Conditions	Normal	Inflammation	Demyelination	Wallerian degeneration	Atrophy (black holes)
Normal	Axon intact Full connectivity	Axon intact Temporary connection failure	Axon intact Slower connectivity	Axon absent No connectivity	Axon absent No connectivity
Histology					
T2w intensity change	=	= or +	+	= or +	+
Diffusion MRI voxels					
Pathologies	-	MS stage 1, ALS, cerebral palsy	MS stage 2	Chronic stroke, TBI	MS, Alzheimer's disease

Fig. 1. Summarizes the various axonal integrities from normal to pathological states as revealed by histological techniques and the associated T2w signal intensity changes seen in conventional MRI exams. The schematic illustrations for the DWI voxel show how the fraction of the isotropic (grey) and the anisotropic component in a DWI voxel can be affected for the different pathologies. The fraction of the isotropic component typically increases with changes in the extracellular space e.g. as for demyelination. The intracellular space (anisotropic diffusion due to restriction) might increase for Wallerian degeneration for a short period of time when the axon is degenerated and myelin sheaths still persist. T2w = T2-weighted; MS = multiple sclerosis; ALS = amyotrophic lateral sclerosis; TBI = traumatic brain injury. Diffusion MRI voxels represent the measured DWI by the fraction of an isotropic (dark gray), i.e., extracellular space and a restricted and hindered component. Courtesy of Matthew Liptrot.

White matter, composed of a high density of myelinated axons, can simply be interpreted as impermeable parallel tubes. Indeed when measured radial to the axons, diffusion is more restricted/hindered by the boundary (cell membranes) than when measured axial to the axons i.e. anisotropic diffusion. Anisotropic diffusion in a voxel is therefore always aligned along with the main direction of the underlying tissue microstructure, as known for the white matter fibre tracts.

The degree of anisotropy is an index highly sensitive to a wide range of microstructural changes. Changes in radial diffusivity (RD) may reflect changes in fibre density (e.g. due to Wallerian degeneration, see next section), cell swelling or change in axon diameter. For example, local loss of myelination can be associated with an increased RD whereas greater fibre density or re-myelination is associated with decreased RD. Axial diffusivity (AD) is mostly sensitive to macroscopic fiber incoherence such as bending, fanning as well as undulating axons [3].

In DWI series of DW image volumes are acquired with a b-value (typical around 1000 s/mm^2), each been sensitized to diffusion (or also known as diffusion encoded) along one (unique) direction in space. Beside the DW image volumes, the DWI dataset often also include a number of non-diffusion weighted images (b-value = 0 s/mm^2) to be used for DTI [4]. Due to the many obstacles in brain tissue, the diffusion process is not really Gaussian as for free water, and the physical diffusion coefficient is therefore referred to as an Apparent Diffusion Coefficient (ADC). Hence, the signal in an acquired DW image relates (via an exponential relation and the diffusion weighting b-value used) to the ADC along that specific direction of the diffusion encoding. The ADC will therefore be different when measured radially or axially to the fibre tract direction.

Using mathematical models, it is possible to map microstructure anisotropy independent of the orientation of fibers and tracts. DTI is such a method that applies the tensor model [5] to the acquired DWI dataset. For compartments with anisotropic diffusion, the tensor has an ellipsoidal shape aligned with the fiber direction, whereas for compartments with isotropic diffusion, the tensor takes on the shape of a sphere (see fig. 1). The diffusion tensor D is spanned by 3 eigenvectors (e_{1-3}), which determine its orientation. For example, e_1 is always aligned with the main fiber direction (axial), whereas e_{2-3} are perpendicular to the fibers (radial). Correspondingly, the shape of the tensor is formed by the 3 values λ_1, λ_2 and λ_3 , which are quantitative measures describing the diffusivity along e_1, e_2 and e_3 , respectively. DTI reflects the average anisotropy within a voxel typically represented by a mixture of compartments, each with different degrees of anisotropy, as described in the previous section.

Clinically, DTI is a powerful diagnostic tool. The most commonly used anisotropic index is fractional anisotropy (FA), normalized between 0 and 1. In addition, DTI provides clinically unique quantitative diffusivity measures of AD (λ_1), RD [$(\lambda_2 + \lambda_3)/2$] and mean diffusivity (MD) [$(\lambda_1 + \lambda_2 + \lambda_3)/3$] (measured in square meter per second) [6]. Although both RD and AD can be more informative than FA and MD, they are rarely used clinically. Note that DTI is a nonspecific measure; therefore, it remains challenging to assess the underlying biological changes associated with DTI

perturbations. Intravoxel incoherence, such as bending, spreading and crossing fibers, impacts on DTI and will result in decreased FA values.

Tractography corresponds to the ability of noninvasively segmenting gross white matter fiber tracts, as opposed to the voxelwise DTI estimates. This can be used for visualization purposes, e.g. to investigate the tract structure around a tumor or to study the microstructure along a fiber tract, i.e. FA, RD, AD, or MD, and to study brain connectivity, i.e. corticocortical connections. Validation studies have shown that fiber projections found with tractography results correlate well with those of invasive tracers [7]. Different tractography methods are available. DTI tractography is the simplest and seemingly a robust tractography method also available on many clinical MRI systems. It draws streamlines along fiber directions identified with the diffusion tensor model. The tractography technique mainly includes two steps. First, the fiber direction(s) in each voxel is (are) found and second, the streamline(s) emanating from a start (seed) region is (are) drawn step by step along the fiber directions until a certain stop criterion has reached as for example the cortex [8]. Note, however, that DTI tractography is based on a single-fiber model (DTI) that often fails in complex regions compared to tractography with multifiber models [9].

The current applications of DWI and tractography in various clinical contexts highlight the high potential of the technique to improve our understanding of damage and recovery after brain injuries. This has been observed in various disorders of the central nervous system such as in spinal cord and traumatic brain injury (TBI), in some forms of vascular dementia, hypoglycemia or stroke [10]. In the next section, we will focus on the main clinical applications of DWI and tractography in two pathologies: stroke and TBI.

Current Clinical Applications

DWI and tractography can provide a new source of clinically relevant biomarkers following brain injuries. Here, we will emphasize the use of DWI and tractography as diagnostic and prognostic tools: from early detection of a local injury to the prediction of outcomes. In addition, DWI and tractography can also be considered as analytic tools: to track the changes in neural tissue accompanying recovery and to get new insights into the neural repair mechanisms.

Diagnosis and Functional Prognosis of Brain Injuries

Example of Stroke

At least 20% of ischemic strokes involve predominantly white matter lesions as a consequence of the occlusion of small penetrating arteries that supply the deep areas of the cerebral hemispheres. Ischemia preferentially alters the intra-axonal environment: the axons are swollen, forming what is known as axonal beading, which causes local diffusion dead space or restricted diffusion.

DWI signal intensity is of particular interest in purely white matter lesions but also in cortico-subcortical lesions. While no abnormalities are typically seen on conventional MR images, DWI shows changes in ischemic brain tissue within minutes after symptom onset, using very short scanning sequences (approx. 2 min, 1 non-diffusion-weighted and 3 gradient directions). Reported sensitivity of DWI in early diagnosis of early infarcts ranges from 88 to 100% and specificity ranges from 86 to 100% [11]. The time course of the ADC signal can also provide temporal information regarding stroke onset: ADC values decline rapidly after the onset of ischemia and subsequently increase to supranormal values within 24 h to 17 days [12].

Besides the primary damage caused by the stroke, DWI can also reveal secondary loss of structural integrity, namely Wallerian degeneration, in chronic stroke patients [13]. DW images show Wallerian degeneration lesions as hyperintense (decreased diffusivity), which presumably represents axonal, intramyelinic or astrocytic swelling that likely introduces a larger restricted diffusion component. This indicates degeneration of axons and their myelin sheaths after injury of a proximal axon or cell body.

DWI data have proven to be a great tool for following disease development and progression, but they can also predict clinical outcome. Large lesions, greater than 30 cm³ have been reported as poor prognosis factor [14]. Significant correlations between the acute DWI stroke lesion volume and both acute and chronic neurologic scores have been demonstrated (including the National Institutes of Health Stroke Scale, the Canadian Neurologic Scale or the Barthel index) [15, 16]. There is also a significant correlation between the acute ADC ratio (lesion ADC to normal contralateral hemisphere ADC) and chronic neurologic assessment scale scores [17].

Although less commonly used in acute care, DTI in acute stroke also reveals a rapid reduction of RD, which correlates with oligodendrocyte swelling, compression of the axoplasm and dendritic injuries. Decreased anisotropy (FA) is often related to the fiber tracts with severe axonal destruction [18]. Motor weakness is one of the most serious impairments after stroke survival. Prediction of an accurate prognosis for motor function in stroke patients is crucial, as it can provide useful information for clinicians, in order to indicate neurological intervention or specific rehabilitation strategies. Reduced FA is associated with poorer outcome, quantified with specific neurological scores. Jang et al. [19] found that the FA ratio at the lesion correlated with the motor Barthel index at 3 months after stroke. The predictive value of DTI for motor outcome in stroke patients has been widely demonstrated, especially for the upper limb functions (for a review, see Jang [20]). Fewer studies examined the structural correlates of recovery of the lower limb. In a recent study, Jayaram et al. [21] reported that asymmetrical FA values between the two hemispheres (reflecting reduced structural integrity of the lesioned corticospinal tract) are associated with greater walking impairment. Jang et al. [19] also demonstrated the ability of the DTI technique to distinguish the primary lesion core from the (secondary) degenerated tract early after stroke. This is very valuable for out-

come predictions, since it provides the opportunity to differentiate irreversibly damaged from restorable tissue where recovery takes place on a longer term.

Finally, in contrast to the local voxelwise DTI indices, tractography enables the investigation of changes along specific tracts or in brain networks. For example, the determination of precisely which white matter pathways have been affected by a stroke can predict some of the symptoms. Right posterior cerebral artery strokes might lead to visuospatial neglect but only when the lesions include the region of the white matter through which the parahippocampal gyrus and the angular gyrus are connected [22]. Similarly, tractography of the language pathways can help understand the pattern of deficits in different types of aphasia [23].

Example of Traumatic Brain Injury

Diffuse axonal injury associated with TBI shows shear strain deformation in the first few hours because rotational acceleration-deceleration ultimately results in axonal disconnection. Subsequent to axonal disconnection, the formation of axonal 'retraction balls' is observed, which is thought to be the result of the accumulation of axoplasm at the site of axonal disconnection [24].

DWI-related biomarkers of regional brain injury can inform clinicians and researchers about the injury severity but also which neurobehavioral systems were injured in patients with TBI. Lesions are more visible on DW images than on conventional T2-weighted images. As a consequence, DWI may be important for the prospective determination of the extent of traumatic injury, the degree of irreversible injury or for long-term prognoses. For example, the number of lesions, characterized by low ADCs, is an indication of cytotoxic edema which then correlates with indices of clinical injury severity [25].

DTI has been used as a tool for in vivo quantification of white matter microstructural alterations following TBI [26, 27]. Sidaros et al. [26] examined the association between white matter DTI indices in late subacute patients with severe TBI and clinical outcome. They found that FA values within the cerebral peduncles and the corpus callosum are reduced in TBI patients in the late subacute phase compared to controls. These values increased over time in patients with a favorable outcome. Some studies also identified significant correlations between DTI indices and cognitive function. The degree of white matter pathology (large areas of reduced FA, increased MD and RD) might predict cognitive deficits (memory or executive function) [28]. Other studies combining DWI and DTI were able to quantify indirectly both edema and damage to the integrity of white matter fiber bundles [29].

Probing Compensatory/Adaptive Mechanisms after Brain Injuries

One key aspect of research in neurorehabilitation is to effectively facilitate recovery and potentially to offer a powerful methodological framework for new neurorehabilitative interventions. To achieve these goals, processes involved in neural repair after brain injury must be characterized to then develop sensitive and clinically mean-

ingful markers of neural repair. In this context, not only can DWI pinpoint an area of pathology, but it can also shed light on longitudinal adaptive mechanisms of neuroplasticity and repair operating during recovery. Brain plasticity is likely to be the main mechanism for recovery and includes multiple substrates (e.g. increased axonal expression of sodium channels, synaptic changes, increased recruitment of parallel existing pathways or 'latent' connections, and reorganization of distant sites).

In stroke patients, increased activity in nonprimary motor areas and in motor areas of the nonlesioned hemisphere is commonly reported and this 'over' activity has an important role in functional recovery [30]. The functional reorganization seems to be predicted by structural damage to motor output pathways. For example, Newton et al. [31] observed that the amount of overlap between the lesion and tracts found with tractography is proportional to the degree of functional overactivation in nonprimary motor areas.

Hence, there is increasing evidence supporting the aberrant use of the existing tracts corresponding to the nonlesioned hemisphere. However, it might be possible that more extensive adaptive changes occur in response to brain damage. Massive axonal rearrangements are likewise seen in primates after brain injury [32]. This remarkable finding suggests that rerouting of axon trajectories could also mediate functional recovery in humans. This raises the challenge to find new *in vivo* markers and DWI-based technologies such as DTI, since tractography has the potential sensitivity for detecting such axonal rearrangements after brain damage.

Future Methodological Challenges and Clinical Applications

DWI is a powerful and clinically sensitive diagnostic tool. Based on the DWI technique, DTI has however some limitations. For example, DTI can only resolve one main direction and indices get incorrect in voxels with crossing and curving fibers (bending, kissing or axonal incoherence). Indeed DTI provides a unique *in vivo* insight into the brain's microstructural environment, but it remains nonspecific. It is impossible to infer whether disruptions in FA and MD parameters are the result of disturbances in axonal membranes, myelin sheath, microtubules, neurofilaments, or other factors. The integration of DWI and tractography with other imaging modalities could help to delineate the biological implications of alterations in white matter fibers. These could include functional-related modalities like fMRI (both resting state and task driven) and more tract-specific investigations of the corticospinal tract via motor-evoked potentials probed by transcranial magnetic stimulation [21]. Finally, magnetization transfer imaging [33] can provide information on myelin loss or remyelination via a ratio between free water molecules and those bound to macromolecules such as myelin.

Many promising imaging modalities have arisen, which in combination can potentially improve the diagnostic specificity and sensitivity. However, these modalities are often too time-consuming for a routine use for most clinical settings where the time needed for the diagnostic workup is a key factor. Clinically, DWI has proven powerful

because of its sensitivity to tissue microstructure. It can be further optimized and adapted for a specific investigation by simply optimizing the sequence parameters, e.g. using active imaging, which is a design-based (sequence) optimization [34]. This approach simply provides a sparse set of optimal sequence parameters giving a priori information about the tissue microstructure of interest (i.e. axon diameter or cell swelling), scanner hardware and maximal scan time. The active imaging approach has been used for generating optimized sequences to obtain axon diameter indices in the living human brain [35], information about neurite orientation [36] and magnetization transfer ratio [37].

Conclusion

DWI and tractography have proved to be useful tools in clinical neuroscience, offering exciting opportunities to test hypotheses that could not previously be addressed in the living human brain and providing in biomarkers of disease severity, progression or functional recovery. Future developments in image acquisition and applications of high and ultra-high fields offer the promise of high-resolution imaging in the clinical setting. These developments should lead to the investigation of more detailed anatomy and will afford greater accuracy and precision in tracing the white matter pathways of the brain.

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Compensatory Contribution of the Contralateral Pyramidal Tract after Experimental Cerebral Ischemia

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Abstract

Many people escape sudden death from ischemic brain stroke, but suffer from severe disabilities such as aphasia and/or paralysis. These survivors of focal brain injury need chronic care to recover from and/or compensate for the impaired sensory and motor functions previously controlled by the focal ischemic core. Functional compensation not only involves the remaining brain areas around the infarction but also the areas contralateral to the stroke lesion, with the need for remodeling of neuronal circuits in some cases. In this review, recent human and animal studies are presented to aid in the understanding of such plasticity in areas contralateral to the stroke lesion providing a new model for rehabilitation. It is well known in the medical field that the intact contralateral hemisphere is recruited for functional remodeling of modalities such as speech. However, the detailed mechanisms underlying these phenomena are less clear. In rodents, *in vivo* imaging techniques combined with other traditional techniques such as electrophysiology and behavior have revealed that functional recovery is achieved by specific synaptic (neuronal circuit) remodeling of the contralateral area in the 1st week after a focal stroke. The intact contralateral hemisphere can therefore potentially adopt a bilateral function, even in adults, following proper remodeling of neuronal circuits. These recent results suggest a possible new pathway using the intact hemisphere's function to recover lost functions stroke patients.

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After stroke, unlike following heart attacks, survivors often remain debilitated and thus severely reducing their quality of life and burdening their families. Stroke is the leading cause of chronic adult disability and the third leading cause of death in Japan (resulting in 130 thousand deaths per year) [1]. In the case of Japan, although the fatality rate has decreased in the last few decades, a large number of survivors still suffer from functional disabilities that lead to the second largest (1.1 million person-years)

loss of disability-adjusted life years [1]. Despite advances in medical care for stroke, more efficacious medical intervention, including research and rehabilitation, are necessary to reduce this health burden. Survivors of focal brain stroke need chronic care to facilitate recovery from/compensation for the specific functions that were impaired by damage to the neural pathways involved. These functions include speech and other sensory and motor functions. Functional compensation utilizes the remaining brain areas in the peri-infarction area but also incorporated brain areas contralateral to the lesion that can be remodeled to adopt new or expanded functions. Rehabilitation strongly contributes to this recovery/compensation process and plays an important role to achieve a good post-stroke quality of life.

In this review, we concentrate on the neuronal adaptations that occur in the contralateral area during recovery from stroke damage. We hope this review provides an enhanced comprehension of the contralateral neuroplasticity and to help facilitate more focused medical care and rehabilitation that ultimately improves recovery of functions.

Contribution of the Area Contralateral to the Stroke Lesion: A Human Case and Animal Models

The fully developed healthy brain is highly flexible during development as new connections are formed and removed through use-dependent processes. Environmental experience from infancy to adulthood and likely in the fully matured brain can markedly affect this plasticity and the resultant function of the human brain. Neuronal circuits in the adult brain are also plastic – being maintained and adapted to life events such as learning new tasks and recovering from brain injury [2]. Recent advances in functional imaging of human brain activity using, for example, positron emission tomography and functional magnetic resonance imaging have revealed the reorganization of the human brain during recovery from stroke showing important changes in the areas contralateral to the injured site [3, 4].

In humans who suffered a focal stroke in the language areas, it is well known that there is some recovery of language by using the nondominant hemisphere, usually the right hemisphere [4]. Some research has also highlighted the contribution of the peri-infarction area for recovery from aphasia. There is also clinical evidence showing that the post-stroke reorganization within the somatosensory system in the contralateral (intact) hemisphere plays an important role in the compensation for impaired functions [5, 6]. Thus, the underlying mechanism of this compensation occurring in the intact hemisphere is important for optimizing the functional recovery of human stroke patients [3].

As with these clinical cases, animal experiments have shown that cortical finger representations adjacent to partly damaged finger representations became enlarged during rehabilitation, while they remained unchanged in the control untreated mon-

keys [7]. This experiment and others have provided evidence that reorganization occurs in the adult nervous system in regions adjacent to a damaged region that leads to plastic changes of the sensory representation of the affected modality. Also in animal models of stroke, unilateral experimental infarctions in the somatosensory cortex (SSC) and motor cortex result in functional and structural changes in the remaining intact contralateral hemisphere. Infarction in the SSC changes the receptive field at the contralateral SSC at 1 week after stroke [8]. After the recovery of motor function that was impaired by cerebral infarction, the topographic map is reorganized and the dendritic branching of layer V pyramidal neurons is increased in the contralateral motor cortex [9], and this is enhanced by an early onset of rehabilitation-like tasks in mice [10]. These results suggest that a change in the underlying neuronal circuits in the contralateral hemisphere may occur during functional recovery from stroke.

Axons sprout from the neurons in the contralateral SSC and motor cortex following stroke, projecting into the deafferented regions of the cervical spinal cord and midbrain that previously received a projection from the now infarcted area [11, 12]. This sprouting can be unequivocally demonstrated, as it results in a novel contralateral projection. Formation of these new axons and branches involves specific molecular events, and these appear to be at least partially distinct from those that regulate axonal growth cone behavior during development [13]. In terms of functional assessment, pharmacological stimulation of axonal sprouting from the contralateral cortex into the cervical spinal cord and brain stem is correlated with improved functional recovery after stroke [14, 15].

Although there is now strong evidence for the contribution of the contralateral (intact) hemisphere to functional recovery after stroke, the nature of the neuronal and circuit remodeling had until recently been less well understood because of the limitations in the resolution of positron emission tomography/functional magnetic resonance imaging. However, recent intense studies using higher-resolution *in vivo* two-photon laser microscopy have revealed a number of neuronal events during the recovery phase after stroke. These include neuronal circuit remodeling (e.g. spine turnover [16]), and glial contribution to synapse remodeling [17] in the damaged hemisphere, as well as neuronal remodeling induced in the contralateral hemisphere, which occurs with different mechanisms and time course [16, 18, 19].

Acute Phase Remodeling Achieves Compensation: The Case of the Area Contralateral to the Lesion

A focal stroke in the SSC in mice results in paralysis and sensory loss in the contralateral side (e.g. left-hemisphere stroke induces right-side paralysis). However, mice show strong functional recovery by 2–4 weeks after stroke, even if the size of the ischemia and damage covered the whole SSC [18]. A key question is: what is happening in the area contralateral to the lesion during this functional compensation?

A focal stroke increases the receptive field of the SSC not only in the peri-infarction area but also the area contralateral to the lesion [8]. After focal stroke, uptake of glucose (radio-labeled glucose; ^{18}F -FDG) is increased in the area contralateral to the lesion [18] indicating that the neuronal (and/or glial) activity of the area is increased. This enhanced activity in the area contralateral to the lesion returns to normal levels by 4 weeks after stroke. What happens to the fine neuronal circuit structures in the contralateral SSC during this time of enhanced brain activity has been observed using the two-photon laser microscopy technique *in vivo*. The turnover of mushroom-type (stable) dendritic spines in the contralateral SSC was increased after stroke, but only at 1 week (fig. 1a) [18]. This time-limited change in the turnover of spines is also seen in the case of stroke within the visual cortex [19]. This restricted period of increased spine turnover is quite different compared with that of the peri-infarction area, where the turnover rate is high even 6 weeks after stroke [16]. Mushroom spines are more stable compared to other spine types (thin and filopodial) being stable for more than a month, or even for a year, in the nonischemic healthy brain [20, 21]. It has also been reported that such mushroom spines become more apparent in response to focal and repetitive neuronal circuit activation and a stimulus that mimics the induction of long-term potentiation [22]. The conclusion is that mushroom spines seem to be necessary for long-term memory and maintaining normal function of neuronal networks. Thus, an increase in the turnover of mushroom spines following stroke induces some kind of adaptation in brain function. Interestingly, smaller spines tend to be preferentially eliminated in the contralateral SSC [Takatsuru and Nabekura, unpubl. obs.] as observed in the SSC of a chronic pain model (enhanced afferent activity [23]). Thus, in readjusted neuronal networks, smaller (weak) synapses could be replaced by newly generated synapses. Note again that the increase in the turnover of mushroom spines in the contralateral area was only induced within 1 week after stroke, i.e. a limited time period.

Following this transient increase in mushroom spine turnover, novel neuronal circuits appear that correlate with ipsilateral limb stimulation. For example, a novel neuronal circuit is detected in the right SSC in the case of left SSC stroke that now responds to the (ipsilateral) right limb stimulation. This finding suggests that the increase in the early turnover of mushroom spine actually induces a change in the neuronal circuit that contributes to the remodeling of brain and functional recovery. After this neuronal circuit remodeling, bilateral processing of somatosensory function is achieved in the intact hemisphere (fig. 1b). In the 2nd week after stroke, local application of 6-cyano-7-nitroquinoxaline-2,3-dione, a potent α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptor antagonist, into the intact hemisphere prevents the behavioral response to stimulation of both the right and left hind limb [18]. Yang et al. [24] reported an increase in mushroom spines in the motor and sensory cortices during motor training or somatosensory stimulation, respectively. In case of motor learning, the number of newly formed mushroom spines was correlated with the performance of motor co-

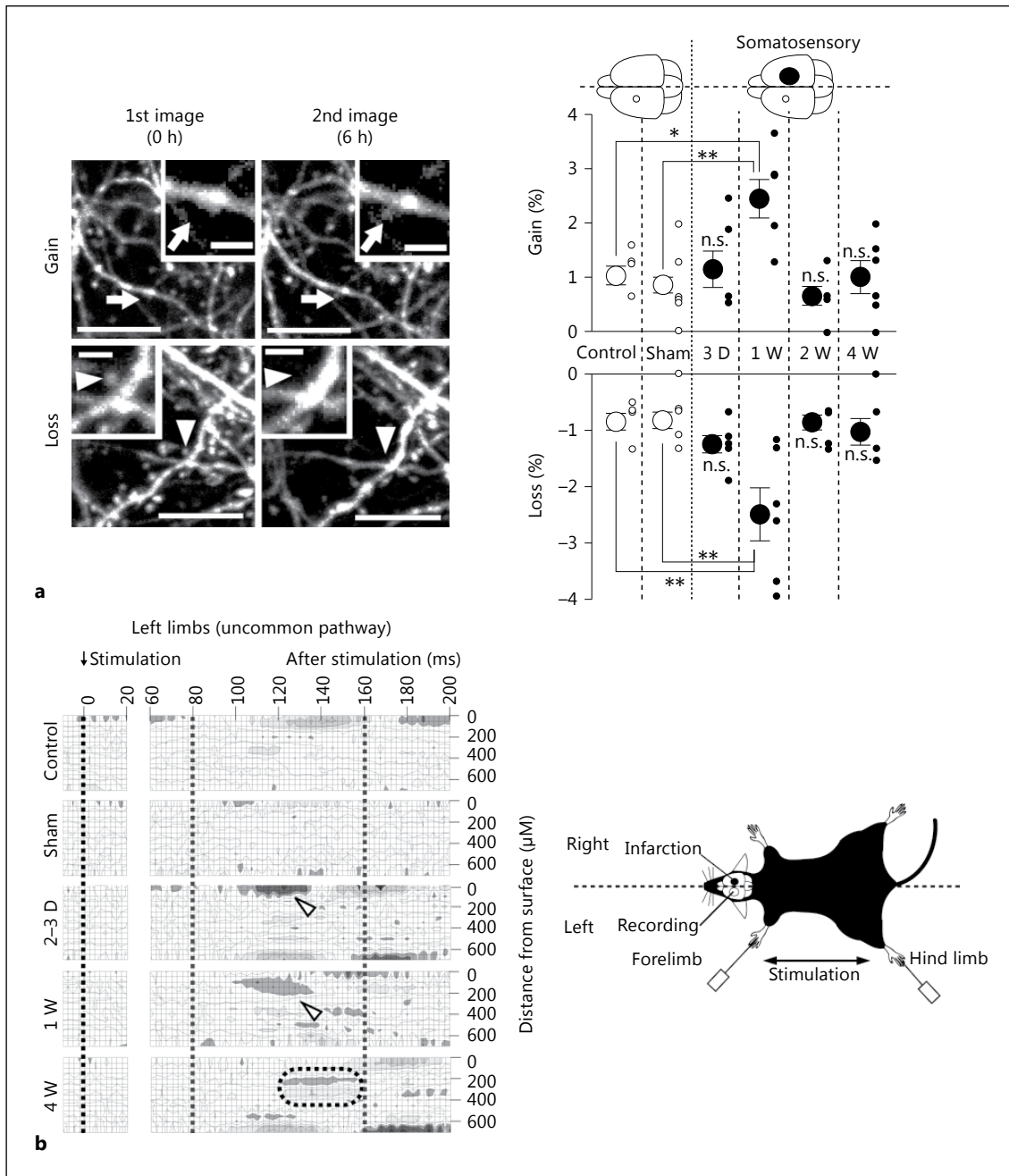


Fig. 1. Structural and functional remodeling in the cortex contralateral to infarction. **a** Left: typical in vivo images of dendritic spines in the SSC taken during the first and second imaging sessions (6 h later). The arrows in the upper panels and the arrowheads in the lower panels show the appearance (gain) and disappearance (loss) of spine. Scale bars, 10 μm . The insets show higher-magnitude images (scale bars, 2 μm). Right: note that significantly greater gain (upper panel) and loss of spines (lower panel) in the SSC contralateral to infarction was limited at 1 week. Error bars represent \pm SEM. n.s. = Not significantly different compared with both control and sham by the Bonferroni test; D =

ordination, and such newly formed mushroom spines had a long survival time. Similarly, in the case of functional recovery from stroke, the over 85% of mushroom spines in the contralateral area newly formed during 2–7 days after stroke survived for 5 days or longer [Y. Takatsura and J. Nabekura, unpubl. data].

Taken together, important structural and functional changes in the neuronal circuit occur in the area contralateral to a stroke lesion during a very limited time period (1 week) after stroke in the animal model (fig. 1). It will now be important to correlate the time course of this sequence of changes (increased activity, increased mushroom spine turnover, neuronal circuit remodeling and functional recovery) to the improved outcome for human stroke patients.

Correlation of Rehabilitation: Acute Is Best?

Rehabilitation is an important process for the recovery from, and/or compensation for, the loss of function following a focal brain injury. To prevent or reduce the sustained loss of function, rehabilitation should be started as soon as possible after stroke. However, it is sometimes difficult to start the rehabilitation in the early post-stroke phase because of the necessity of acute medical care. Thus, when is the appropriate timing to start the rehabilitation?

The consensus from human imaging studies is that the most successful recovery occurs in individuals that exhibit relatively normal lateralized patterns of sensory activation in the hemisphere in which the stroke has taken place. It is said that patients with larger strokes who often show bilateral cortical activation typically have less complete recovery [25, 26]. However, the timing of rehabilitation has not been reported in those cases and there is some possibility that less complete recovery was related to a delayed or inappropriate timing of rehabilitation.

Animal studies indicate that many of the genes and proteins that are important for neuronal growth, synaptogenesis and the proliferation of dendritic spines are expressed at their highest levels during early brain development. Similarly, there is an increased expression of these genes for a limited period following stroke [27, 28]. Furthermore, animals given early rehabilitation resulted in significant recovery, whereas animals given delayed treatment exhibited little improvement. Notably, early enrichment increased the dendritic branching of layer V cortical neurons, whereas enrich-

days; W = weeks. **b** A focal infarction in the right SSC induced the appearance of new neuronal circuits in the left SSC. Current source density maps of electrical responses in the SSC (left), contralateral to infarction in the right hemisphere, to stimulation of the left limbs. The dashed circle represents the spatial and temporal distribution of the strongest sink and source of the current source density. Note that the neuronal circuit activity in response to left limb stimulation in stroke mice gradually developed and became particularly distinct at 4 weeks. W = Weeks; D = days. Adopted and modified from Takatsuru et al. [18].

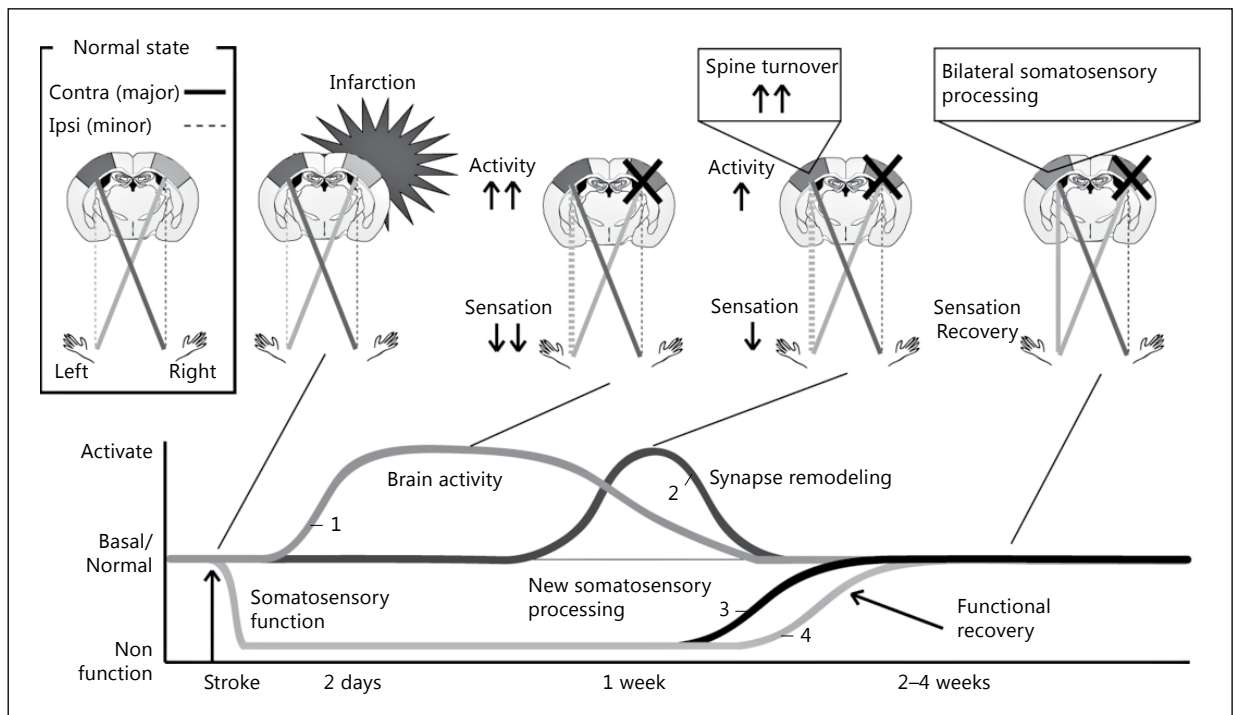


Fig. 2. Schematic illustration of the time course of plasticity and remodeling of the contralateral (intact) hemisphere following a stroke in mice, which can ultimately result in some compensation for the loss of sensory function. Immediately after a stroke in the SSC, the peripheral sensations from the side contralateral to the ischemic area are lost. During the acute post-stroke phase (within 2 days after stroke), the neuronal activity in the brain contralateral to infarction is increased (line 1). This increase in neuronal activity is followed by an increase in the turnover rate of mushroom spines in the intact SSC. This dendritic remodeling occurs during a limited time window, i.e. about 1 week (line 2). Subsequently, a new electrical pattern of neuronal circuits in response to ipsilateral sensory stimulation is gradually generated in the intact hemisphere (line 3). These new neuronal circuits contribute to a compensation or recovery of behavioral function which had been impaired by the infarction (line 4). We propose that rehabilitation plans should account for these different post-stroke phases – making new synapses and/or using new synapses, reorganization of functional circuits – and thus, could differ depending on these time phases of remodeling.

ment that was delayed until 30 days after stroke had no effect [10]. An early onset of rehabilitation achieves good clinical results [29]. These data provide strong evidence for a critical period after stroke, during which the brain is most receptive to modification by rehabilitative experience, and suggest that earlier therapy is better.

As a precaution, other animal studies have shown that very early, intensive therapy may have detrimental effects and exacerbate brain injury through overuse of the affected limb [30, 31]. However, if we think about the timing of the structural plasticity in mice after stroke, mushroom spine turnover in the contralateral area is not seen very early after stroke (2 days) but becomes transiently apparent at 7 days after stroke

[18]. Hence, discrete rehabilitation strategies may have individual time windows during the rehabilitation process after stroke. In mice, brain activity is high in the contralateral area at 2 days after stroke, but not yet structurally adapted plastic (which is seen at 7 days). There seems to be an individual time window for any contralateral remodeling and functional recovery, such as a ‘making new synapses’ phase, a ‘using new synapses’ phase, and ‘reorganization of circuits’ function (fig. 2). It is important to think about these specific time windows, and to assess more precisely ‘what happens during stroke recovery in mice’ may lead to advances in post-stroke care and rehabilitation.

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Compensatory Contribution of the Contralateral Pyramidal Tract after Stroke

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Abstract

Stroke is a leading cause of long-term disability with early accelerated followed by gradual recovery during the first 6 months after the ictus. The most important mechanism concerning early recovery is thought to be brain plasticity provided by anatomical and functional reorganization of the central nervous system after injury. Recent advances in noninvasive, functional brain imaging techniques provided some insight indicating the contribution of ipsilateral uncrossed corticospinal tracts in motor recovery after stroke. Since motor tracts vary considerably among subjects, the ratio of contralateral corticospinal tract fibers and their interhemispheric control versus the amount and function of ipsilateral corticospinal tract fibers may affect the scale of motor recovery after stroke. Further studies are needed to clarify the mechanisms of motor recovery after stroke in humans.

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Brain injury after stroke causes damage to intrinsic neural networks affecting the function of motor, sensory, cognitive and other functional domains. Motor dysfunctions including dysphagia, hemiparesis and gait disturbance are important aspects of activities of daily living and affect the long-term psychosocial welfare. It is well known that some deficits, such as hemiparesis or imbalance, often recover spontaneously, suggesting that inherent plasticity within neural networks compensates for lost motor function.

While many patients achieve independence in their activities of daily living after 6 months, the most significant recovery in motor function happens within the first month regardless of the initial stroke severity. Mild to moderate stroke patients recover within the first month, while moderate to severe stroke patients continue to recover for prolonged periods after stroke [1].

Several mechanisms have been proposed to explain the pattern of recovery after stroke. We believe that the main mechanism implicated in early recovery after

stroke is an inherent blueprint of pre-existing anatomical pathways and functional rearrangement of networks within the central nervous system. This rebuilding process may involve various mechanisms, including (1) redundant pathways that perform similar functions being able to substitute one another if one pathway has been damaged, (2) unmasking of silent pathways, and (3) sprouting of fibers from surviving neurons with formation of new synapses [2–4]. The temporal profile of recovery observed very likely reflects the variant mechanisms involved. Short-term changes are probably related to functional enforcement of existing circuits, such as unmasking of silent synapses [5, 6], whereas long-term changes involve other processes, such as axonal regeneration or synaptic sprouting. When damage to the system is only partial, complete recovery may occur within the system. More extensive damage, however, requires the recruitment of a functionally related system.

Role and Plasticity of the Pyramidal Tract

The pyramidal tract, which is mainly involved in motor function, consists of descending nerve fibers that originate in the cerebral cortices (Brodmann's areas 4, 6 and others) and extend longitudinally through the bulbar pyramid into the spinal cord. Anatomically, 75 and 90% of the corticospinal fibers cross the midline in the lower medulla or upper cervical cord forming 3 separate corticospinal tracts on each side [7]. The largest one is, naturally, the lateral corticospinal tract that crosses in the medullary decussation and extends caudally in the dorsolateral fasciculus to the last sacral segment. The fibers of this tract terminate mainly on the ipsilateral motoneurons of the ventral horn of the spinal cord. The anterior or ventral corticospinal tract consists of fibers that do not cross in the medullary decussation and extend caudally only until the upper thoracic cord. These fibers are known to decussate at various levels of the cervical cord through the anterior white commissure to largely terminate on contralateral motoneurons within the medial portion of the ventral horn. Additionally, a third uncrossed corticospinal tract, which varies individually in size and may be a compact, well-delineated tract, is known to exist [7]. Whereas the function of this third tract has not been elucidated, it may be dormant, and activated only in case of damage to the central motor control system. We speculate some role within early motor recovery after stroke in humans.

It is well established that motor recovery in children goes beyond that seen in adults. In fact, prenatal brain damage, such as congenital hemiplegia, induces extensive reorganization of the corticospinal tract particularly of transient projections to the ipsilateral spinal cord. Studies using transcranial magnetic stimulation (TMS) revealed that topographic rearrangement of representations from the primary motor cortex occurs in both, paretic and nonparetic limbs in patients with congenital hemiplegia [8, 9].

Preserved Ipsilateral Corticospinal Tract Function Is a Plausible Mechanism of Motor Recovery after Stroke

Brain plasticity implies adjustment of neural function over time, which in turn results in behavioral adaptation after brain injury. Behavior-related plasticity can be quantified by repeated computation of the distribution of neural activity following sequential task repetitions. Comparison of patterns of activation as a function of time identifies brain areas in which time-related modification of activity appears. This concept has been applied to functional brain imaging studies examining changes of brain function during the acquisition of motor skills [10]. Similarly, the recovery of brain function after stroke has been studied using noninvasive functional imaging techniques, such as positron emission tomography, functional magnetic resonance imaging (fMRI) and electrophysiological techniques represented by TMS. In the early 1990s, the significance of bilateral cerebral and cerebellar regional activation involving the ipsilateral sensorimotor cortex and motor pathways in motor recovery of stroke patients was discovered [11–13]. Technological improvements in MRI methodology made fMRI the predominating functional brain study since the late 1990s. The principle of fMRI is based on blood oxygenation levels in comparable brain regions between rest and activated states. When neurons become active, local blood flow to those brain regions increases, and oxygenated blood displaces deoxygenated blood around 2 s later. This rises to a peak over 4–6 s before falling back to the original level (and typically undershooting slightly). Deoxygenated hemoglobin is paramagnetic as opposed to oxygenated hemoglobin being resistant to magnetism. This difference leads to an improved MR signal that can be mapped to reveal which neurons are active at a time. Using blood-oxygenation-level-dependent technology, it could be shown that the contralateral primary sensorimotor cortex is activated during passive movement of the paretic limb in patients with hemiparetic strokes [14]. The results indicated that in patients recovering from hemiparesis contralateral motor pathways became involved in the reorganization of motor function possibly involving the contralateral brain regions via uncrossed corticospinal tracts or other indirect uncrossed pathways. In contrast, stroke patients with infarcts not involving the primary motor cortex exhibit a linear relationship between recovery scores and task-related brain activation in many parts of the associated motor system [10]. It can be speculated that differences among patients are likely the result of variable anatomical damage and cognitive parameters such as motivation, concentration and attention. Moreover, TMS studies revealed that motor evoked potential amplitude correlates with the extent of hand motor recovery after subcortical stroke when stimulating the affected side of the cerebral cortex [15, 16]. In summary, motor recovery is best when motor cortices remain structurally preserved, functionally connected, and can magnify information processing. While there is no doubt about the contribution of cerebral reorganization to functional recovery after stroke, the processes and factors affecting it remain elusive. A detailed sequential analysis of stroke patients re-

Table 1. Clinical features of the patients who showed hemiparesis ipsilateral to the lesion side caused by the second stroke attack

Authors, year	Age, years	Sex	Symptoms of 1st SA	Lesion site of 1st SA	Type of 1st SA
Kim [17], 1999	64	male	Rt hemiparesis + sensory deficit	Lt IC + basal ganglia	hemorrhage
	68	male	Rt hemiparesis + sensory deficit	Lt IC + thalamus	infarction
	53	female	Rt hemiparesis + sensory deficit	Lt IC + thalamus	infarction
Ago et al. [18], 2003	59	male	Lt hemiparesis	Rt IC + basal ganglia	hemorrhage
Song et al. [19], 2005	62	female	Lt hemiparesis	Rt IC + thalamus	hemorrhage
	41	male	Lt hemiparesis	Rt CR	infarction
Yamamoto et al. [20], 2007	74	male	Rt hemiparesis + aphasia	Rt CR	infarction
	76	male	Rt hemiparesis + aphasia	Lt CR + T-P lobes	infarction
	64	male	Rt hemiparesis + dysarthria	Lt CR + T-P lobes	infarction
Our case, 2013	83	female	Lt hemiparesis	Rt CR	infarction

SA = Stroke attack; Rt = right; Lt = left; mRS = modified Rankin Scale score; IC = internal capsule; CR = corona radiata; T-P = temporoparietal; O = occipital; F-P = frontoparietal.

covering from motor deficits with highly sophisticated functional imaging techniques may help to reconcile therapeutic strategies to enhance motor recovery in the future.

Role of the Ipsilateral Corticospinal Tract in Patients with Motor Recovery after Stroke

Recently, several patients with a prior recovered hemiparesis after stroke were reported to experience worsening of their motor function after a new contralateral stroke recurrence. The important unifier is the lesion site in all these cases [17–20]. These cases reveal that the ipsilateral, uncrossed corticospinal tract may have helped to com-

mRS score after 1st SA	Duration between two SAs	Symptoms of 2nd SA	Lesion site of 2nd SA	Type of 2nd SA
2	3 months	exacerbation of Rt hemiparesis	Rt thalamus + O lobe	infarction
1	7 years	exacerbation of Rt hemiparesis	Rt F-P lobes	hemorrhage
1	1 year	exacerbation of Lt hemiparesis	Rt pons basis	infarction
2	5 years	exacerbation of Lt hemiparesis	Lt CR	infarction
2	1 month	exacerbation of Lt hemiparesis	Lt CR	infarction
0	1 month	recurrence of Lt hemiparesis	Lt CR	infarction
2	11 years	exacerbation of Rt hemiparesis	Rt thalamus	infarction
1	11 years	exacerbation of Rt hemiparesis	Rt CR	infarction
1	4 years	exacerbation of Rt hemiparesis	Rt CR + basal ganglia	infarction
0	3 years	recurrence of Lt hemiparesis	Lt CR	infarction

compensate motor recovery after the earlier stroke. Table 1 shows the previous case reports including a patient that we recently investigated.

In what follows, we will present the features of this patient in more detail. An 83-year-old right-handed female with hypertension was admitted to our institute (National Cerebral and Cardiovascular Center) because of suddenly developed left hemiparesis, dysarthria and sensory disturbance; 1.5-tesla MRI revealed an acute small infarcted lesion in the left corona radiata on diffusion-weighted images (fig. 1a). Previously, the patient had experienced left hemiparesis caused by right corona radiata infarction as shown on a brain computed tomography scan (fig 1b). The previous left hemiparesis fully recovered within several months after the first stroke, and no residual motor weakness remained. Her recurrent left hemiparesis recovered once again fairly well (modified Rankin Scale score: 2) after the second stroke. We

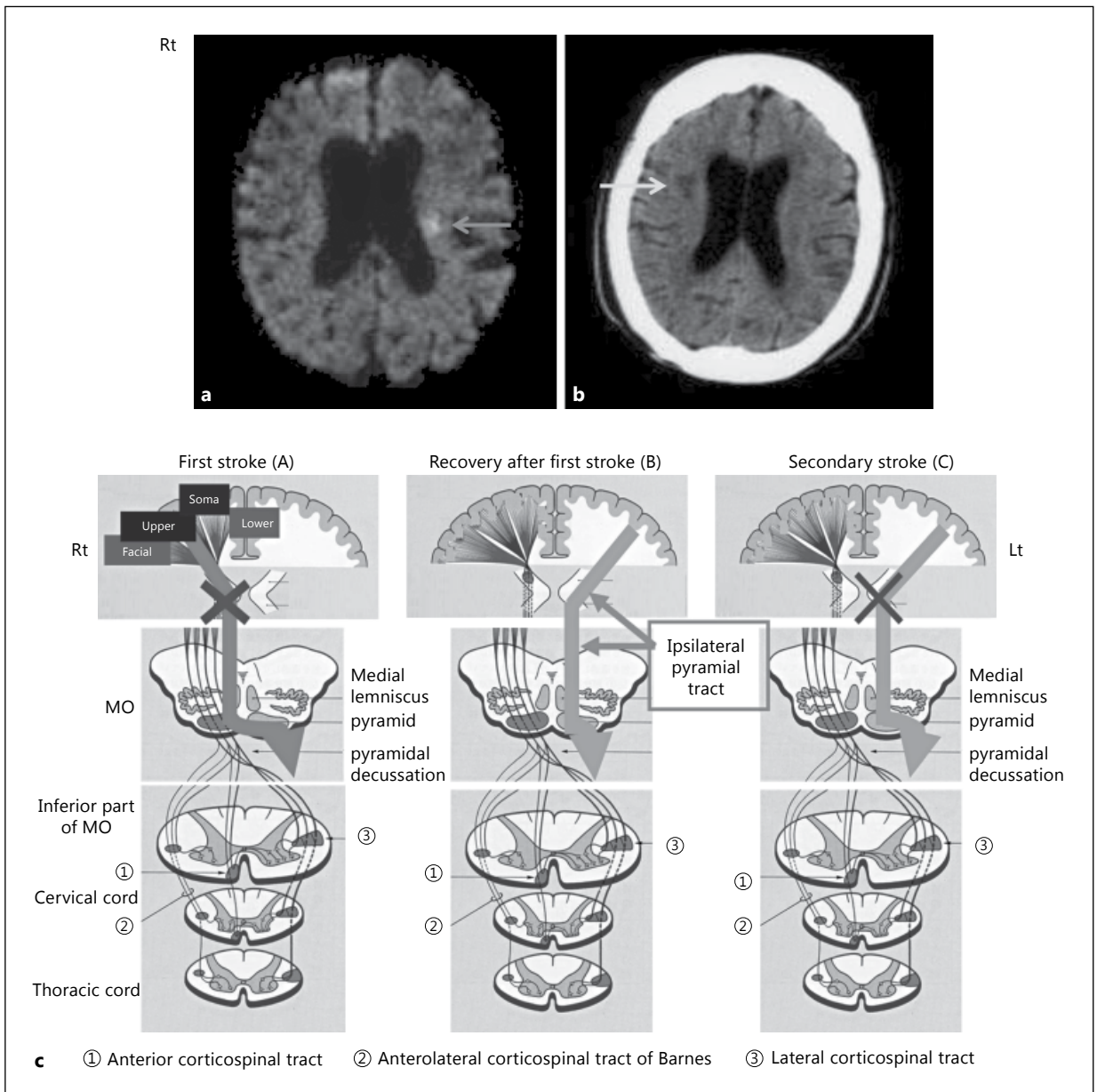


Fig. 1. a, b Our patient’s neuroimages. **a** Diffusion-weighted image reveals the recent infarct lesion in the left corona radiata (arrow). **b** Brain computed tomography scan reveals the past infarct lesion in the right anterior corona radiata (arrow). **c** A plausible mechanism of motor recovery in our patient. The right pyramidal tract had been damaged in the corona radiata at the initial infarction and left hemiparesis occurred (A). However, her motor deficit was fully recovered by the compensatory role of the left, contralesional corticospinal tract (B). Recently, a second infarction damaged the left corticospinal tract in the left corona radiata, resulting in left hemiparesis (C). Rt = Right; MO = medulla oblongata; CST = corticospinal tract.

suspect that the left hemiparesis caused by the first stroke at the right corona radiata recovered completely due to the compensation with the uncrossed left-sided corticospinal tract. During the second stroke at the left corona radiata, the compensating uncrossed left-sided corticospinal tract was damaged, and hence left hemiparesis again manifested. Interestingly, the patient did not show right hemiparesis during the second stroke in spite of damage to the left corona radiata. Presumably, the second stroke affected only the left-sided uncrossed corticospinal tract and did not involve crossing the corticospinal tract controlling right motor function (fig. 1c).

Several possible explanations have been proposed in terms of the relationship of the ipsilateral corticospinal tract with motor recovery following stroke. (1) The ratio of uncrossed corticospinal fibers in the whole pyramidal tract seems to vary between 10 and 25%, but there is considerable variability of the ratio [7]. A higher ratio may correspond to a superior recovery of hemiparesis after stroke. Reports of ipsilesional hemiparesis after cerebral injury in some patients imply the possibility of primarily dominant ipsilateral corticospinal fiber architecture [21, 22]. (2) The lesion size and topography of brain injury are crucial for motor recovery. At least 60% of neural fibers of the pyramidal tract originate in the primary motor cortex, premotor frontal and supplementary motor areas. Most of the corticospinal fibers from the primary motor cortex cross at the decussation, while corticospinal fibers from premotor, frontal and supplementary motor cortices connect with the ipsilateral primary motor cortex, contralateral homologous areas, and to various levels of the spinal cord via both sides of the corticospinal tract [23]. Furthermore, premotor frontal and supplementary motor areas promote reticulospinal tract function via rich projection from those cortical areas to brainstem reticular formation [24]. Fibers of the reticulospinal tract descend to the spine and send axonal branches to spinal gray matter to control proportional movements of proximal limb muscles. Motor deficit caused by the localized lesion at the primary motor cortex is considered to show good recovery in adults as well as children. (3) The activity of motor cortices can be modulated via transhemispheric connection to the contralateral motor cortices [25]. Low-frequency repetitive TMS applied to the motor cortex inhibits motor cortical excitability in the homonymous motor representation of the opposite hemisphere. Therefore, the presence of a balance of reciprocal inhibitory projections between both hemispheres has been proposed. Recent studies have suggested that this balance is disturbed during voluntary movement of the paretic hand in patients with cortical infarcts. Specifically, more severely paretic patients demonstrate greater interhemispheric inhibition between the primary motor cortices than those less affected [26]. Therefore, it is conceivable that decreasing the inhibiting effect of the ipsilateral, intact, motor cortex may enhance motor recovery of the paretic hand. Recent case studies have revealed that inhibitory regional TMS of the unaffected hemisphere is effective even in chronic stroke patients with severe motor deficits [27]. Similarly, regional TMS of the contralesional primary motor cortex can improve motor function in patients with subcortical infarction, presumably by balancing neural activity in both hemispheres documented by fMRI [28]. The durability of these effects

and enhanced clinical outcomes remain to be shown. Brain activation patterns observed with constraint-induced movement therapy (which forces the use of the paretic limb by movement restriction of the intact extremities) also support the concept of hemispheric neural activity balance [29]. Alternatively, the concurrence of compensatory uncrossed corticospinal tracts and interhemispheric inhibition may limit the potential for recovering motor function after stroke.

Using TMS and fMRI (simple finger tapping) techniques, two patterns of corticospinal reorganization have been proposed in congenital hemiparesis [30]. The premotor pattern observed in patients with only minor lesions and mild hand paresis shows activation of the premotor areas in the affected hemisphere. The primary pattern observed in the more severely affected patients shows activation of the primary sensorimotor region in the contralateral hemisphere. While the premotor pattern may forecast beneficial reorganization, the primary pattern may reflect detrimental adaptations, such as disinhibition of ipsilateral pathways and interhemispheric inhibitory connections. Clinical observation suggests that both patterns occur simultaneously supporting a potential contradiction of anatomical and functional preconditions for motor recovery. We suggest that the clinical observations along with the activation patterns observed in recent fMRI studies limit a role for ipsilateral corticospinal tract reorganization in motor recovery after stroke. The degree of motor recovery depends foremost on the extent of the damage, while early recovery is destined by the individual makeup of uncrossed versus crossed projections.

Conclusion

The role of the ipsilateral, uncrossed pyramidal tract in motor recovery after stroke remains elusive. Interaction of this tract with environmental factors, drugs and genes is still unknown. From the clinical point of view, we suspect that the ipsilateral, uncrossed corticospinal tract may be a compensatory asset in motor recovery of hemiparesis in some stroke patients. Further research is needed to substantiate this proposed mechanism to determine the impact of rehabilitation approaches for motor deficits.

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Regeneration of Neuronal Cells following Cerebral Injury

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Abstract

Stem cells possess a definitive role in neuronal rejuvenation following a cerebral injury. Whether endogenous, from the neurogenic niches of the subventricular zone and subgranular zone, or recruited from the bone marrow through peripheral circulation, accumulating evidence demonstrates that stem cells ameliorate the consequences of cerebrovascular events, particularly cerebral ischemia. In this chapter, we review milestone studies implicating the role of stem cells in response to disease. Furthermore, we outline specific mechanisms of action along with their clinical potential as therapeutic treatments for ischemic stroke.

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The idea that stem cells may reconstitute regions of neuronal damage has prompted much research interest in using bone marrow (BM) as a donor source for transplantation therapy in neurological disorders, notably stroke. The heterogeneous mixture of cells populating the BM includes: hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and very small embryonic-like stem cells (VSELs). A collection of *in vivo* and *in vitro* research suggests these cells may mobilize into the peripheral blood (PB) upon cerebrovascular injury and respond by secreting essential growth factors for survival [1], along with possibly undergoing neuronal differentiation on exposure to inducing regimens [2].

As stroke remains a primary cause of death worldwide, this chapter pursues the possibility of the aforementioned cell lines to afford brain plasticity and remodeling [3]. In the clinic, minimally invasive intravascular transplantation is an appealing approach for stem cell therapeutic measures. However, this scheme requires concerted mechanisms to ensure that cells, or their secreted therapeutic molecules,

reach the site of injury. The mechanisms involved in migration, homing, isolation, and the potential therapeutic effects of these cells will be discussed within this theme.

Hematopoietic Stem Cells

In addition to the defining feature of repopulating ablated BM [4], HSCs can also migrate to PB in response to injury. During homeostasis, HSCs are quiescent and low in number, a characteristic attributed to chemokine regulation. Yet, in response to injury, these cells can become motile, with increased migration into blood circulation [5]. Stromal cell-derived factor 1 (SDF-1; also termed CXCL12) contributes to an essential chemoattractant pathway via the receptor CXCR4 [5]. When SDF-1 is active, HSCs cross the endothelial blood-BM barrier and populate the PB [6]. The SDF-1/CXCR4 interface is highly expressed in several stem cell niches, notably the brain endothelium [7]. With the central nervous system (CNS) contributing to HSCs motility, conditions of stress (i.e. stroke) can amplify recruitment of HSCs into the brain [4, 5].

One such mechanism of CNS control in the migration of BM-derived HSCs is the induction of cytokines. Recently proposed, the neurotransmitter catecholaminergic signaling pathway may promote HSC mobilization through sympathetic secretion into the blood or via a more paracrine fashion from the BM nerve endings [4, 8]. This neurotransmitter-mediated interaction is bidirectional. Accumulating evidence suggests human HSCs can affect the nervous system and modulate its action. The homing of BM-derived stem cells through the catecholaminergic system involves multiple signaling pathways, including Wnt and β -catenin, as well as specific migratory molecules, such as membrane-bound enzyme MT1-MMP and SDF-1, which all contribute to proliferation, increased motility, and engraftment capability of CD34 HSCs [8]. In terms of clinical stroke data, it is noted that following human acute stroke, the extent of PB immature hematopoietic CD34+ (HSCs) mobilization directly correlates with the recovery of function [9]. Following neurorestorative events such as neoangiogenesis, the upregulation of SDF-1 within ischemic tissue will recruit CXCR4+ HSCs from PB.

HSC mobilization may also serve an integral role in early host repair mechanisms for many other neurological disorders. Endogenous reparative responses have been seen in pathological conditions such as: elevated BM CD34+ HSCs accompanying chronic spinal cord injury, cord blood (CB) CD34+ cells reducing heat stress symptoms upon injection, delays in disease progression of amyotrophic lateral sclerosis upon injection of human CB mononuclear cells into mice, and CB mononuclear injection decreasing β -amyloid deposits in animal Alzheimer models. With the experimental evidence surmounting, influence of the CNS in the mobilization of HSCs suggests potential for the maintenance and repair of the nervous

system upon insult. Furthermore, HSCs have been proposed as an ideal donor graft source because of their safety and efficacy profile in the clinical treatment of other diseases [10].

Mesenchymal Stem Cells

MSC transplantation has been utilized in experimental stroke models and demonstrates improvement in functional recovery of neurological deficits induced by cerebral ischemia. The following sections within this topic will expand upon potential mechanisms that may mediate the therapeutic effect of MSCs in cerebrovascular incidents.

The proposition of stem cell differentiation into neuronal cells remains controversial. Upon transplantation, via intravenous, intracarotid, or intracerebral delivery, the graft survival is modest at best, therefore adequate levels for differentiation seem unlikely [11]. A more plausible mechanism involves the production of trophic factors such as: hepatocyte growth factor (HGF), vascular endothelial growth factor, nerve growth factor, brain-derived neurotrophic factor, basic fibroblast growth factor (FGF-2), and insulin growth factor 1, which may each activate ischemic brain endogenous repair through particular mechanisms [12]. For example, early increases (1 h after stroke) could increase blood-brain barrier (BBB) leakage, exacerbating ischemic cell damage, but when administered 48 h after stroke, vascular endothelial growth factor could enhance angiogenesis in the ischemic border zone to improve recovery [13].

HGF has demonstrated an influential role in vascularization. Upon treatment with HGF, the amelioration of BBB destruction without exacerbating cerebral edema, decreased intracranial pressure, and induction of angiogenesis have all been reported. Although it seems unlikely that MSCs differentiate into neurons themselves, research indicates that transplantation with MSCs may promote migration and induction from subventricular zone and subgranular zone neurogenic sites within the brain to regions of ischemia [12]. This process of neurogenesis appears to be regulated by the neurotrophic factors being secreted by the transplanted MSCs.

A limitation of human MSCs is their lack of telomerase activity, leading to a population doubling of approximately 18, with decreased expectations upon passaging of the stem cells [14]. A mechanism to circumvent this issue is the use of retroviral transfection of human HSCs with the human telomerase gene, termed hTERT-MSCs [15]. Expanding gene manipulation of human MSCs, transfection of genes such as FGF-2, HGF, and brain-derived neurotrophic factor has also been incorporated into hTERT-MSCs before transplantation to extend and increase neurotrophic efficacy [15].

As an alternative to genetic manipulation, studies have also utilized trophic factors as adjuvants with MSC delivery. Studies show that transplantation with brain-derived neurotrophic factor markedly improved stroke recovery in the animal models [16]. The use of other adjuvants, such as cell-permeable inhibitor of caspases (Z-VAD),

enhances graft survival and behavioral recovery when intracerebrally infused with MSCs into the region of ischemia [17]. Additionally, intravenous infusion of MSCs with a nitric oxide donor (DETA/NONOate) demonstrates enhancement of vessel perimeter and endothelial cell proliferation, leading to improved functional recovery in stroke animals [18]. Nitric oxide donor adjuvants have also contributed to increased subventricular zone neurogenesis alongside vascular endothelial growth factor and basic fibroblast growth factor expression within ischemic regions [18]. The use of grafted MSCs may also impart benefits by way of glial cell proliferation including neuron remyelination as well as synaptogenesis and a reduction in apoptosis.

As previously mentioned, the SDF-1/CXCR4 chemoattractant pathway serves as a homing signal for stem cell populations. In the nonhematopoietic system, SDF-1 similarly serves as a signal from injured organs to influence migration of CXCR4 cells. SDF-1 expression is regulated by the hypoxia-responsive transcription factor HIF-1 (hypoxia-inducible factor 1). With transplanted MSCs expressing CXCR4, the SDF-1 gradient pattern associated with the hypoxia gradient provides a signal for attracting both HSCs and nonhematopoietic stem cells [19] to migrate from the periphery to the site of ischemic injury.

Endothelial Progenitor Cells

Although hematopoietic in origin, EPCs can be found in the PB of adults on the one hand as well as derived from umbilical cord blood (UCB). In pioneering studies, transplanted EPCs isolated from human UCB, populated endothelial neovascularization in regions of ischemia. The ability of EPCs to participate in re-endothelialization during neovascularization makes EPCs an exceptional candidate for management of cerebrovascular disease.

Currently, EPCs display a variety of markers for isolation such as: CD31, VE-cadherin, E-selectin, eNOS, and von Willebrand factor [20]; however, substantial evidence suggests that only the CD34+ EPCs from BM or UCB are capable of differentiating into mature endothelium [21]. A contributing factor to the lack of clearly defined methods for cell isolation may be the rare prevalence of EPCs in adult PB (0.01%). Until recently, neovascularization, the formation of new blood vessels, was thought to occur exclusively from proliferation and migration of pre-existing endothelial cells; this process is known as angiogenesis. Juxtaposing neovascularization, vasculogenesis (also known as vascularization) is the differentiation of endothelial cells from precursor cells and was thought to only occur in the embryo during development. Yet, current evidence suggests that BM-derived EPCs in circulation are capable of homing to neovascularization sites for proliferation and differentiation of subsequent endothelial cells [22].

Over the last few years, clinical research has suggested that circulating EPCs as a biomarker may predict clinical outcome of cardiovascular disease, with low EPC

counts correlating to more severe functional impairments. Expanding upon this observation, clinical studies have also been initiated to assess the higher risk for atherosclerotic events in populations with lower EPC numbers. In terms of clinical applications for neurovascular disease, the observational studies are limited and with notable discrepancies.

The primary mechanisms of stroke pathogenesis remain unclear; however, there is mounting evidence that implicates an immunological attack upon the brain and/or its vasculature, which provides a novel therapeutic stroke target involving EPCs. This immunological attack could result in altered interendothelial junction integrity, leading to vascular endothelial damage and breakdown of the BBB. Therefore, restoration of this barrier through EPC therapy may serve to abrogate the consequences of stroke pathogenesis.

Very Small Embryonic-Like Stem Cells

Present in a variety of adult organs, specifically the brain, VSELs express several progenitor stem cell markers. These very tiny stem cells can be mobilized into the PB following tissue and organ injuries. Human VSELs, smaller than an erythrocyte, belong to the nonhematopoietic fraction of leukocytes (Lin-/CD45 cells) expressing CD34, CD133, and CXCR4 antigens [23]. Due to their low constitution of PB, special flow cytometric protocols have been established for identification. VSEL phenotypic markers include: CD45 (mouse and human), positive expression of Sca-1 (mouse), CXCR4, CD133, and CD34+ (mouse and human), positive progenitor stem cell markers (i.e. Oct-4, Nanog, and SSEA), and express markers of epiblast/germ line stem cells [23]. In addition to PB, purified VSELs can be isolated from BM.

With the notion being that VSELs are epiblast-derived stem cells deposited early in embryonic development, these stem cells may present as a good candidate for tissue rejuvenation and regeneration. The ease of harvesting should also be considered as a therapeutic potential. The patients' own BM, stored UCB, and mobilized PB are sources readily accessible in harvesting VSELs for autologous transplantation. With respect to allotransplantation, histocompatible-related or unrelated donors could serve as another source. Yet, despite the ease of harvesting these cells, expansion strategies must be employed due to the relatively low number of cells yielded.

Treatment strategies for the acute and subacute stage (time 0–1 week after injury) appear to provide the best opportunity to initiate therapeutic intervention. Due to this immediate need for intervention, purifying these cells from BM aspirates, UCB, or mobilized PB through multiparameter staining and regular high-speed sorting may not be feasible [24]. To counter this challenge, the Ratajczak group [25] proposed a relatively short and economical three-step method for isolation that allowed approximately 60% recovery of the initial number of Lin-/CD45-/CD133+ UCB-VSELs.

This novel procedure takes 2–3 h per UCB unit (ideally applicable using BM aspirates as well as mobilized PB) and should produce VSELs freshly isolated from BM, PB, or UCB that are precommitted to neurological lineage in *ex vivo* cultures [25].

Conclusion

The developmental biology research elucidating stem cell plasticity has served as the impetus for advancing regenerative medicine in many neurological disorders, including stroke. Some of the most commonly explored cell lines include: HSCs, MSCs, EPCs, and VSELs, all with specific therapeutic potential. Each of these cell lines does, however, impart its own individual challenges.

The ability of HSCs to develop into differentiated neurons has yet to be determined. Opposing this notion, transdifferentiation may be explained as a transient change in phenotypic expression induced by neural tissue-derived spherical membrane fragments called microvesicles. These fragments, also termed exosomes, may transfer neural cell surface receptors, mRNA, and miRNA to the HSCs employed for regeneration [26].

An emerging concern in the use of MSCs involves the potential to cause neoplastic tumor formation upon deposition into the brain. Similar to the initial impression of HSC differentiation, it was challenged whether MSCs are able to develop into neuronal cells. One possible explanation for this finding was *in vitro* contamination in the cell culture media that may alter the morphology of MSCs [27]. Therefore, the working postulate is that, upon homing of the stem cell to the site of injury, the production of trophic factors influences the microenvironment. Evidence that grafted stem cells do not persist after delivery and are rapidly eliminated supports this proposal.

More recently, my research group has explored EPC transplantation for repair of the BBB after stroke [28–30]. The working hypothesis suggests that tissue plasminogen activator may exacerbate the breakdown of the already vulnerable BBB. Currently, much of the stroke therapy implemented does not consider the capacity of BBB damage after stroke. It is our contention that if EPC transplantation promotes restoration of the vascular endothelium, the clinical effects could be far reaching and substantially help a large population of patients that may be excluded from the current 3-hour guideline for tissue plasminogen activator.

Lastly, another appealing therapy for stroke is the use of VSELs. A prominent restriction in cell therapy is their ability to cause embolism, especially accompanying the large quantity necessary for a therapeutic effect. This caveat makes the use of VSELs appealing. Because the isolation and expansion of this cell line may be more tedious and longer, the use of allogenic transplants and faster expansion protocols are to be considered.

In summary, the plethora of accumulating stem cell research is quickly translating into clinical trials. The use of HSCs, MSCs, EPCs, and VSELs all appear to provide

specific insight into treating neurological disease from many facets. However, it is important to acknowledge that these mechanisms are yet to be fully determined and there is still a gap in our translational laboratory-to-clinic understanding of stem cell therapy. Therefore, as the research transcends theory and progresses into treatment, we must ensure that systematically designed preclinical studies precede initiation of clinical trials to allow rigorous investigations as to the safety and efficacy of these stem cells.

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Translational Challenge for Bone Marrow Stroma Cell Therapy after Stroke

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Abstract

There is increasing evidence that the transplanted bone marrow stromal cells (BMSC) significantly promote functional recovery after central nervous system (CNS) damage in the animal models of various kinds of CNS disorders, including cerebral infarct. However, there remain several challenges before considering BMSC transplantation for patients with ischemic stroke. In this review, therefore, the authors discuss what should be clarified to establish cell transplantation therapy in the clinical setting and describe their scientific contributions in this matter. The BMSC have the ability to alter their gene expression profile and phenotype in response to the surrounding environment and to protect the neurons by producing certain neurotrophic factors. They also promote neurite extension and rebuild the neural circuits in the injured CNS. The BMSC can be expanded in vitro using the animal serum-free medium. Pharmacological modulation may accelerate the in vitro proliferation of the BMSC. Using in vivo optical imaging technique and MRI, the transplanted BMSC can noninvasively be tracked in the living animal for at least 8 weeks after transplantation. It is urgent to develop a clinical imaging technique to track the transplanted cells in the CNS and evaluate the therapeutic significance of BMSC transplantation in order to establish it as a definite therapeutic strategy in the clinical setting in the future.

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Ischemic stroke still has a high incidence all over the world and can oftentimes cause long-standing disability. There are a few if any drugs that are effective to protect or repair the central nervous tissue (CNS) tissue in the clinical setting in spite of numerous numbers successful applications in animal models [1]. However, recent discoveries strongly suggest that cell transplantation therapy may promote functional recovery after various kinds of CNS disorders, including ischemic stroke. A variety of cell types have been studied as cell source of transplantation in animal models of CNS

disorders, including embryonic stem cells, neural stem cells, induced pluripotent cells, and bone marrow stromal cells (BMSC). Of these, the BMSC may have the most enormous therapeutic potential, because they can be harvested from the patients themselves without posing ethical or immunological difficulties [2, 3]. Although the results obtained from basic research are quite encouraging, a variety of questions or problems still remain to be solved [4, 5]. In this review, we present recent progress in basic and translational research on the field of BMSC transplantation for ischemic stroke, and critically discuss what should be clarified to establish BMSC transplantation therapy as a scientifically proven method in the clinical setting.

Basic Aspects of Bone Marrow Stromal Cell Transplantation

There is increasing evidence that the transplanted BMSC enhance functional recovery by differentiating into the neural and endothelial cells and/or by producing various kinds of cytokines or growth factors that can restore the host neurons [5, 6]. They differentiate into fat, bone, and cartilage, but can also transdifferentiate into embryologically unrelated tissues, including neural cells [7, 8]. It may sound strange that the BMSC have the ability to differentiate into neural cells. However, the BMSC per se express the genes related to neuronal and glial cells [9]. They can also have the potential to modify their gene expression profile under certain experimental conditions [9] and start to differentiate into neurons without evidence of cell fusion [6]. Furthermore, the BMSC are also known to produce some neuroprotective or neurotrophic factors and support the survival of the host neural cells [10]. It is quite close to their primary function that BMSC generally support the homing and proliferation of the hematopoietic cells in the bone marrow [11]. The conditioned medium of BMSC significantly promotes neurite outgrowth from the dorsal root ganglion [12]. More interestingly, when the BMSC are co-cultured with the neurons exposed to glutamate, they significantly increase their release of soluble neuroprotective factors such as nerve growth factor and brain-derived neurotrophic factor and ameliorate the neuronal injury [6]. According to these observations, the BMSC may consist of heterogeneous cell populations and protect and/or repair the damaged CNS through multiple mechanisms [6].

Recent *in vivo* studies have gradually elucidated their actions in the injured brain. According to new research, the engrafted BMSC maintain their aggressive proliferation property even after intracerebral transplantation into the infarct brain [13]. They migrate towards the infarct tissue through chemokine systems such as monocyte chemoattractant protein 1, stromal cell-derived factor 1 and hepatocyte growth factor [14–16]. Numerous numbers of experimental studies have proven that the engrafted BMSC express the proteins specific for neurons, astrocytes, and endothelial cells in the peri-infarct area [17–20]. Alternatively, they may support the survival of the host neurons through their paracrine production of soluble factors (see above).

However, it still remains to be defined how the engrafted cells contribute to functional recovery after cerebral infarct. A recent study has shown that the engrafted BMSC express γ -aminobutyric acid receptor and improve the binding potential for ^{125}I -iomazenil in the peri-infarct area [21]. They also improve glucose metabolism in response to sensory stimuli when transplanted into the rat cold injury model [22]. Furthermore, an ^{18}F -fluorodeoxyglucose PET study has very recently shown that the BMSC markedly improve the recovery of glucose metabolism in the peri-infarct neocortex, when stereotactically transplanted into the infarct brain at 7 days after ischemia [23].

According to a recent study by Liu et al. [24], the BMSC may enhance axonal sprouting from the survived cortical neurons in the peri-infarct area. Furthermore, Chiba et al. [25] have recently found that the BMSC are integrated into the neural circuits of the host spinal cord and promote functional recovery. These biological properties of BMSC may play a key role in the enhancement of functional recovery after ischemic stroke.

Translational Aspects of Bone Marrow Stromal Cell Transplantation

As described above, the observations in basic experiments are quite encouraging, and some clinical trials of BMSC transplantation have already been started in patients with ischemic stroke. Bang et al. [26] intravenously injected autologous BMSC into 5 patients with severe neurological deficits due to ischemic stroke at 5–9 weeks after the onset, and preliminarily concluded that autologous BMSC infusion is a feasible and safe therapy that may improve functional recovery. Honmou et al. [27] intravenously transplanted BMSC into 12 patients with ischemic stroke 36–133 days after stroke. Very recently, Lee et al. [28] performed an open-label, observer-blinded clinical trial of 52 patients with ischemic stroke, and followed them up for up to 5 years. These authors conclude that intravenous transplantation of autologous BMSC could be a safe and effective strategy for ischemic stroke.

However, there are many variables that may affect the efficacy of BMSC transplantation in the clinical setting. They include donor cell factors (safety, autologous or allogeneic, ex vivo cell expansion), patient factors (age, stroke type), treatment factors (interval since onset, delivery route, cell dose), and validation factors (neurological assessment, imaging) [29, 30].

First, allogeneic cells permit ‘off the shelf’ use even within 24 h after onset, but they imply long-term medication of immunosuppressants. Autologous BMSC from patients themselves would be ideal as donor cells for restorative medicine, but require several weeks for ex vivo expansion. Therefore, it should be scientifically proven that the BMSC can enhance functional recovery after ischemic stroke even when transplanted several weeks after the onset. More importantly, it would be critical to establish a feasible protocol to ‘safely and rapidly’ expand the BMSC. Thus, BMSC

have been cultured in medium including fetal calf serum (FCS) in the majority of animal experiments and even clinical trials [26]. However, FCS carries the potential risk of prion, viral, or zoonoses contamination. Alternatively, autologous serum is employed to expand the BMSC, but this may require a large amount of serum [27]. Very recently, human platelet lysate has been proven useful to expand BMSC as an alternative substitute. The human BMSC expanded with the FCS-free, platelet lysate-containing medium retain their capacity of migration, survival and differentiation, and significantly promote functional recovery when stereotactically transplanted into the infarct brain. Therefore, platelet lysate may be a clinically valuable and safe substitute for FCS in expanding human BMSC to regenerate the infarct brain [17, 20, 31].

Second, BMSC were transplanted within 24 h or 7 days after the insult in the majority of animal studies, whereas they were usually transplanted several weeks (or even several months) after stroke onset in previous clinical trials [26–28]. In order to resolve this problem, pharmacological modulation may be useful to promote *in vitro* proliferation of cultured BMSC to shorten the interval between stroke onset and cell therapy. For example, granulocyte colony-stimulating factor significantly enhances the proliferation and growth factor production of the cultured BMSC and accelerates functional recovery by BMSC transplantation into the infarct brain [32]. Such pharmacological modulation would be essential in considering autologous stem cell therapy for older patients with ischemic stroke, because adult stem cells, including BMSC, suffer from ageing effects and reduce their self-renewal and differentiation capacity [33]. A very recent study has also demonstrated that granulocyte colony-stimulating factor significantly promotes the proliferative capacity of BMSC harvested from the aged rats [34]. These observations should be taken into consideration when establishing the treatment protocol in the clinical setting.

Third, the BMSC can be transplanted directly, intravenously, intra-arterially, or intrathecally. Although direct, intracerebral, or stereotactic injection permits most efficient delivery of the donor cells to the damaged tissue, a less invasive procedure would be more favorable. Intravenous or intrathecal transplantation is attractive because of its noninvasive, safe technique for the host CNS, but has been reported to result in less pronounced cell migration and functional recovery than direct cell transplantation [35]. Alternatively, the intra-arterial injection of BMSC may be valuable to noninvasively deliver them to the damaged CNS [19, 36]. Therefore, an optimal transplantation technique should be developed leading to maximally safe and efficient results. Currently, there are limited numbers of studies that directly compare the therapeutic effects of these delivery routes under the same conditions. It is an urgent need to test the effects of each delivery route on functional recovery after cerebral infarct. Recently, Kawabori et al. [37] transplanted BMSC into the infarcted brain, directly or intravenously, at 7 days after the insult, which is a clinically relevant timing. The authors conclude that intravenous administration of

BMSC has limited effectiveness at a clinically relevant timing and intracerebral administration should be chosen for patients with ischemic stroke. Furthermore, they directly transplanted the BMSC (1×10^5 or 1×10^6) into the infarct brain at 1 or 4 weeks after the insult, and found that earlier transplantation requires a smaller number of donor cells for beneficial effects [38]. These observations strongly suggest the importance of timing, delivery route, and cell dose to yield therapeutic effects of BMSC transplantation for ischemic stroke. Similar translational research should thoroughly be conducted to establish a scientifically proven protocol prior to the start of clinical testing.

Finally, it is essential to develop techniques to serially and noninvasively track the fate of the transplanted cells in the host CNS. A cell tracking technique would also be important as a 'biologically relevant end point' [1]. Magnetic resonance imaging, nuclear imaging, and optical imaging are candidate modalities. The donor cells can be identified on magnetic resonance imaging by labeling with a superparamagnetic iron oxide agent [20, 39]. On the other hand, the optical imaging technique may also serve future technology to visualize the BMSC engrafted in the damaged CNS. Quantum dot emits near-infrared fluorescence with a longer wavelength (800 nm) that can easily penetrate the living tissue. A very recent study has shown that quantum dot-labeled BMSC can be clearly visualized under *in vivo* fluorescence imaging through the skull and scalp for at least 8 weeks when transplanted into the infarct brain of rats [18, 19]. Imaging technology is valuable to assess the effects of BMSC transplantation on the function of the host brain. As described above, ^{18}F -fluorodeoxyglucose PET may be a useful tool to visualize the beneficial effects of BMSC transplantation for ischemic brain in the clinical setting [22, 23].

Conclusion

Recent studies have strongly suggested the therapeutic potential of BMSC transplantation for ischemic stroke. While mostly still in the experimental stage, the authors would like to emphasize that further translational studies are warranted to establish it as a scientifically proven strategy in the clinical setting.

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Experimental Evidence and Early Translational Steps Using Bone Marrow Derived Stem Cells after Human Stroke

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Abstract

Neurogenesis is principally restricted to the subventricular zone of the lateral ventricle wall and the subgranular zone of the hippocampal dentate gyrus in physiological situations. However, neuronal stem cells are known to be mobilized into the post- and peristroke area and we have demonstrated that appropriate support of these stem cells, achieved by therapeutic angiogenesis, enhances neuroregeneration followed by neuronal functional recovery in an experimental stroke model. We also found that neural stem cells are mobilized in patients after stroke, as well as in animal models. Based on these observations, we have started cell-based therapy using autologous bone marrow-derived stem/progenitor cells in patients after stroke. This review summarizes the findings of recent experimental and clinical studies that have focused on neurogenesis in the injured brain after cerebral infarction. We also refer to the challenges for future cell-based therapy, including regeneration of the aged brain.

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Stroke is the third leading cause of death in developed countries after heart disease and cancer [1], and the leading cause of disability worldwide. More than 50% of stroke survivors are unable to completely recover and 20% of stroke patients require assistance with their daily activities [2]. Although thrombolysis can improve the functional outcomes of stroke patients, patients must be treated within 3 h (or 4.5) of the onset of a stroke [3] and no definitive treatment exists after that period other than rehabilitation. To improve functional recovery after stroke, clinical trials of various drugs have been conducted but have achieved either only mild or nonsignificant therapeutic effects, or have sometimes even had serious adverse effects [4, 5]. Thus, development of novel and safe therapies is eagerly awaited.

Recently, a number of studies have focused on cell-based therapies to promote the neuronal regeneration in the ischemic brain [6–8]. In this chapter, we present current

basic and clinical findings that focus on therapeutic neurogenesis after stroke. We also refer to a novel cell-based therapy that may enable regeneration of the aged brain.

Neuronal Regeneration Is Activated after Cerebral Ischemia

Neuronal tissue in the central nervous system is well known for its limited reparative/regenerative capacity. Physiologically speaking, neurogenesis is principally restricted to the subventricular zone of the lateral ventricle wall and the subgranular zone of the hippocampal dentate gyrus, where unique niche architectures permit continuous neurogenesis [9, 10]. In pathological situations, recent studies using experimental models have revealed that endogenous neurogenesis is activated around injured areas where neurogenesis does not occur under normal conditions [11]. Consistent with these findings, histopathological studies in stroke patients have pointed out the presence of neural stem/progenitor cells in the post-stroke human cerebral cortex, and that the peak in endogenous neurogenesis occurs approximately 1–2 weeks after a stroke [12]. These findings indicate the potential for a novel therapeutic strategy using injury-induced neurogenesis for functional recovery in patients with cerebral infarction.

Angiogenesis Is Essential for the Survival of Injury-Induced Neuronal Stem Cells

The post brain-injury neurogenic response eventually yields only a very small number of mature neurons, as most of them die after the initial boosting [11]. To achieve functional recovery by endogenous neuroregeneration, appropriate support for their survival is essential and angiogenesis has been proposed as the key element in this [7]. In the adult songbird, testosterone-induced angiogenesis leads to neuronal recruitment into the higher vocal center [13]. In the adult rat, endogenous neurogenesis and neovascularization occur in proximity to one another in the cortex following focal ischemia [14]. Moreover, angiogenesis and neurogenesis have been shown to use the same molecules for their regulation; sphingosine-1-phosphate, for example, plays a critical role in neurogenesis and angiogenesis during embryonic development [15]. This accumulating evidence indicates a close relationship between the vascular system and neurogenesis in the central nervous system, and recent studies have focused on the promotion of neurogenesis in association with angiogenesis [6].

Cell-Based Therapy to Enhance Neurogenesis in Ischemic Brain

To achieve angiogenesis in ischemic tissue, an approach using bone marrow-derived mononuclear cells, a rich cell source of both hematopoietic stem cells and endothelial stem/progenitor cells, has been proposed. Local transplantation of bone marrow-

derived mononuclear cells in experimental models of limb ischemia significantly induces angiogenesis and releases ischemic stress in experimental models [16]. Based on these results, clinical trials were initiated, and a cure for ischemic ulcer, with significant angiogenesis in ischemic limb, has been reported [17]. The potential for transplantation of bone marrow-derived mononuclear cells to myocardial ischemia patients was also investigated and demonstrated a therapeutic effect in experimental models. Clinical trials were initiated in patients with ischemic heart disease and the therapeutic potential for improvement in regional perfusion and heart function has been reported [18].

Based on these experimental and clinical findings, we investigated the effect of intravenous transplantation of bone marrow-derived mononuclear cells [19] and hematopoietic stem cells [7] in an experimental model. As a result, we found the following three effects: (a) cell therapy enhances neovascularization at the border of the ischemic zone; (b) neovascularization is essential for the survival of injury-induced neuronal stem cells, and (c) supporting the survival of endogenous neurogenesis improves functional outcomes [19]. The positive effect of bone marrow-derived mononuclear cells was negated by administration of an anti-angiogenesis reagent [19]. It is noteworthy that survival of transplanted cells was rarely observed, despite significant activation of angiogenesis by cell therapy. These findings indicate that the differentiation of the stem cells into endothelial cells in the ischemic brain is not essential for angiogenesis after stroke and therapeutic angiogenesis could be a novel therapeutic strategy to enhance functional recovery after stroke.

To examine the effects of the mobilization of hematopoietic stem cells from bone marrow by drug administration, granulocyte colony-stimulating factor was given in an experimental stroke model and found to impair functional recovery with brain atrophy and with exaggerated inflammatory response at the border of the ischemic region [20]. This result suggested that the mobilization of bone marrow cells, including both granulocytes and hematopoietic stem cells, by granulocyte colony-stimulating factor might augment the inflammatory response consequent to ischemic tissue damage. We also investigated the effect of intravenous transplantation of bone marrow-derived mesenchymal stem cells in an experimental stroke model but found only a mild or nonsignificant effect on functional recovery (unpublished data), though mesenchymal stem cells have the potential to suppress excessive inflammation [21].

In a preclinical trial, we investigated the appropriate cell numbers and optimal therapeutic time window using a highly reproducible murine stroke model [22] and found that administration of a relatively small number of bone marrow-derived mononuclear cells had a significantly beneficial effect on the regeneration of injured brain tissue [23]. Analysis of the therapeutic time window revealed that administration of bone marrow-derived mononuclear cells at 24 h after stroke had a mild or nonsignificant effect on regeneration following ischemia, but administration of these cells between day 2 and day 14 after the ischemic event had a significantly positive effect. This result may be attributed to the time lag between the onset of stroke and the peak of neurogenesis [12].

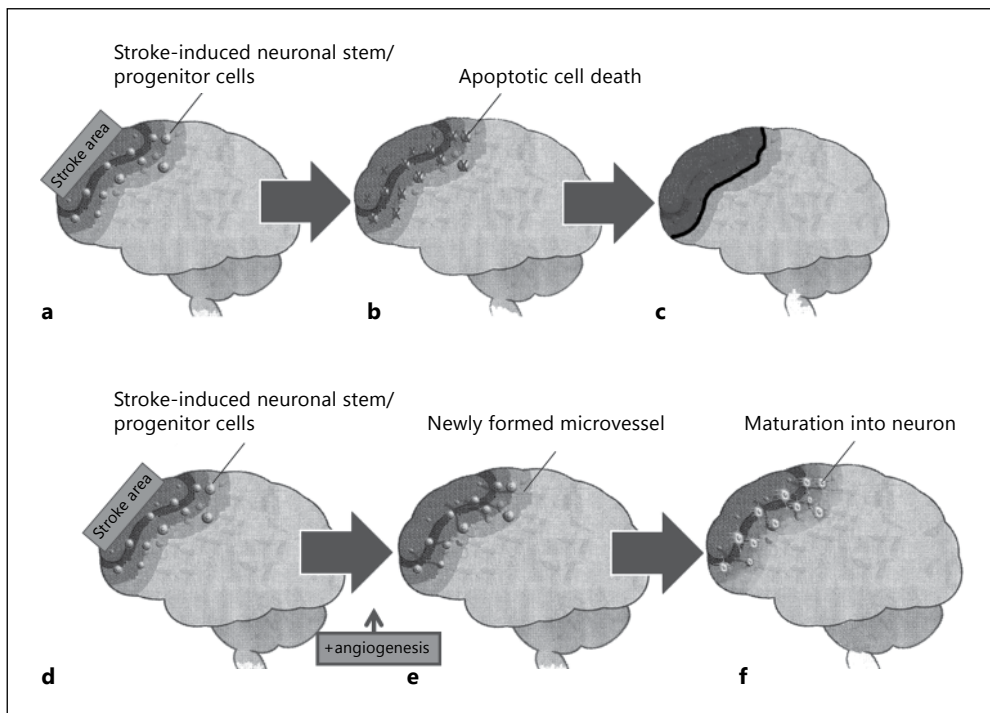


Fig. 1. Schematic representation of cell-based therapy for patients with cerebral infarction. **a–c** Neurogenesis after stroke without therapeutic angiogenesis. Endogenous neurogenesis is activated around the stroke area (**a**). However, stroke-induced neuronal stem/progenitor cells do not survive because of the lack of an appropriate environment (**b**), and do not contribute to functional recovery (**c**). **d–f** Neurogenesis with angiogenesis. Stroke-induced neuronal stem/progenitor cells (**d**) survive in an environment with angiogenesis (**e**). Neuronal stem/progenitor cells differentiate into mature neurons and contribute to functional recovery (**f**).

Clinical Trials to Enhance Neurogenesis in Patients after Stroke

Based on these results, we initiated a clinical trial to enhance neurogenesis and functional recovery through activating angiogenesis in patients with cerebral infarction. A schematic representation of this therapy is shown in figure 1. Our clinical trial is an unblinded, uncontrolled phase 1/2a study (ClinicalTrials.gov Identifier: NCT01028794). The major inclusion criteria are patients with cerebral embolism, day 7 after stroke, a National Institutes of Health Stroke Scale (NIHSS) score of more than (or equal to) 10, and an improvement in the NIHSS score of less than (or equal to) 5 since admission. On days 7–10 after stroke, either a 25-ml (low-dose group, n = 6) or a 50-ml (high-dose group, n = 6) aspiration of bone marrow cells was performed. These mononuclear cells were purified by Ficoll-Paque Premium (GE-Healthcare, USA) and administered intravenously on the day of the bone marrow aspiration. The primary outcome measures are improvement of the NIHSS score at 30 days after

treatment and frequency of change for the worse on the NIHSS at 30 days after treatment, compared with historical control. Though this clinical study is currently still underway, we have already treated 11 patients (6 in the low-dose and 5 in the high-dose group), and no side effects or safety problems have been observed to date. Results related to the therapeutic effects of the treatment are expected in a year. Similar clinical trials are being carried out in other countries, including the USA, UK, Brazil and Spain, with promising results [24, 25]. Though the route of administration (intravenous or intra-arterial) and cell source (bone marrow mononuclear cells or CD34-positive cells) vary, no side effects or safety problems with cell therapy have been reported. The current status of most of these ongoing clinical trials can be searched through <http://clinicaltrials.gov/>.

Future Cell-Based Therapy for Prevention of Cerebrovascular Diseases

Previously, we have shown that patients with cerebrovascular disease have a decreased level of circulating bone marrow-derived immature cells, the latter associated with impaired cerebrovascular function [26] and impaired cognition [27], whereas increased levels of bone marrow-derived immature cells are associated with neovascularization of the ischemic brain [28]. In addition, we have demonstrated that partial rejuvenation of bone marrow stem cells in aged rats improves vascular function and reduces ischemic damage after induction of stroke in stroke-prone spontaneously hypertensive rats [29]. Furthermore, we investigated the effect of bone marrow-derived stem cells on white matter damage in a mouse model of cerebral hypoperfusion and found that administration of bone marrow-derived stem cells has a significant protective effect against white matter damage by enhancing cerebral blood flow via the activation of nitric oxide synthase [30]. These findings clearly indicate that bone marrow-derived stem/immature cells have the potential to improve microvascular circulation and prevent cerebrovascular diseases, and the challenge to find novel strategies using autologous, allogeneic or induced pluripotent stem cell-derived hematopoietic stem cells to regenerate the aged brain is ongoing.

Conclusion

Currently, for patients after stroke, there is no specific recovery-targeted treatment other than physical and cognitive rehabilitation techniques after the period of thrombolysis. However, accumulating evidence indicates significant activation of neurogenesis after stroke, and utilization of the stroke-induced neuronal stem cells, we believe, will become a major therapeutic target for the acceleration of functional recovery. The mechanism that links angiogenesis and neurogenesis cannot be attributed to a single molecule or signaling pathway. It is likely that multiple cytokines,

growth factors, and cell adhesion molecules are involved, and the balance between these molecules may determine the fate of injured brain tissue. Careful, step-by-step investigation will lead to more efficient neurogenesis with a longer therapeutic time window. Experimental and clinical research focusing on neuroregeneration is needed to enhance functional recovery in patients after stroke.

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Therapeutic Drug Approach to Stimulate Clinical Recovery after Brain Injury

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Abstract

The identification of strategies by which the central nervous system (CNS) can transform itself in response to injury has incited the systematic exploration of methods to enhance neurological recovery after CNS injury. Several pharmaceuticals have been shown to promote such recovery; however, more rigorous clinical trials are necessary to establish their clinical relevance. The major impediment for these strategies in the clinical arena is the astounding heterogeneity surrounding neuroplasticity and regeneration. Tolerance to injury and varied rates of recovery are likely governed by genetic and environmental factors that remain largely elusive. The extraordinary complexity of the neural networks in the CNS impedes the assessment of 'plain' pharmacological interventions in therapeutic trials. 'Proof-of-principle' studies of pharmacological interventions enhancing neuroplasticity or regeneration may therefore at first focus on surrogate markers, such as functional MRI, magnetoencephalography and diffusion tensor imaging, or investigate seemingly more uniform systems, such as spinal cord injuries. The discovery that experimental adult CNS lesions can essentially regenerate has rejected the conviction that adult axon injury is always permanent and spurred research into determining whether the circumstances under which such regeneration occurs can be created in human CNS injury. The hostility of the microenvironment preventing axonal regrowth has been linked to key molecular targets involving myelin-associated factors and glial scar components. While the mechanisms involved are better understood now and potential therapeutic targets are identified, the crucial question whether manipulating the molecular regulation of axonal repair is feasible and will benefit patients remains uncertain. While factual repair of brain tissue may still be years away, research into the mechanisms of adaptation after brain injury offers more tangible return on the short run.

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Traumatic and vascular brain injuries are among the leading causes of adult disability in the developed world [1, 2]. In recent years, basic and clinical research results have triggered re-emphasis on recovery from chronic disability following brain and spinal cord injuries. Basic science discoveries have determined mechanisms underlying neurological recovery while clinical research identified redress processes that occur within the injured brain or spine during recovery [3, 4]. Therapeutic perspectives

related to these discoveries have spurred much clinical interest in this area. In general, neuroregeneration, consisting of axonal reconstruction and/or collateral sprouting, is distinguished from neuroplasticity, the ability of nervous tissue to adapt to injury by reorganizing its intrinsic network to regain and/or modify functionality. When recovering from brain and spine injury, these mechanisms may occur simultaneously, at various intensities and at different time periods after injury. While initial clinical improvement is thought to be mediated by neuroplasticity accompanied by moderate sprouting from collateral axons, persistent neurological deficits are believed to indicate the lack of axonal regrowth/sprouting and/or maladaptive plasticity. Given the extraordinary size and redundancy of the neural circuitries, many brain lesions can be completely corrected by plasticity, while others, depending on extension and/or location, may not respond to system adaptations alone. Furthermore, it is likely that genetic factors affect tolerance to injury, or modify neuroplasticity, axonal regrowth or sprouting resulting in heterogeneity of recovery after brain injury [5, 6]. This chapter assumes a proportionate clinical deficit according to location, extension, patient characteristics and acute treatment-related factors that triggers a uniform cascade of adaptive/reparative responses. The corresponding clinical deficit is the baseline variable from which complete or incomplete recovery may occur as a result of neuroplasticity and neuroregeneration.

Mechanisms Underlying Clinical Recovery

Variability in recovery from brain injury is substantial across human patients. The mechanisms for this disparity are largely unknown but experimental and human studies suggest a broad range of underlying molecular and physiological events. Recently, brain mapping studies in human patients recovering from brain injury have provided insight into the plasticity on a systems level and may help to tailor and monitor responses to rehabilitative strategies [4]. Rather than being driven by functional deficits alone, treatments may need to be tailored according to the lesion topography and the temporospatial activation pattern detected by functional MRI. Reorganization of central nervous system (CNS) networks after injury awards recovery by alterations in interhemispheric lateralization [7], activity of association cortices linked to the injury and/or reorganization of cortical representational maps [8]. Recovery afforded by neuroplasticity alone usually remains incomplete, such as known from spastic hemiplegia after stroke or paraplegia after spinal cord injury. Deficiencies deriving from axonal disconnections cannot be rectified by reorganization alone. Strategies to recover or replace neurons consumed by the injury and/or disease processes are ultimately required to fully restore the preinjury function. In this regard, patient characteristics, including demographics, behavioral experiences, and genetics greatly influence recovery and are important covariates to be considered in future trials of restorative agents/devices that target recovery from brain injury.

Genetic Variability

In recent years, much research has been conducted to explain the heterogeneous recovery from brain injury. Variations in recovery from brain injury may be related to genetic polymorphisms within the systems generating plasticity and regeneration or depend on variability in tolerance to the inciting brain injury. Studies assessing the genetic variants of dopaminergic, serotonergic neurotransmission, or genes involved in synaptic plasticity, including brain-derived neurotrophic factor and kidney- and brain-expressed protein, have revealed influences on cognitive domains [9, 10]. These polymorphisms may explain various clinical responses to pharmacological therapies and physical or cognitive training after brain injury. So far, research in this area is still in its infancy; however, future investigations may help to tailor individual rehabilitative efforts depending on biologically distinct genetic patterns. In addition, variability in myelin-associated inhibitors, such as the Nogo-A family of proteins, and astroglial scarring, involving chondroitin sulfate proteoglycans, influence neural regeneration. In a genomic association study, two single nucleotide polymorphisms in close proximity to *NINJ2*, encoding ninjurin-2, were associated with an increased risk of stroke. Ninjurin-2 is a transmembrane protein that has been induced and shown to promote axonal regeneration in peripheral nerve injury models [11]. In the CNS, it is constitutively expressed at low levels by radial glia and may when induced counteract inhibition of axonal regeneration and sprouting by myelin-associated inhibitors, in particular Nogo-A. Experiments with knockout mice suggest that the level of ninjurin-2 expression may affect the tolerance of the brain to injury [12].

Plasticity

The ability of nervous tissue to reorganize its network in response to intrinsic or extrinsic stimuli by retooling connections and functions is the hallmark of neuroplasticity. Plasticity after brain injury describes changes in the intrinsic network incited by damaged fibers and correlated functional loss. It can be conceived on different levels, from molecular to cellular to systems and cognition. It appears during development and in response to the environment, including socialization, learning, injury or recovery. In principle, neuroplasticity can be adaptive or maladaptive. When adaptive, neuroplasticity may result in functional gains, such as recovery after brain injury. When maladaptive, it may result in deficiencies, such as reflex sympathetic dystrophy following peripheral nerve damage or dystonia after brain injury. Clinical neuroplasticity has most intensively been studied in motor recovery after stroke [7, 13]. Connective adaptations within the injury-diminished network occur via abundant molecular changes affecting synaptic connections of residual neurons aiming to renovate the corrupted circuitry. Spontaneous motor recovery after stroke illustrates that injury to a region in the motor network can induce intrahemispheric

changes, such as changes in the representational maps, while the interhemispheric balance can shift to enhanced activity in relation to movement at the same time. Similar adaptations have been demonstrated in recovery from aphasia and neglect although the underlying networks employ different anatomic pathways. Many examples of plasticity in the human brain involve biochemical changes in synaptic activity without anatomic reorganization. Neuroplasticity during recovery in patients can be exposed by brain activity mapping using functional MRI or magnetoencephalography. Exposing brain activity patterns during spontaneous recovery after brain injury may reveal temporospatial motifs of alternate or latent pathway recruitment and provide insight into mechanisms of favorable versus failing rehabilitation. These advanced techniques to detect and associate brain activity may also provide a window into the mechanism of pharmacological approaches to recovery after brain injury [14]. Monitoring brain activity over time during recovery may also establish temporal patterns and help defining time windows for various candidate drugs. Pharmacological approaches affecting neuroplasticity are thought to facilitate recovery by modulating certain neurotransmitter and neurotrophic systems altering synaptic plasticity and assist in the progress of resuming lost capacities. In spite of the above-outlined self-repair mechanisms of adaptive reorganization, adult human brain and spinal cord damage commonly produces persistent deficits with only limited recovery. Persistent deficits result from the restricted capacity of the adult human brain and spinal cord to re-extend and rearrange severed axonal connections and replace expired neurons.

Regeneration

The term ‘neuroregeneration’ describes the growth of injured or damaged axons over longer distances and longer periods of time. Sprouting refers to growth from injured or damaged fibers or from intact fibers over shorter distances. Regeneration is the most time-consuming of these mechanisms taking weeks to months to produce functional improvements while sprouting and particular plasticity may occur within days or even hours of injury. Injury-related axonal damage opposes neuroregeneration by triggering a cascade of axonal deterioration comparable to apoptosis after nonlethal neuronal damage. Presumptive mechanisms, including poor axonal transport, mitochondrial dysfunction and calcium influx have been identified. Four responses delineate possible axonal responses to injury and may occur both concomitantly or sequentially. (a) A wide range of insults triggers axon degeneration impairing the prospects of recovery [15]. Proactive axonal death programs and genetic factors may determine the scope of deterioration but need to be understood in more detail before they can be affected. (b) Ordinarily, only minimal if any axonal regeneration occurs after injury. Axonal growth can be stimulated after various injuries under experimental conditions [16]. Axons severed by a disease process may extend themselves from the dis-

tal severed end or regrow as collaterals from the intact proximal segment. Adjacent neuronal networks, not involved in the primary injury, can stimulate collateral pathways. Finally, altered sensory input and even injunction from nonneuronal tissue damage can stimulate axonal regrowth. (c) Axonal regeneration after injury is time sensitive. Chronic adjustments may depend on the contingency of certain early modifications within the network. Without proper initiation, long-term repair may result in functional discord. (d) In spite of the often meandering course within the CNS, regrowing fibers are able to connect with proper targets in experimental studies. Although many mechanisms of axonal regeneration are elusive at this time, further research into the development of therapeutics to spur axonal growth and halt stagnation by axonal degeneration at the same time are needed to move the field into the clinical arena [3]. Promoters of axonal regrowth are being identified and provide possible therapies to stimulate regeneration [17]. In addition, inhibitory proteins derived from myelin and the astroglial scar enacting as major barriers for successful regeneration of injured CNS neurons can be targeted as well [18].

Stimulating Plasticity by Neuropharmaceuticals

Cellular and molecular events underlying neuroplasticity include specific neurochemical processes accessible to pharmacological interventions. All of the agents in the current clinical evaluation represent accidental discoveries and systematic research is still in its infancy. Several medications already in clinical use for other indications and even naturally occurring intermediates have been shown to manipulate cellular and synaptic pathways possibly assisting in neuroplasticity after brain injury, especially when used to augment task-specific training [19]. The administration of selective serotonin reuptake inhibitors has been shown to improve clinical motor function in parallel with an increased activation of the ipsilateral motor cortex [20]. In contrast, similar pharmacological manipulation of cellular and synaptic pathways may also retard neuroplasticity as shown for dopamine antagonists [21] or GABA stimulants [22]. Pharmacological facilitation of recovery has mostly been studied in the motor system and recently extended to other areas, such as language recovery and return of wakefulness from coma. In addition to pharmaceuticals involved in synaptic transmission, several cytokines, experimentally known to affect survival of brain cells, have been found to peak during brain development and to be upregulated after brain injury. Erythropoietin (EPO) and its variants and granulocyte colony-stimulating factor (G-CSF) may exert beneficial effects during recovery by modulating synaptic repair, stimulating neurogenesis, and enhance neuronal differentiation. Enhanced hippocampal responses during memory retrieval have been demonstrated by functional MRI in normal volunteers [23]. In this chapter, clinical studies of agents affecting neurotransmission and network modulation after brain injury are reviewed.

Amantadine

Amantadine hydrochloride is one of the most commonly prescribed medications for patients with prolonged disorders of consciousness after traumatic brain injury. The acute phase of recovery from severe traumatic brain injury shows a brief period of neuronal excitability followed by a long period of hypoexcitability with depletion of neurotransmitters, including dopamine. Amantadine may facilitate dopaminergic activity and promote favorable neurobehavioral effects by interacting with dopamine-dependent nigrostriatal, mesolimbic and frontostriatal circuits mediating arousal, drive and attentional functions [24]. A recent international, multicenter, randomized controlled trial involved 184 patients in a vegetative or minimally conscious state 4–16 weeks after traumatic brain injury receiving inpatient rehabilitation (ClinicalTrials.gov NCT00970944). Patients were assigned to receive amantadine increasing weekly from 100 mg twice daily to 200 mg twice daily in weekly 50-mg increments or placebo for 4 weeks and were followed for 2 weeks after treatment was discontinued. Amantadine accelerated the pace of functional recovery during active treatment but did not result in improved functional recovery 2 weeks after discontinuation. In particular, the brief treatment interval of only 4 weeks leaves the important question unanswered whether treatment with amantadine, as compared with placebo, improves the long-term outcome or simply accelerates recovery to an injury-defined level of function [25].

Catecholaminergics

Amphetamine exerts its behavioral effects by modulating several key neurotransmitters in the brain, including dopamine, serotonin and norepinephrine. The activity of amphetamine throughout the brain appears to be region specific and closely related to the brain's reward circuitry. In spite of the notion that amphetamines enhance the effects of motor training in healthy volunteers, clinical trials in stroke patients showed heterogeneous results. Five controlled trials demonstrated a tendency to an improved outcome when dexamphetamine was paired with physiotherapy and administered within 3–60 days after stroke. The most recent small double-blind placebo-controlled study randomized 16 hemiparetic patients within 60 days after stroke to amphetamine plus physiotherapy versus placebo plus physiotherapy. Dexamphetamine (10 mg oral) or placebo were administered 2 days per week prior to physiotherapy over a 5-week period and motor function and activities of daily living were assessed before, during and 6 and 12 months after treatment. Significant improvements in motor scales and activities of daily living suggest augmentation of physiotherapy by amphetamines [26]. For language recovery, 21 aphasic patients with subacute ischemic infarction were randomly assigned to receive either dexamphetamine (10 mg oral) or a placebo. Patients were enrolled between days 16 and 45 after onset and were treated on a 3-day/4-day schedule for 10 sessions. Thirty minutes after drug/placebo administration, patients received a 1-hour session of speech/language therapy. There were no differences between the drug and placebo groups before treatment. In contrast, by 1 week after treatment, there was a significant gain in the dexamphetamine group; however, this difference was lost after

6 months [27]. So far, clinical studies have been small, included only highly selected patients, and have not addressed possible confounding effects of the drug on mood and untreated depression. Furthermore, increased blood pressure and cardiac arrhythmias during amphetamine treatment do not support routine use of this drug in patients after stroke at this time. Levodopa, which is metabolized to dopamine in the brain and converted to norepinephrine, may have advantages because it avoids most of the clinical adverse events seen with amphetamines. In a randomized controlled trial, the effect of 100 mg oral levodopa daily enhanced the effects of goal-oriented physiotherapy in terms of motor recovery from stroke irrespective of stroke severity [28].

Cholinergics

Impaired cholinergic transmission is frequently seen in cognitive dysfunction after traumatic brain injury. Injury of cholinergic projections from the nucleus basalis of Meynert to the cortex are thought to impair the ability of cortical networks to encode verbal and sensorimotor memory. Therefore, impairment within the cholinergic transmission may result in cognitive dysfunction and impaired response to rehabilitation. Learning new motor skills by motor memory formation is enhanced by the acetylcholinesterase inhibitor tacrine in volunteers. The involvement of the cholinergic system in memory encoding has been demonstrated in various domains, including sensorimotor improvements in hemiplegic stroke patients [29]. In a recent pilot study of patients with cognitive impairment after right-hemispheric stroke randomly assigned to daily donepezil 5 mg versus placebo over 4 weeks significant improvements in the Mini-Mental State Examinations along with increased activation in both prefrontal areas, both inferior frontal lobes and the left inferior parietal lobe were shown [30]. So far, several small trials confirm the involvement of the cholinergic system in recovery from brain injury with encouraging results for acetylcholinesterase inhibitors in various rehabilitation settings.

Serotoninerigics

The effect of a single dose of fluoxetine on the cerebral motor reorganization has been investigated in a series of 8 patients suffering from pure motor subcortical stroke. Each patient served as its own control and was studied with functional MRI performing an experimental active motor task with the affected hand under fluoxetine and placebo condition. Under fluoxetine, during the active motor task, hyperactivation in the ipsilateral motor cortex could be demonstrated along with improved motor skills. No such changes were seen after a passive movement of the paretic hand under fluoxetine. The researchers conclude that the serotonergic system amplifies the pyramidal neurons in generating the experimental active motor task. They also suggest that chronic fluoxetine application may boost physiotherapeutic results in patients with pure motor strokes [20]. The modulating effect of serotonin on motor recovery after stroke has been convincingly shown in a recent double-blind randomized placebo-controlled trial by the same researchers (ClinicalTrials.gov NCT00657163). One hundred and eighteen patients who suffered a stroke with hemiparesis or hemiplegia were

randomly assigned to fluoxetine (20 mg daily) or placebo for 3 months. The patients had moderately severe strokes with predominant motor deficits and were enrolled within 5–10 days after stroke onset. At the end of the study, day 90, the overall motor scores in the fluoxetine group were significantly improved resulting in a 17% increase of functionally independent patients in the fluoxetine group [19].

Signaling Molecules

A large number of cytokines are involved in neuronal survival and regeneration. Recent research on neuroprotection and neuroregeneration has focused on two hemopoietic factors, EPO and G-CSF, and resulted in phase II clinical trials. These cytokines are studied in various clinical scenarios with most experience coming from trials in acute stroke patients. Disappointing results of the pivotal clinical studies outlined below have curtailed further research with these proteins.

Erythropoietin

EPO and EPO receptors are expressed in the nervous system peaking during brain development and have been found to be upregulated following experimental brain injury in the adult brain. EPO and some of its variants can cross the intact brain barrier, stimulate neurogenesis and differentiation and activate neurotrophic, antiapoptotic, antioxidant and anti-inflammatory signaling. The availability for human use for the treatment of renal anemia along with these attractive preclinical properties made EPO a prime candidate for clinical use in brain injury, neuroinflammatory and degenerative conditions. A series of studies in stroke patients were initiated as early as 1997. After safety and preliminary efficacy in patients with middle cerebral artery strokes within 6 h from onset, a phase IIb study enrolled 522 patients [>60% pretreated with intravenous tissue plasminogen activator (tPA)] receiving EPO intravenous infusion within 6 h from symptom onset and at 24 and 48 h (40,000 IU each) (ClinicalTrials.gov NCT00604630). The primary outcome measures, including the modified Rankin scale and Barthel Index on day 90, were neutral. In addition, there was an increased risk of serious complications: death, intracerebral hemorrhage, brain edema and thromboembolism in the EPO arm in particular when cotreated with intravenous tPA. The increased death rate in the recombinant tPA population remains unexplained and may result from a combination of (as yet unknown) factors and/or potential recombinant tPA-EPO interactions [31].

Granulocyte Colony-Stimulating Factor

G-CSF has long been used to counteract neutropenia for example after chemotherapy or mobilizing hematopoietic stem cells from the bone marrow in stem cell transplantation. G-CSF inhibits apoptosis in neurons, stimulates neurogenesis, and enhances vascular regeneration in experimental brain ischemia. Similar to EPO, G-CSF is released by neurons in response to ischemia. Systemically administered G-CSF passes

the intact blood-brain barrier and decreases infarct size in experimental models. In addition, G-CSF is not only acutely protective but also improves functional recovery after stroke when given delayed. Early clinical trials established safety over various doses [32]. A phase II randomized controlled trial enrolled 328 patients (>60% pre-treated with intravenous tPA) within 9 h from stroke onset receiving 135 µg/kg G-CSF over 72 h intravenously (ClinicalTrials.gov NCT00927836). The results, adjusted for baseline characteristics age, stroke severity and infarct volume, were neutral for clinical and imaging outcome parameters. There were no safety concerns [33].

Stimulating Regeneration by Neuropharmaceuticals

Although it is still debated whether axonal regrowth takes place over longer distances (>1 mm) in CNS injury models at all, several recent observations suggest it may occur even in humans. A longitudinal study in patients with severe traumatic brain injury monitored with diffusion tensor imaging during rehabilitation determined microstructural white matter changes of clinical relevance during long-term follow-up. In two subsequent diffusion tensor imaging exams, fractional anisotropy increased in the internal capsule and in the centrum semiovale primarily in patients with clinical improvement [34]. Such an increase in fractional anisotropy in patients recovering from traumatic brain injury suggests that axonal regeneration takes place and may play a role in clinical recovery. It is not known what mechanisms induce the increases in fractional anisotropy and whether the patients recover as a result of it, but this invites more research on drugs stimulating axonal recovery to enhance recovery after brain injury. Obviously, much more research is needed and it is not clear whether drug candidates have a favorable side effect profile, can be effectively applied and reveal clinically relevant benefits.

Neutralizing Axonal Growth Inhibitors

Myelin-associated inhibitors signal through the Nogo receptor present on the growth cone of a regenerating axon [35], and inhibitory extracellular matrix molecules associated with the glial scar have been shown to act through the Rho signaling pathway to inhibit axon regeneration. Rho activation and downstream activation of Rho-associated kinase leads to a collapse of the growth cone scaffold and to axon growth arrest. The convergence of inhibitory signaling pathways on the Rho pathway makes it an attractive target for regenerative therapies. At this point, early clinical studies are ongoing in patients with spinal cord injury.

Nogo-A is a protein present in CNS myelin that inhibits neurite growth. Models of spinal cord injury and unilateral sensorimotor cortex lesions demonstrate that treatment with function-blocking antibodies of Nogo-A results in an upregulation of

growth-specific proteins, enhanced regenerative and compensatory sprouting of fibers, and the formation of new functional connections in the spinal cord and brainstem. Behavioral tests showed marked improvements of functional recovery in the Nogo-A-antibody-treated spinal cord or brain-injured animals. From a clinical perspective, a phase I clinical trial applying anti-Nogo-A antibodies to patients with acute spinal cord injury has been completed (ClinicalTrials.gov NCT00406016) and a multicenter phase II trial has started enrollment in summer of 2012. The phase I safety trial enrolled more than 50 patients within 2 weeks after complete and incomplete spinal cord injury. The drug was applied intrathecally via a pump or repeated injections over various time periods between 24 h and 4 weeks. There were relevant side effects ascribed to anti-Nogo-A antibodies [36].

A Rho activation inhibitor derived from C3 transferase (Cethrin) has been shown to reduce the lesion extent and improve functional recovery in rodent models of spinal cord injury. A recent phase I clinical trial found safety and feasibility of intrathecal applications of escalating doses of Cethrin (0.3–9 mg) in patients with spinal cord injury (ClinicalTrials.gov NCT00500812). Patients with complete and incomplete cervical and thoracic spinal cord injury within 1 week of spinal cord injury were recruited in this observational study. The study revealed an overall acceptable safety profile of the compound over the range of doses tested and a preliminary efficacy signal suggesting favorable responses in cervical cord injury at doses of 3 mg. It is also intriguing that improvements in the motor scale continued up to 1 year after injury suggesting clinical efficacy [37]. A phase II study is planned.

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Rehabilitation and Plasticity

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Abstract

Therapies for effective neurorehabilitation are in part based on brain mechanism commonly described as neuroplasticity. These therapeutic approaches emphasize the re-learning of functionality that was lost due to the injury through reorganization of neural circuits in the remaining intact tissue. Important elements of these therapies are intensive and repetitive training, motivation and potentially interactive devices (therapy “robots”) and supportive therapies such as brain stimulation or plasticity inducing medications. Because neuroplasticity-based interventions are complex and multifactorial optimized treatment protocols have to be developed before large clinical trials can provide the evidence of efficacy.

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Neurorehabilitation is a quickly developing field accelerated by advances in neurosciences and rehabilitation engineering. In contrast to acute care medicine with its goal to cure, neurorehabilitation aims at minimizing disability and accelerating the return to an independent life. Naturally, it has to focus on all the deficits and dysfunctions of a patient that stand against these goals. Typically, physical (movement), communicational and cognitive deficits occur and interfere with each other. Therefore, the classical neurorehabilitative approach combines physio- and occupational therapy with speech and language and cognitive training, the latter typically provided by neuropsychologists.

Training, i.e. repetitive engagement in challenging exercises, is still the mainstay of neurorehabilitative treatment protocols. Training shares certain neural mechanisms with healthy learning, i.e., what is commonly described as neuroplasticity, although it is unclear in how far these processes overlap or share common mechanisms.

Whether training can be effectively enhanced by plasticity-encouraging interventions such as brain stimulation or certain drugs remains to be proven by clinical research.

What is known from experience as well as science is that higher intensity is more effective than lower intensity [1, 2], although this may not hold true for early time points after injury (in this case, stroke) [3]. High-intensity training requires excellent patient compliance and motivation, the major limiting factors for successful training.

Mechanisms of Training

Animal Models

Like motor learning in healthy people, rehabilitative training after a lesion to the motor cortex leads to modifications in motor cortices adjacent to the lesion. Motor representations change in size and shape [4, 5] and new connections form within and between sensory and motor cortical networks [6]. The representations of complex movement patterns that can be evoked by long high-frequency pulses to the injured hemisphere – a possible reflection of a memory trace for complex movements – are lost after a cortical lesion. With rehabilitative training they recover; the degree of restoration of complex movement patterns evoked by brain stimulation correlates with behavioral recovery [7].

Plasticity also occurs in the uninjured hemisphere contralateral to the lesion. Biernaski and Corbett [8] reported enlarged dendritic trees in the uninjured hemisphere in animals that were trained in an enriched environment after a focal lesion to the motor cortex as compared with control animals that received standard therapy. Indirect evidence for plastic modification was also found in other brain areas that are involved in motor control. In the cerebellum of rats that were subjected to repeated treadmill training after a middle cerebral artery occlusion, the 25-kDa synaptosomal-associated protein and glial fibrillary acidic protein – markers of synaptic plasticity – were upregulated as compared with untrained controls [9]. Similarly in the thalamus, synaptic plasticity indexed by synaptophysin expression was increased in the damaged hemisphere in animals that were trained on a rotarod (skilled locomotor training) as compared to a treadmill (locomotor training of lower difficulty) or no training [10]. In the striatum, expression levels of glutamate, brain-derived neurotrophic factor, and p-synapsin I were increased by treadmill training after middle cerebral artery occlusion [11]. However, the type of motor training certainly plays a role in the pattern of plastic modification across the rat brain: whereas forelimb precision movement training (typically single pellet reaching tasks) depends more on cortical plasticity, locomotor training likely induces reorganization in larger motor control networks including the cerebellum, basal ganglia and thalamus.

Studies in Humans

In humans, evidence for plastic modification of neural circuits comes from imaging and electrophysiology. Task-related activation in motor cortices or, in case of cortical lesions, the peri-infarct cortex is modified during the course of recovery. After corticospinal tract infarction, initial overactivation of the contralesional motor cortex (ip-

ilateral to the moving hand) reverts to ipsilesional (contralateral) dominance as recovery progresses [12, 13]. The presence of motor potentials evoked from the lesioned hemisphere using transcranial magnetic stimulation (TMS) and corticospinal tract integrity in diffusion tensor magnetic resonance imaging predicted the response to arm training in chronic stroke survivors [14]. Interfering with the contralesional hemisphere by TMS did not affect reaction times with the paretic hand in stroke survivors with varying degrees of recovery at least 2 years after stroke, but interfering with the ipsilesional motor cortex did [15]. This emphasizes the role of the ipsilesional hemisphere (contralateral to the moving limb) for successful recovery.

In predicting therapy gains, the degree of injury to descending tracts from primary motor and premotor cortices is an important surrogate marker. It correlated with gains achieved after hand robotic therapy [16]. Bilateral arm training improved the arm impairment in chronic stroke survivors. This improvement was paralleled by an increase in activation of bilateral premotor cortices after as compared to before training [17]. As compared with conventional physiotherapy according to neurodevelopmental principles, bilateral arm training was as effective but showed a different neural response: where conventional physical therapy had no effect on brain activation, bilateral training led to an increase in bilateral premotor cortex activation [18]. This indicated that different therapies operate through different mechanisms even if they induce a comparable behavioral response.

Therapeutic Principles

Timing

When to start rehabilitation after stroke or trauma is a matter of debate. It seems that forcing an animal to train too early increases the infarct volume [19]. On the other hand, stroke induces the expression of specific proplastic genes defining an optimal time period in which training should be most effective [20].

In humans, using high-intensity constraint-induced movement therapy (CIMT; 3 h of training based on shaping principles plus immobilizing the intact limb for 90% of the waking hours for 10 days) starting around 10 days after stroke results in adverse outcomes as compared with standard occupational therapy and standard CIMT (2 h of shaping plus 6 h of immobilization per day) [3]. In contrast, Bernhardt et al. [21] suggested that early – within 24 h – mobilization, i.e., getting the patient out of bed, improves the outcome 3 months after the stroke. This finding awaits confirmation in a larger sample.

Intensity

Many studies have compared different training therapies but have shown similar therapeutic responses. Experience suggests that the intensity of training is a major determinant of the training effect. Additional leg training resulted in better walking ability and arm training in better dexterity as compared with immobilization in subacute

stroke survivors enrolled within 14 days of stroke onset indicating that the intensity of training counts [10]. Similarly, a community-based physical activity program improved walking velocity, balance and quality of life measures as compared with standard care also emphasizing training intensity [22]. On the other hand, as mentioned above, high intensity early after stroke may result in adverse functional outcomes [3].

Motivation

The main obstacles that prevent high-intensity training are fatigue and motivation. Fatigue can be counteracted by sufficient interruptions and rest periods. Motivation requires a motivating environment, positive feedback from the therapist or a training device that specifically addresses motivational aspects, e.g. by providing rewarding schemes in a gaming environment. How to optimally use motivational therapy to improve training outcomes is still unclear.

Elements of Effective Training

Little is known about which training principles are most effective and for whom. Active training is better than passive movement, but also mental imagery of movement has a training effect in combination with active training [23]. Bilateral training is similarly effective as unilateral training when looking at the population mean; individual patients may respond better to one or the other [18]. It remains unknown which factors predispose a patient to respond to a specific treatment.

One of the largest randomized controlled trials (n = 222 participants) in neurorehabilitation showed that CIMT is superior to conventional care in patients between 3 and 9 months after a stroke [24]. CIMT was developed on the basis of the finding that disuse of an extremity leads to functional deterioration. The constraint element enforces active movement of the affected limb by immobilizing the unaffected. While this trial clearly demonstrated the lasting benefits of CIMT, the controlled comparison depends on the nature of conventional care. In most countries and medical systems, conventional care in the time frame of 3–9 months is limited to low-frequency outpatient therapy, e.g. physiotherapy and occupational therapy once a week. It seems expected that such low intensity is less effective than CIMT which engages patients in training for several hours per day. Hence, it is unknown whether it is simply the intensity that renders CIMT more effective, or it is the strictly unilateral nature of the training, or any other element of therapy.

Progression is another therapeutic principle that may be a key to success. Adding to the complexity, the loading or the velocity of training provides a constant stimulus for learning as the patient continues to improve [25].

Therapy Using Robots

Robots were initially developed as assistants to the therapist enabling highly repetitive, uniform passive movements. In patients with spinal cord injury, robotic gait training may be superior to standard overground training [26], but further studies are needed to con-

firm these differences. For patients with brain lesions, various studies using different upper or lower extremity robotic training devices were performed and showed inconsistent results ranging from inferiority to equality as compared with conventional physiotherapy [27, 28].

Robots, however, enable a form of standardized precision training that differs from training under the direction or with the assistance of a therapist. Robots may complement therapist-based training. Precision movement training is often implemented in a computer game. Gaming environments may also increase the motivation for training by introducing reward or competition with others. Finally, robots may partially support the patient's movement (assistance mode) or may perturb the patient's movement, e.g. by applying forces that counteract the patient. Whether assistance or perturbation per se are effective training elements remains to be investigated.

Supportive Therapies

If training is performed in a setting in which plastic reorganization of the brain is facilitated, its effects may be higher, faster and/or longer lasting. Repetitive TMS (rTMS) or transcranial direct current stimulation provide such a setting. They likely work by modulating cortical excitability (in the case of rTMS by increasing or decreasing excitability depending on the frequency of the repetitive stimulation) [29]. As compared with sham stimulation, 10 sessions of rTMS as an adjunct to conventional physical and occupational therapy starting 5–10 days after stroke improved disability (as measured by the Barthel index) and impairment (NIHSS) [30]. In a small randomized trial, rTMS in combination with task-oriented walking exercises improved gait symmetry more than walking exercises alone (combined with sham stimulation [31]. Transcranial direct current stimulation in combination with conventional physical therapy improved arm impairment and activity of daily living function more than physical therapy alone (combined with sham stimulation) [32]. Positive effects of rTMS and transcranial direct current stimulation were also reported for the treatment of aphasia [33, 34].

Drugs potentially improve the effects of rehabilitative training interventions. One larger trial in 118 acute ischemic stroke patients found that adding fluoxetine to standard rehabilitation improves motor impairment at 3 months [35]. The mechanisms by which this selective serotonin reuptake inhibitor exerts this effect are unknown.

Evidence in Neurorehabilitation

Neurorehabilitative interventions are multidimensional often combining different treatments such as exercise and brain stimulation. Finding optimal therapy protocols is much more complex than in pharmacological therapy because many parameters need to be optimized, such as timing, intensity, duration, and rest periods. The design of a clinical trial in neurorehabilitation is further complicated by the fact that

valid control interventions or groups are difficult to define. Because there is no placebo pill to a training therapy, it is difficult and often impossible to double/single-blind the trial. This leaves the investigation vulnerable to placebo effects, e.g. seeing an expensive robot equipment may introduce a placebo effect over the conventional physical therapist. In addition, the outcome measures and scales for movement, speech language and cognitive function all have their shortages. There is little agreement which scale best reflects a clinically relevant treatment effect. Because training interventions require large amounts of therapist time, trials in neurorehabilitation are expensive.

These factors all contribute to the fact that little evidence exists in neurorehabilitation today. Large and expensive trials are risky as long as optimal treatment protocols are not known. Small studies with less than 100 participants are common. Clinical guidelines therefore remain brief and leave much room for personal experience as well as treatments that can still be considered experimental.

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A Brain-Computer Interface to Support Functional Recovery

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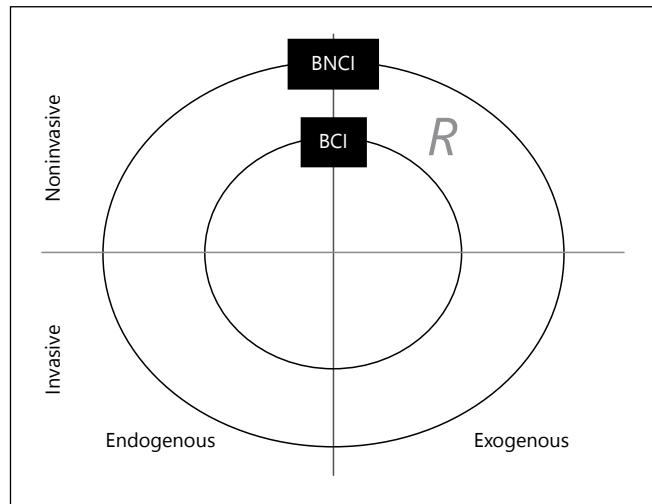
Abstract

Brain-computer interfaces (BCI) register changes in brain activity and utilize this to control computers. The most widely used method is based on registration of electrical signals from the cerebral cortex using extracranially placed electrodes also called electroencephalography (EEG). The features extracted from the EEG may, besides controlling the computer, also be fed back to the patient for instance as visual input. This facilitates a learning process. BCI allow us to utilize brain activity in the rehabilitation of patients after stroke. The activity of the cerebral cortex varies with the type of movement we imagine, and by letting the patient know the type of brain activity best associated with the intended movement the rehabilitation process may be faster and more efficient. The focus of BCI utilization in medicine has changed in recent years. While we previously focused on devices facilitating communication in the rather few patients with locked-in syndrome, much interest is now devoted to the therapeutic use of BCI in rehabilitation. For this latter group of patients, the device is not intended to be a lifelong assistive companion but rather a ‘teacher’ during the rehabilitation period.

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Brain-computer interfaces (BCI) in the broad sense refer to the ability to use signals measured in the brain to control computers. The field is growing rapidly these years with many new applications and a rapid growth in the number of publications. A recent roadmap of the future of BCI is the result of an international collaboration [1]. The roadmap divides the broad term BCI into pure BCI, in which only the brain signal is interfaced, and the more heterogeneous group of BNCI (brain neuronal computer interaction), in which other measures like muscle signal and gaze direction are taken into account. In this chapter, we will use BCI in the broad sense including knowledge of other modalities than electroencephalography (EEG), i.e. muscle activity, which is important when using BCI in rehabilitation.

Fig. 1. Most of the various forms of BCI belong to one of the eight groups on the figure. The area labeled 'R' refers to typical rehabilitation use. In this field, the recording devices are places on the outer surface of the body and the recorded signal is triggered from an external source rather than being spontaneously generated (top right corner of the figure). Since other body signals play a crucial role, the outermost circle best describes the recorded signals.



There is a number of existing and potential nonmedical uses of BCI like education, gaming, neuroeconomics, safety and security not dealt with here. In the medical field, BCI may be used in mental state monitoring or detection of certain events like craving in addicts, which is also not discussed here. This chapter focuses on the use of BCI in motor-impaired patients.

Running a Brain-Computer Interface

A typical source of brain signals for BCI is EEG due to high temporal resolution, ease of moving around and relatively low cost. Several groups have worked with other systems like functional magnetic resonance imaging, magnetoencephalography and near-infrared spectroscopy. But most research now and in the nearest future is based on EEG signals.

EEG signals are either recorded from electrodes placed during surgery (invasive) or adhered to the skin (noninvasive) (fig. 1). There are advantages and disadvantages of both approaches. The invasive techniques give a much better signal-to-noise ratio and allow for stable recordings for weeks and months. On the other hand, placing of the electrodes is costly and risky and there is an important long-term risk of infections and bleeding. The surface electrodes, on the other hand, are risk free and easily adhered to the skin but procedures involving hair removal or skin abrasion among other things may take up to 20 min every time the system is set up. Furthermore, the use of extracranial electrodes is associated with a decreased signal-to-noise ratio and there is a risk of slight imperfection of electrode placement which greatly deteriorates the signal.

The recorded EEG shows large variations over time depending on sleep, level of arousal, anxiety and number of artifacts generated within or outside the body. When

no specific external stimuli are applied and the subject does not perform specific tasks, we talk about spontaneous EEG. The spontaneous EEG pattern changes to a task-specific pattern when the subject performs a task. Such a task could be imagining a movement or actually performing a movement. Typical patterns in these conditions are event-related synchronization or event-related desynchronization, which appear over the contralateral motor cortex [2]. Such patterns are referred to as endogenous.

EEG patterns may also be secondary to external events. We call those exogenous. The external event can be a simple signal initiating or modifying a specific task or it can be repeated stimulation like flickering symbols. In the first case, the external event helps defining the time at which to look for a physiological signal change. In the case of repeated stimulation, simple often short-lasting EEG changes in response to each stimulus leads to a so-called evoked potential. One way to use these evoked potentials is to compare the timing of objects flickering with different frequency to the recorded potentials.

Use for Disabled People

A large number of medical conditions are associated with disabilities. Some rare conditions like amyotrophic lateral sclerosis and locked-in syndrome are associated with a normal brain unable to take command of any muscles. For these patients, any way to make themselves understood by others is paramount. Many of the first BCI systems were designed with these people in mind. A large number of persons suffer a stroke, traumatic brain injury or cerebral palsy. They may have a range of different problems from language and cognition to motor performance and with varying degrees of disability. Thus, their need for BCI also varies a lot. Now that BCI are becoming cheaper, more flexible and more powerful, many of these persons for whom we previously saw no use of BCI may well benefit from these systems in the future.

In recent years, BCI have moved out of the laboratories and into the hospitals and homes of disabled people. The central task is to improve the lives of the disabled through widely different approaches. One approach is that the BCI is used as a personal assistive technology (PAT) to help the patients with activities of daily living like communicating with people around them or via the Internet, controlling a wheelchair or drawing the curtains. The other approach is therapy based with the goal to train the motor system in disabled patients so that they can eventually give up the BCI and interact with their surroundings naturally. Thus, the goal is not to provide motor control or facilitate communication, but to produce permanent or lasting behavioral changes. Neither the PAT-based nor the therapy-based use of BCI can stand alone and has to be integrated with existing empowerments and therapies.

BCI may be a useful tool for accelerating motor rehabilitation. Motor rehabilitation of patients after for instance stroke often involves motor imagery and assisted move-

ments. If a BCI is included, it may augment the rehabilitation process. A physiotherapist often facilitates the rehabilitation process by passive movements accompanied by asking the patient to imagine the movement. A skilled therapist will be able to evaluate the actual movements and feedback to the patient. However, even the most skilled therapist is unable to monitor the brain activity associated with this process without a BCI. Thus therapist and patients can obtain additional information if a BCI is employed demonstrating which brain activity is associated with a given training activity. Thus, the patient not only (re-)learns to activate the muscles but he also learns what constitutes the optimal brain activity associated with the movement. The motor activity may be directly observed or measured as electrical muscle signals or accelerometer output. Other possible approaches involve BCI directly controlling a training robot [3, 4].

An unfortunate real-world limitation in rehabilitation of stroke patients is the amount of resources available for the individual patient. Much research indicates that many patients would benefit from more training than they actually get [5]. When the patient is left to do training on his own, the BCI may play an important role in inducing compensatory plasticity. The data recorded may be turned into time-frequency maps of event-related potentials or focus on specific frequency bands which are considered effective tools to monitor motor imagery and may effectively be combined with actual motor performance [6, 7].

Not even the most intense training program combined with optimal brain plasticity and treatment leads to full recovery in all patients. When patients do not recover fully, they may benefit from PAT helping them do things they will never be able to do again in any other way. Thus, the purpose of PAT is not training the patient but assisting him with daily activities at whatever level he is currently functioning.

Our Brain-Computer Interface Efforts

In a fruitful ongoing collaboration between the University of Copenhagen, Rigshospitalet and the Technical University of Denmark, a number of BCI applications have been developed. Many of the applications may serve as useful assistive devices for disabled persons or serve as a basis for BCI-based therapy. Table 1 summarizes some of the results we have obtained.

The use of endogenous signals to control a spelling device was inspired by Hex-o-Spell from the Berlin group [8]. The original approach was based on a 2-class movement of the hands. The left hand signal turned a dial on a hexagon with groups of letters in the corners. The right hand signal lengthened the dial until a group of letters was selected. Then a new hexagon appeared with individual letters from the selected group placed at each corner, and a selection was performed in a similar way. The speed was 6–8 letters per minute corresponding to a little more than 1 word per minute and accuracy was acceptable. We improved this method in several ways. Organizing the letters so that the more common were reached first could increase the speed, but re-

Table 1. BCI collaborative results

Subject	Endogenous	Results
Real and imaginary movements	yes	Similar results can be obtained whether movement is actually performed or just imagined
Three-class movement BCI	yes	Adding imaginary feet movements significantly increased data transfer
Training imaginary movements	yes	Training has a positive effect on performance in many subjects; however, even more subjects are likely to benefit if the classification algorithm is adaptive. Some subjects remain BCI illiterates
Optimizing algorithms when the subject does not use the BCI	yes	By off-line delayed training of systems on individual data acquired in a given subject, better performance could be obtained in subsequent on-line sessions
Spelling device	yes	Improved spelling program using 3 imaginary movement modalities
Environmental control	yes	TV remote control was easily integrated into BCI system
Stimulation of brain signals	yes	Artificial EEG signals perform as well as natural. Thus optimal extraction and classification paradigms may improve the individual device while the subject is otherwise engaged. This of course does only partially substitute the human training
High-speed visual evoked potential-based BCI	no	Up to 112 bits/min obtained in various paradigms of environmental control and simple computer games like packman
Going from computer screen to purpose-built stimulus devices	no	Hardware-encoded stimulation in real-time BCI leads to more robust systems in tested subjects
Stimulus frequency	no	Using various encoding schemes for timing of stimulus appearance, we found that Kasami sequences were superior to commonly used time-shifted sequences

quired more learning since the order was no longer alphabetical. By adding a 3rd condition, i.e. movement of the right foot, we were able to switch between the spelling hexagon and another hexagon with suggestions for how to complete the word, similar to what you see on telephone dictionaries. We used the large Danish text corpus 'Korpus.dk' containing 56 million words collected between 1990 and 2000 [9]. This allowed us to speed up spelling and come closer to 2 words per minute with no loss of accuracy.

The use of a BCI is rapidly increasing these years based on low cost, ease of use (e.g. wireless BCI) and more and more applications. It is important to keep on improving the reliability of BCI to meet the moment-to-moment needs of the user [10]. While we previously thought of medical BCI as a PAT for few severely handicapped patients, it looks as if we are going in the direction of BCI as a natural empowerment and interface in rehabilitation of a large number of people suffering a stroke and other acute neurological conditions.

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Novel Methods to Study Aphasia Recovery after Stroke

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Abstract

The neural mechanisms that support aphasia recovery are not yet fully understood. It has been argued that the functional reorganization of language networks after left-hemisphere stroke may engage perilesional left brain areas as well as homologous right-hemisphere regions. In this chapter, we summarize how noninvasive brain stimulation can be used to elucidate mechanisms of plasticity in language networks and enhance language recovery after stroke. We first outline some basic principles of transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). We then present evidence from studies in healthy volunteers for a causal role of the right hemisphere in different language functions. Finally, we review recent studies that used TMS or tDCS to promote language recovery after stroke. Most of these studies applied noninvasive brain stimulation over contralateral right-hemisphere areas to suppress maladaptive plasticity. However, some studies also suggest that right-hemisphere regions may beneficially contribute to recovery in some patients. More recently, some investigators have targeted perilesional brain regions to promote neurorehabilitation. In sum, these studies indicate that language recovery after stroke may integrate left- as well as right-hemisphere brain regions to a different degree over the time course of recovery. Although the results of these preliminary studies provide some evidence that noninvasive brain stimulation may promote aphasia recovery, the reported effect sizes are not striking. Future studies on larger patient collectives are needed to explore whether noninvasive brain stimulation can enhance language functions at a level that is clinically relevant.

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Aphasia is a severely disabling consequence of stroke that typically results from injury to cortical and subcortical structures perfused by the left middle cerebral artery [1]. Estimations suggest that more than 20% of patients suffering a stroke develop aphasia. While most patients show some degree of spontaneous recovery within the first months after stroke, the majority of patients with post-stroke aphasia are left with some degree of chronic deficit for which current rehabilitative treatments are marginally effective

[2]. Recent studies suggest that noninvasive brain stimulation techniques such as transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS) may be of potential benefit in aphasia research in two ways. First, noninvasive brain stimulation may be used in evaluating the neural mechanisms of compensation following aphasia after stroke. Secondly, due to their potential to modulate cortical excitability and plasticity, these techniques may provide effective means in facilitating treatment by promoting adaptive processes when combined with language therapy.

Shaping Language-Related Neural Processing with Transcranial Magnetic Stimulation

TMS is a noninvasive tool for electrical stimulation of the human cortex (for reviews, see Barker et al. [3] and Siebner et al. [4]). TMS provides a means of transiently disrupting ongoing neuronal processing in the stimulated cortex and thus permits to draw causal conclusions regarding the contribution of the stimulated area to a specific brain function [5].

Repetitive TMS (rTMS) refers to the application of prolonged trains of stimuli which are either given continuously as long trains at a constant rate (continuous rTMS) or intermittently as repetitive bursts (i.e., intermittent or burst-like rTMS).

There are two different possibilities of applying (r)TMS to investigate cognitive functions: TMS can either be applied during the task ('online') or shortly before the task ('offline'). Online TMS may consist of single pulses or short high-frequency trains given at distinct time points during a task to perturb intrinsic neuronal activity in the stimulated area (the 'virtual lesion' approach). An important advantage of TMS-induced lesions relative to studies of structural lesions is that there is insufficient time for functional reorganization to occur during online TMS and thus, the acute 'lesion' effect should not be confounded by chronic processes mediating functional recovery locally and at the systems level [5]. Of note, the term 'virtual lesion' implies that TMS always induces an impairment of brain functions. However, TMS-induced disruption of neuronal activity in one area may also lead to a paradoxical improvement or facilitation in task performance.

In contrast to online TMS, offline TMS involves longer rTMS protocols that are applied shortly before subjects perform an experimental task. Usually, the interventional rTMS protocol is 'inhibitory' [6]. This means that the rTMS intervention induces a lasting suppression of neuronal excitability in relevant areas. This conditioning approach bears some analogies to acute stroke, because inhibitory offline TMS gives rise to an acute adaptive reorganization within the nonstimulated functional loops of the networks to compensate for the TMS-induced suppression of neuronal activity in those components of the network that have been perturbed with TMS. It needs to be borne in mind that the local excitation induced in the stimulated cortical area can spread transsynaptically to remote brain regions via corticocortical and corticosubcortical projections. Hence, the behavioral changes induced by the TMS pulse

may not only be caused by local neural excitation at the site of stimulation, but also by indirect excitation of connected cortical areas.

In sum, online and offline TMS represent complementary approaches: while online TMS acutely disrupts a specific function, offline TMS impairs cortical processing beyond the time of stimulation and thus can be used to induce and examine the capability of brain networks to undergo acute reorganization. Due to the possibility to change cortical excitability beyond the duration of stimulation, offline rTMS protocols are of particular relevance for the use in neurorehabilitation.

Shaping Language-Related Neural Processing with Transcranial Direct Current Stimulation

tDCS modulates cortical excitability by application of weak direct electrical currents (1–2 mA) between two electrodes applied to the scalp over a relatively extended period of time (e.g. 5–20 min). Depending on the orientation of the stimulation electrodes and the direct current polarity, tDCS has been shown to elicit polarity-dependent changes in cortical excitability, presumably due to direct current-induced changes in the resting membrane potentials. In the motor cortex, anodal tDCS may increase motor-cortical excitability while cathodal tDCS may decrease excitability [7]. Emerging evidence suggests that the effects of tDCS can persist beyond the period of stimulation, and changes in task performance were reported for up to 6–12 months after intervention [8].

An attractive feature of tDCS is the apparent lack of any significant side effects when using standard protocols. For instance, tDCS has not been reported to provoke seizures in nonacute neurological deficits since intervention protocols are well below the threshold of tissue damage [9]. In contrast to TMS, it is relatively easy to blind the patient and examiner to the type of tDCS which enables a double-blind study design. Therefore, compared with TMS, tDCS may be a viable option for stimulation of the perilesional cortex where the threshold to induce seizures is lower. This makes tDCS an appealing form of neurostimulation in chronic stroke populations. Like TMS, tDCS may be applied before a certain task (offline) or during task processing (online). We also wish to mention that additional transcranial stimulation techniques such as alternative direct current stimulation or transcranial random noise stimulation have been introduced in recent years which may also be suited as interventional tools to modulate the impaired language system after stroke.

Right-Hemisphere Contributions to Language Processing in the Healthy Brain

There is consensus about a dominant role of the left hemisphere in language processing and most of the studies investigating language functions in the healthy brain with noninvasive brain stimulation techniques were restricted to left-hemisphere

brain regions. However, recent TMS studies provided some evidence that the right hemisphere is causally involved in different aspects of language processing, including phonological decisions, reading, and the processing of paralinguistic features such as (emotional) prosody or metaphor processing (for a review, see Hartwigsen and Siebner [10]).

Adopting a novel dual-site approach in which TMS was applied either unilaterally over homologous areas in the left or right hemisphere or simultaneously to both hemispheres, we recently tested the functional relevance of the previously reported activation of the left and right supramarginal gyrus (SMG) for phonological judgments [11]. We thus applied 10 Hz online rTMS over either the left, right or bilateral SMG while subjects performed phonological judgments (does a word have 2 or 3 syllables?) or semantic judgments (does a word represent a natural or man-made item?).

We hypothesized that if right supramarginal activation is redundant to phonological processing, then task performance should only be impaired when online TMS was applied over the left SMG but not the right SMG. In contrast, if the right SMG also contributes to phonological decisions, then task performance should also be disrupted with TMS of right SMG. The simultaneous application of TMS over the left and right SMG enabled us to test whether both areas can compensate a virtual lesion to the respective other area. We expected that if phonological decisions are possible with either the left or right SMG, the lesion effect should be greater when TMS was applied over both the left and right SMG. In contrast, if the left and right SMG are equally necessary for efficient phonological decisions, the effect of TMS should be the same irrespective of whether it was applied unilaterally or bilaterally.

TMS relative to an ineffective sham procedure significantly impaired task performance for phonological decisions, but not for the semantic task independent of the stimulated hemisphere. Additionally, we found that both hemispheres were equally sensitive to the disruptive effect of TMS. Together, these findings indicate that both hemispheres are equally necessary for intact phonological processing. Furthermore, the results suggest that efficient phonological judgments require both the left and the right SMG in healthy subjects without any evidence that both areas can compensate a lesion to the respective other area.

In sum, these results indicate that the involvement of right-hemisphere language areas is not limited to aphasia recovery after stroke but is also essential for phonological processing in healthy subjects.

Other studies reported increased task-related neuronal activity in right-hemisphere areas during language processing after the contralateral homologous area had been suppressed with offline rTMS [12]. These studies show how offline TMS can be used to investigate short-term plasticity in language networks in the healthy brain which might ultimately contribute to the understanding how such networks reorganize after long-term disruption such as occurring after stroke.

With respect to potential beneficial effects of right-hemisphere noninvasive brain stimulation on language functions, Ross et al. [13] recently demonstrated that anodal

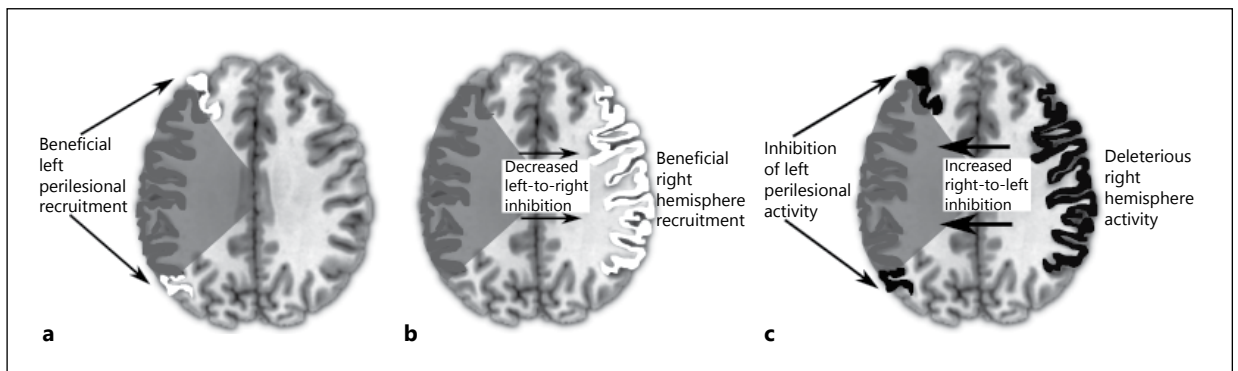


Fig. 1. Different accounts of plasticity in language systems in chronic aphasia. **a** After unilateral left-hemisphere stroke (in gray), some language functions may be subserved by perilesional areas (in white). **b** Right perisylvian areas (in white) may be recruited to subservise some language functions, a process facilitated by decreased transcallosal inhibition of the right hemisphere by the damaged left hemisphere. **c** By contrast, right-hemisphere activity may be deleterious. Released from interhemispheric inhibition, right-hemisphere structures (in black) may exert an increased inhibitory influence on left perisylvian areas, impeding functional recovery of lesional and perilesional areas in the left hemisphere (in black). Modified and reprinted from Hamilton et al. [2], copyright (2011), with permission from Elsevier.

tDCS over the right anterior temporal lobe improved naming possibilities in 15 healthy subjects. These results suggest that anodal tDCS may have some potential to promote language recovery in patients with post-stroke aphasia.

Reorganization of Language Networks after Stroke – Evidence from Repetitive Transcranial Magnetic Stimulation Studies

The majority of rTMS studies in post-stroke aphasia used low-frequency rTMS to inhibit contralateral homologous areas (for an overview, see Chrysikou and Hamilton [14]). These studies were based on the assumption that following left-hemisphere stroke, the right hemisphere is released from transcallosal inhibition and thus exerts an increased transcallosal inhibitory effect on perilesional regions of the left hemisphere, thereby suppressing language-related left-hemisphere activity. According to this interhemispheric inhibition model, improvements in language function after a left-hemisphere lesion can be achieved either by inhibition of right-hemisphere activity or via facilitation of left-hemisphere activity [15] (fig. 1a, c). For instance, in 4 patients with chronic aphasia after stroke, Naeser et al. [16] showed that repeated daily application of 1 Hz rTMS over the right anterior inferior frontal gyrus significantly improved picture naming abilities for up to 2 months after treatment. More recent studies suggest that the reported benefits in naming after 1 Hz rTMS of the contralesional right anterior inferior frontal gyrus in patients with non-

fluent aphasia generalize to other language abilities like spontaneous speech and auditory comprehension [17].

Of note, not all aphasic patients benefit from suppression of right-hemisphere activation and lesion location might be a critical determinant of recovery success. It was further suggested that aphasia recovery might depend on the extent to which some areas are affected or spared by stroke [18].

While many studies demonstrated that suppression of right-hemisphere activity after left-hemisphere stroke may promote recovery, evidence from other TMS studies suggests that the right hemisphere might also be critically contributing to language performance in some patients [19, 20] (fig. 1b). This may indicate that although recovery from post-stroke aphasia seems to depend more on an effective integration of available perilesional left-hemisphere regions, right-hemisphere areas may be successfully integrated in some cases [19]. While the role of the contralesional right hemisphere in language recovery is more controversial than that of the left hemisphere, a beneficial contribution of right-hemisphere areas to the successful reorganization of language networks after stroke is compatible with the results from previous neuroimaging studies (for a review, see Saur and Hartwigsen [21]). It has been argued that additional factors such as premorbid laterality of language representation, the time course of recovery, and the lesion size are important determinants that might influence the successful integration of right-hemisphere activity during post-stroke reorganization of language networks [14].

A recent study explored the possibility of facilitating cortical activity in the left hemisphere to improve language recovery. Szaflarski et al. [22] applied intermittent theta burst stimulation over Broca's area in 8 chronic stroke patients with aphasia. In 6 of the 8 patients, semantic fluency was improved after intermittent theta burst stimulation, and subsequent functional magnetic resonance imaging data demonstrated a leftward shift in language-related activation. These preliminary results suggest that the application of facilitatory protocols over left-hemisphere perilesional areas may enhance language recovery.

In sum, the potential of TMS to promote aphasia recovery after stroke remains to be determined as the results of the above-cited studies are heterogeneous and limited by the small number of patients included. Moreover, it is unclear whether TMS should target perilesional left-hemisphere areas or contralateral right-hemisphere areas to enhance neurorehabilitation. The use of TMS in clinical settings is further limited by the fact that stimulation can be unpleasant, especially if frontal regions like Broca's area are targeted at high intensities. While the high focality of figure-of-eight-shaped TMS coils allows for the functional segregation of subareas, it requires anatomical specific hypotheses with respect to the precise desired stimulation site (e.g. anterior vs. posterior part of Broca's area). Since accurate placement of the TMS coil over the cortical area of interest and continuous monitoring of the coil throughout the TMS experiment is crucial to assure high spatial accuracy, TMS may not be used simultaneously with language therapy.

Table 1. tDCS studies in aphasia

Authors	n	Lesion location	Months after stroke	Aphasia type	Stimulation site	Stimulation protocol	Task	Outcome
Monti et al. [23]	8	L frontal L frontoparietal L frontotemporoparietal	24–96	Broca's global	L Broca	1 session: 10 min, 2 mA offline, anodal, cathodal, sham	picture naming	Improved accuracy after cathodal but not anodal tDCS (no follow-up)
Baker et al. [24]	10	L frontal L frontoparietal L frontotemporoparietal L temporal L temporoparietal	10–242	anomic Broca's	L frontopre-central	5 days: 20 min, 1 mA online, anodal, sham	picture naming	Improved accuracy after anodal vs. sham tDCS 1 week after treatment
Fiori et al. [31]	3	L frontal/subcortical L frontotemporoparietal	21–71	Wernicke's	L Wernicke	5 days: 20 min, 1 mA online, anodal, sham	picture naming	Improved accuracy after anodal vs. sham tDCS on day 5, n = 2: 3 weeks after treatment
Floel et al. [27]	12	L frontal, L subcortical L frontotemporal L frontotemporoparietal L temporal L temporoparietal	14–260	Broca's Wernicke's amnesic	R temporo- parietal junction	3 days: 2 × 20 min, 1 mA online, anodal, cathodal, sham	picture naming	Improved accuracy for anodal vs. cathodal tDCS 2 weeks after treatment
Fridriksson et al. [32]	8	L posterior cortical or subcortical	10–150	fluent	L frontal	5 days: 20 min, 1 mA online, anodal, sham	picture naming	Faster reaction times after anodal vs. sham tDCS in 7/8 patients 3 weeks after treatment
Kang et al. [25]	10	L frontal L frontotemporal L frontotemporoparietal L temporoparietal L subcortical	6–180	Broca's anomic global	R Broca	5 days: 20 min, 2 mA online, cathodal, sham	picture naming	Improved picture naming accuracy after cathodal relative to sham tDCS
Vines et al. [28]	6	L frontal	15–120	Broca's	R inferior frontal gyrus	3 days: 20 min, 1.2 mA online, anodal, cathodal, sham	verbal fluency	Increased fluency with anodal vs. sham tDCS (no follow-up)
You et al. [26]	21	L frontal, L temporal L frontotemporal L frontoparietal L frontotemporoparietal L temporoparietal L parietal, L subcortical	16–38	Broca's Wernicke's	L Wernicke anodal, R Wernicke cathodal	10 sessions in two weeks: 30 min, 2mA online, 3 groups with 7 patients: anodal, cathodal, sham	verbal comprehension	Improved verbal comprehension for cathodal vs. anodal or sham tDCS

L = Left; R = right.

Reorganization of Language Networks after Stroke – Evidence from Transcranial Direct Current Stimulation Studies

To date, only a few studies have applied tDCS in post-stroke aphasic patients to facilitate treatment in language recovery (see table 1 for details). The majority of these studies investigated the effects of cathodal tDCS over perilesional left-hemispheric regions to facilitate picture naming. For instance, Monti et al. [23] showed that cath-

odal tDCS significantly improved picture naming in 8 ischemic stroke patients with aphasia. It was concluded that the effect of cathodal stimulation may be a downregulation of overactive inhibitory cortical interneurons in the lesioned hemisphere that ultimately give rise to increased activity and function in the damaged left hemisphere. In contrast, several other investigators have reported improved language performance after either anodal stimulation of the left hemisphere or cathodal stimulation of the right hemisphere [24–26]. Recently, two studies found significant language improvement after anodal tDCS over the right hemisphere [27, 28]. These preliminary results suggest that an upregulation of right-hemisphere activity may be beneficial for language recovery in some patients.

The above-cited studies show that tDCS might be of potential benefit in promoting aphasia recovery after stroke. However, the results are heterogeneous and it is currently impossible to judge the value of tDCS as a means to enhance neurorehabilitation of language. For instance, it remains to be determined whether anodal or cathodal tDCS should be applied to perilesional left-hemisphere regions or contralateral right-hemisphere areas.

With respect to the usability of tDCS in clinical settings, an important advantage of tDCS compared with TMS is the relatively easy application and the absence of any severe side effects if tDCS is applied within the published safety guidelines. Finally, tDCS can be administered concurrently with language therapy and may be easily applied in double-blind settings.

A Hierarchical Model of Aphasia Recovery after Stroke

While many of the studies discussed above were based on the assumption that suppression of activity in the ‘overactive’ right hemisphere after left-hemisphere stroke may promote language recovery, others provided some evidence that the right hemisphere might play a beneficial role in aphasia recovery. Consequently, it was argued that language recovery is a dynamic process that may involve a variety of plastic changes in both hemispheres [2]. In their hierarchical model of language recovery, Heiss and Thiel [29] assumed that with small left-hemisphere lesions, complete or near-complete language recovery may be achieved by restoration of normal patterns of activation in left-hemisphere language networks. If lesions of the left hemisphere damage important language centers, perilesional regions may be recruited to subserve language functions. However, when left-hemisphere networks are more severely impaired, the right hemisphere appears to be capable of assuming some functions. While right-hemisphere recruitment for language tasks may contribute to overall language recovery in severely affected patients, the remodeled language network in these patients is likely inefficient compared with premorbid intact left-hemisphere perisylvian regions. The hierarchical model of effective aphasia recovery is based on the assumption that best recovery is achieved when left-hemisphere language networks recover

normal function. According to this model, good recovery is achieved when perilesional left-hemisphere areas compensate for damaged left-hemisphere language regions. In contrast, a preferential recruitment of the right hemisphere during language tasks is associated with limited recovery from aphasia because of limited right-hemispheric processing capacities with respect to language.

Conclusions and Future Directions

Recent studies provide some first evidence that noninvasive brain stimulation techniques might be beneficial in promoting aphasia recovery after stroke. However, although these preliminary results are interesting, they should be interpreted with caution and the therapeutic potential of the various transcranial cortex stimulation protocols still remains to be determined. The reported findings are inconsistent and partly contradictory. For instance, there is strong variation across studies with respect to the outcome of different stimulation protocols (e.g. anodal vs. cathodal tDCS) and the optimal stimulation site (i.e., perilesional left-hemisphere regions vs. right hemisphere homologues). Further, previous studies only included a small number of patients (usually less than 10 patients per group) and the reported effect sizes were moderate. Some studies lacked control groups and subject blinding. The literature on noninvasive brain stimulation in aphasia recovery is further limited by the fact that there are no long-term investigations on the duration of the reported effects. Future studies should investigate larger patient collectives to explore whether noninvasive brain stimulation can enhance language functions at a level that is clinically relevant beyond the time of stimulation. Here, the combination of brain stimulation and language therapy seems promising. These studies should also address the optimal time point of intervention (i.e., before, during or after language therapy). Finally, it might also be promising to use multifocal approaches allowing for the simultaneous application of TMS over multiple brain sites within specific language networks.

One possible explanation for the partly inconsistent findings across different studies is that language recovery is a dynamic process that involves both hemispheres at different times to different degrees [30]. Future studies should systematically investigate the use of different protocols over the time course of recovery to advance the current knowledge about critical areas for specific language functions during language reorganization. For instance, it might be worthwhile to apply facilitatory protocols over right-hemisphere homologue regions in the subacute phase after stroke while an enhancement of left-hemisphere functions may be more beneficial in the chronic phase after stroke.

A possible way forward is to use multimodal approaches combining different methods, such as functional and structural imaging with transcranial stimulation techniques in both healthy volunteers and stroke patients with aphasia to identify critical networks for different language functions across the time course of recovery [21].

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Role of Repetitive Transcranial Magnetic Stimulation in Stroke Rehabilitation

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Abstract

In recent years, efforts have focused on investigating the neurophysiological changes that occur in the brain after stroke, and on developing novel strategies such as additional brain stimulation to enhance sensorimotor and cognitive recovery. In the 1990s, repetitive transcranial magnetic stimulation (rTMS) was introduced as a therapeutic tool for improving the efficacy of rehabilitation for recovery after stroke. It is evident that disturbances of interhemispheric processes after stroke result in a pathological hyperactivity of the intact hemisphere. The rationale of using rTMS as a complementary therapy is mainly to decrease the cortical excitability in regions that are presumed to hinder optimal recovery by low-frequency rTMS delivered to the unaffected hemisphere, while high-frequency rTMS delivered to the affected hemisphere facilitates cortical excitability. However, the exact mechanisms of how rTMS works are still under investigation. There is a growing body of research in stroke patients investigating the effect of rTMS on facilitating recovery by modifying cortical and subcortical networks. Clinical trials applying rTMS already yielded promising results in improving recovery of sensorimotor and cognitive functions. Altogether, in combination with conventional therapeutic approaches, rTMS has a potential to become a complementary strategy to enhance stroke recovery by modulating the excitability of targeted brain areas. In future studies, emphasis should be placed on selecting patient populations to determine whether treatment response depends on age, lesion acuteness, or stroke severity. Furthermore, it is important to identify parameters optimizing the beneficial effects of rTMS on stroke recovery, and to monitor their long-term effects.

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Stroke is a leading cause of disability and the burden of stroke is borne disproportionately by older people who have a greater incidence and prevalence of ischemic stroke than younger individuals. For each successive 10 years after 55 years of age, the stroke rate more than doubles in both men and women; 65% of all strokes occur in individuals older than 65 years. Five million survivors are left permanently disabled, with complications including motor (50–83%), cognitive (50%) and language impairments

(23–36%), as well as psychological disturbances (20%) [1]. Estimates indicate that 33–42% of patients still require assistance for activities of daily living 6 years after stroke, and that 36% of patients remain disabled after 5 years [2].

Recovery after stroke is complex. Many interventions have been developed to support recovery of impairment and associated functions, and a number of randomized controlled trials and systematic reviews investigated their effectiveness in stroke rehabilitation [3].

Additionally to established therapies (e.g. constraint-induced movement therapy, robotic-assisted strategies, repetitive task training, cognitive training and speech therapy), a noninvasive brain stimulation technique, such as transcranial magnetic stimulation (TMS), has been developed. This stimulation interacts with spontaneous brain activity and influences sensorimotor and higher-order cognitive abilities.

In the 1980s, TMS was originally introduced in clinical neurophysiology for the evaluation of the functional state of the corticospinal pathway [4].

In the 1990s, technological advances allowed the delivery of rhythmic trains of magnetic pulses in a rapid sequence up to a 100-Hz repetition rate, which was referred to as repetitive TMS (rTMS). It was reported that rTMS interacts with cortical activity more effectively than TMS. In recent years, rTMS has been rapidly developed as a potential therapeutic tool in many other clinical fields [5].

Principles of Repetitive Transcranial Magnetic Stimulation and Biological Aspects

The primary property of magnetic stimulation is its ability to penetrate all body structures, allowing stimulation of regions below layers of bone (e.g. the brain).

Single-pulse TMS applied on the scalp overlying the primary motor cortex (M1) assesses the excitability and conductivity of corticospinal motor pathways. This approach has primarily been applied in studies of movement physiology in patients with neurological disorders and in postlesion follow-up studies of plastic cerebral reorganization. Paired-pulse techniques have been shown to provide measures of intracortical facilitation and inhibition as well as corticocortical interactions, which are important when evaluating changes in functionality. When multiple stimuli of TMS are delivered in trains, one can differentiate conventional and patterned protocols of repetitive stimulation. For conventional protocols, there is agreement on the term rTMS. Application of rTMS influences neural excitability of selected brain areas. Low-frequency rTMS of ≤ 1 Hz was found to suppress while high-frequency rTMS of ≥ 5 Hz was observed to facilitate local neural activities [6]. Nevertheless, it is speculated that low-frequency rTMS to the nonaffected hemisphere reduces interhemispheric inhibition towards the affected hemisphere, leading to facilitation of beneficial functional reorganization in the affected hemisphere [7]. Patterned rTMS refers to a repetitive application of short rTMS bursts at a high inner frequency, which are separated by short pauses of no stimulation. Theta burst stimulation

(TBS) is the most commonly used method of patterned rTMS. In TBS, short bursts of 50-Hz rTMS are repeated as a continuous or intermittent train at a rate in the theta range (5 Hz). The excitatory and inhibitory effects of this type of stimulation can be manipulated by continuous or intermittent delivery of these theta bursts over time [8]. The TBS protocol has been used to modulate motor thresholds [8] and cognitive functions [9]. Recently, quadripulse stimulation, which is able to induce long-term changes in cortical excitability, has been added to the patterned rTMS procedures [9].

Moreover, it was reported that 1-Hz cortical inhibitory effects on a stimulated area were dependent on both GABA and NMDA receptor system activity, whereas high-frequency stimulation might rely on the same system but have opposite effects. Both, long-term depression and long-term potentiation have been postulated as likely mechanisms to explain the persistent effects of rTMS on cortical activity.

The use of rTMS in the clinical practice depends mainly on its ability to transiently interact with the stimulated neural network rather than its ability to modulate cortical excitability. Therefore, rTMS can be used with two distinct approaches: on-line stimulation (rTMS is applied during the performance of a task) and off-line stimulation (rTMS is applied before). In general, it is assumed that on-line rTMS induces an alteration of cortical activity within a specific targeted area that can significantly impair performance, and the effect of on-line rTMS is short-lived. In the case of off-line stimulation, rTMS affects the modulation of cortical excitability and aims to change the cognitive and motor performance.

Nevertheless, modification of the activity of a neural network by rTMS carries important behavioral implications for neurorehabilitation, which will be considered later. It was reported that the effects induced by several off-line rTMS approaches were site specific, but not site limited. Thus, the long-term consequences induced by sustained repetitive brain stimulation were most likely due to activity changes in a given network of cortical and subcortical areas rather than a local inhibition or excitation of an individual brain area. In other words, brain stimulation can modulate the ongoing properties of a neuronal network by facilitation or reduction of its activity. Since the brain mainly operates through flexible and interactive distributed networks, we can expect that the modification of a node of the network would affect the entire network.

Repetitive Transcranial Magnetic Stimulation Modifies Sensorimotor and Cognitive Recovery

The most common impairment caused by stroke is motor impairment, appearing as a limitation or loss of function in motor control or a limitation in mobility. Therefore, the focus of stroke rehabilitation is often on the recovery of impaired movement and the associated functions based on the paradigm of motor learning. In recent years, ef-

forts have focused on investigating the neurophysiological changes that occur in the brain after stroke, and on developing novel strategies such as additional brain stimulation to enhance motor recovery. In particular, rTMS is known as a therapeutic tool for improving the efficacy of rehabilitation for motor recovery after stroke. In addition to producing effects on cortical excitability, stroke may affect the balance of transcallosal inhibitory pathways between primary motor areas in both hemispheres: the affected hemisphere may be disrupted not only by the infarct itself but also by the resulting asymmetric inhibition from the unaffected hemisphere. Therefore, rTMS could be used therapeutically to restore the balance of interhemispheric inhibition after stroke. According to the interhemispheric competition model, there are two therapeutic strategies for improvement of motor function using rTMS: downregulation of the excitability of the primary motor cortex in the nonaffected hemisphere with low-frequency stimulation, and upregulation of excitability of the primary motor cortex in the affected hemisphere with high-frequency stimulation [6]. All in all, studies have not determined if the facilitation of the affected hemisphere [10] or the inhibition of the unaffected contralateral hemisphere [11–13] is more effective in improving the hampered function.

The downregulation strategy has been proven to be effective in consecutive multi-session trials for acute and chronic stroke in children and adults. On the other hand, the upregulation strategy has rarely been applied, primarily due to safety concerns, since it was thought that high-frequency rTMS would increase the risk of seizures. Nevertheless, Corti et al. [14] recently reviewed the evidence regarding the safety, and efficacy of high-frequency rTMS to the motor cortex of the affected hemisphere was collated. The studies included in this systematic review investigated the concurrent effects of rTMS on the excitability of corticospinal pathways and upper-limb motor function in adults after stroke. The authors concluded that rTMS applied to the affected hemisphere is a safe technique and could be considered as an effective approach for modulating brain function and contributing to motor recovery after stroke. Moreover, some researchers studying the motor cortex have suggested that the stimulation of both areas would be the most effective strategy [15].

Another frequent motor symptom following stroke is dysphagia. Up to one third of patients experience swallowing problems in the period immediately after a stroke. Most patients recover swallowing ability within a few weeks, but the extent of recovery varies widely from patient to patient. Previous studies using TMS have demonstrated the presence of a direct corticobulbar projection to swallowing muscles from the motor cortex [16]. The projection is bilateral, but is often asymmetric, independent of handedness. Hamdy [16] has suggested that if a stroke affects the dominant swallowing hemisphere, then dysphagia is more likely to occur than if the nondominant hemisphere is affected. Several lines of evidence show that the cortex retains its potential for reorganization after stroke, both in the damaged and undamaged hemispheres. This offers the possibility to modify the corticobulbar network by stimulation.

A recent study reported that 5 daily sessions of rTMS over the esophageal motor cortex of the affected hemisphere improve clinical recovery of swallowing functions in patients with acute monohemispheric stroke, and that, compared with the sham group, this recovery was maintained for at least 2 months [17]. In addition, the electrophysiological measures on 10 patients who received real rTMS indicate that the recovery is associated with an increase in the excitability of the corticobulbar projections from both hemispheres. Furthermore, Khedr and Abo-Elfetoh [18] have shown in a randomized controlled study that active rTMS applied to each hemisphere (affected and unaffected) – compared with sham rTMS – improved swallowing function in patients with acute lateral medullary or other brainstem infarctions. This improvement was maintained over 2 months of follow-up.

Cognitive impairment is a frequent consequence of stroke, with estimates of 50% of patients presenting cognitive impairment in the early phase after stroke [1], and up to 32% of patients demonstrating persistent cognitive impairment up to 3 years after the onset of their first stroke [19].

Approximately 40–81% of stroke patients after stroke demonstrate hemineglect, and this symptom is sustained in approximately one third of these patients [20]. Hemispatial neglect can result from lesions to different structures within an extended attentional network, such as the inferior and posterior parietal lobe, the superior temporal lobe, the inferior frontal lobe, basal ganglia and thalamus, and connecting fiber tracts [21]. Furthermore, hemineglect interferes with the rehabilitative process and is associated with a poor functional outcome.

rTMS – as a noninvasive technique modulating cortical activity – gains growing importance in the field of hemispatial neglect treatment. In neglect patients after stroke, it has been proposed that neuronal activities in contralesional homologous regions are increased due to a loss of active interhemispheric inhibition [5]. In line with this finding, it has been reported in few small studies that reducing activity in the left nonaffected parietal lobe by applying inhibitory low-frequency rTMS can improve hemineglect by reducing abnormally increased interhemispheric transcallosal inhibition from the nonaffected to the affected cortex [22, 23].

In a recent study by Song et al. [24], the effects of repeated applications of low-frequency rTMS over the posterior parietal cortex were investigated in a total of 14 right-hemispheric neglect patients. Seven patients were treated with rTMS during 2 weeks, twice a day, whereas 7 patients had no stimulation. Repeated application of low-frequency rTMS resulted in a significant improvement lasting 2 weeks, whereas no improvement was found in the control group without stimulation. In this study, confounding learning effects were controlled and spontaneous remission was accounted for. However, since the study did not include a sham stimulation group, it is difficult to exclude unspecific placebo-like effects.

Newly developed protocols such as TBS present shorter stimulation times and their repeated application can significantly prolong the effects on cortical excitability. The repeated TBS application has thus a promising future as rehabilitative approach in

neglect [25]. Moreover, in a recent review it was concluded that rTMS is a promising approach to reduce the interhemispheric imbalance in neglect patients and to ameliorate symptoms [26].

Aphasia is a frequent consequence of stroke with serious effects on the patient's autonomy. Although speech therapy significantly improves language and communication deficits particularly in very early stroke recovery, residual aphasia has a multifactorial impact on quality of life and participation.

With reference to the theory of transcallosal disinhibition [27], recent studies in stroke patients with chronic aphasia suggest that the restoration of the left-hemispheric language network by inhibition of the overactive right homologous frontal speech areas with rTMS as a complementary treatment is linked to better recovery in language and communication deficits [28]. Moreover, a recent functional imaging study proposed that inhibitory rTMS of the right-hemispheric Broca homolog together with subsequent speech therapy prevents establishing right-hemispheric lateralization and that this normalization of the activation pattern might be accompanied by better clinical improvement [29].

In a recent article, Naeser et al. [30] concluded that new rTMS studies suggested that the use of 1-Hz rTMS for a series of at least 10 rTMS treatments results in significant improvement in naming, and often in phrase length during propositional speech. These improvements are long lasting, up to 2 months, or even as long as 2 years, after TMS. Moreover, when rTMS is combined with speech therapy, additional improvement has been observed, beyond rTMS alone.

Although the stimulation protocol varies (table 1), most of the mentioned studies concluded that rTMS is an effective, safe and feasible complementary therapy for stroke rehabilitation.

The idea behind rTMS is that modification of the cortical excitability leads to a reorganization of the functional network responsible for the impaired function. The function may be restored by mechanisms that involve structural as well as functional changes of the neuronal circuits. Following the loss of a part of the neural population after stroke, a reduction of excitability of cortical neurons within the affected area might induce a depression of the circuit underlying the function, resulting in an impaired function. Thus, rTMS can induce a partial recovery of sensorimotor and cognitive abilities, which may be due to a strengthening of the synaptic activity of the surviving neurons in the stimulated network.

Another aspect is that areas – connected or adjacent to the lesion – become 'silent' due to diaschisis, and therefore lesion-induced effects are weakening the synaptic activity resulting in silent synapses. In line with this, rTMS might induce a readjustment of an intact but 'functionally' suppressed area due to a reduction in synaptic strength. On the other hand, strengthening the synaptic activity by applying rTMS leads to more effective processing within the functional network.

Finally, it has been suggested that lesion-induced plasticity might be stronger when it occurs shortly after stroke, and this plasticity becomes weaker as more time

Table 1. Studies mentioned in the article evaluating the effects of rTMS in stroke patients

Authors and years	Number of patients	Time after stroke	Sham control	Stimulation site	Stimulation parameter	Frequency of stimulation	Results
<i>Hemiparesis</i>							
Chang et al. [10], 2012	28 (18 real and 10 sham)	7–26 days	yes	affected hemisphere primary motor cortex	1,000 pulses at 10 Hz, 90% MT	10 sessions over 2 weeks	rTMS led to a significant motor improvement
Kakuda et al. [11], 2012	204	more than 1 year	no	nonaffected hemisphere primary motor cortex	1,200 pulses at 1 Hz, 90% MT	22 sessions over 15 days (2 sessions every day)	significant motor improvement, changes were seen up to 4 weeks after discharge in 79 patients
Seniów et al. [13], 2012	40 (16 real and 17 sham)	less than 3 months	yes	nonaffected hemisphere primary motor cortex	1,800 pulses at 1 Hz, 90% MT	multiple sessions over 3 weeks (1 session every day)	rTMS led to no significant motor improvement
<i>Dysphagia</i>							
Khedr et al. [17], 2009	26 (14 real and 12 sham)	acute stage	yes	nonaffected hemisphere esophageal cortical area	300 pulses at 3 Hz, 120% MT	5 sessions over 1 week	rTMS led to a significant improvement in swallowing
<i>Neglect</i>							
Koch et al. [23], 2008	15 (10 with neglect and 5 without neglect)	1–6 months	no	nonaffected hemisphere parietal cortex (P3)	600 pulses at 1 Hz, 90% MT	1 session	rTMS significantly reduced hyperexcitability within the left hemisphere only in neglect patients
Song et al. [24], 2009	7	3–8 weeks	no	nonaffected hemisphere parietal cortex (P3)	450 pulses at 0.5 Hz, 90% MT	20 sessions over 2 weeks (2 sessions every day)	rTMS improved visual spatial neglect
<i>Aphasia</i>							
Weiduschat et al. [29], 2011	10 (6 real and 4 sham)	subacute stage	yes	nonaffected hemisphere Broca homolog	600 pulses at 1 Hz, 90% MT	multiple sessions	rTMS led to a significant improvement in the Aachen Aphasia Test

MT = Motor threshold. Stimulation site reported according to the international 10–20 EEG system.

is over. Several experiments have reported that plasticity changes were not caused by rTMS alone; they also require a focused rehabilitation procedure. Therefore, the best way to facilitate recovery is to stimulate the area and activate the network supporting the specific function. This approach can be achieved by combining exogenously induced plasticity by applying rTMS with a specific training-induced plasticity like focused rehabilitation procedures.

Altogether, rTMS – which as a technique is able to noninvasively modulate cortical activity – gains growing importance in the field of stroke recovery. The possibility of noninvasively interacting with the functioning of the brain and its plasticity mechanisms opens new scenarios in the neurorehabilitation field.

Conclusion

A growing body of research in stroke patients indicated that cortical and subcortical networks are involved in sensorimotor and cognitive dysfunction and that rTMS can facilitate recovery of motor and cognitive functions. The literature provides evidence for the disturbance of interhemispheric rivalry processes as a central pathophysiological mechanism in sensorimotor and cognitive dysfunctions, resulting in a pathological hyperactivity of the intact nonaffected hemisphere. Thus, a reduction of this pathological hyperactivity by means of inhibitory rTMS seems to be an effective approach to improve sensorimotor and cognitive symptoms after stroke. However, it is still debatable which paradigm of rTMS (downregulation with low-frequency rTMS vs. upregulation with high-frequency rTMS) should be applied to enhance recovery after stroke.

Clinical trials applying rTMS already yielded very promising results. The results of rTMS studies suggest that rTMS has a potential role in terms of facilitating motor and cognitive recovery after stroke, and thus findings support the merits of noninvasive cortical interventions as adjuvant strategies during sensorimotor and cognitive rehabilitation. In recent studies, it has been shown that particularly the repeated application of newly developed stimulation protocols such as TBS seems to be able to disproportionately prolong the positive stimulation effects by means of significantly shorter stimulation times than conventional protocols. However, further research is needed to assess stimulation effects not only on clinical testing, but also in terms of disability improvement. Beneficial effects of rTMS on recovery after stroke in the framework of prospective, randomized, double-blind, sham-controlled clinical trials with larger sample sizes are needed to validate this novel therapeutic approach. Moreover, possible interactions in the combination of rTMS with conventional therapeutic approaches should also be assessed.

In future studies, emphasis should be placed on selecting patient populations to determine whether treatment response depends on age, lesion acuteness, or stroke severity. Additional aspects need to be considered, such as the timing of rTMS ap-

plication after stroke, the duration of the rehabilitation protocol, the type of frequency as well as the ideal area that should be stimulated. Furthermore, it is important to identify parameters optimizing the beneficial effects of rTMS on stroke recovery, and to monitor their long-term effects.

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Influence of Therapeutic Hypothermia on Regeneration after Cerebral Ischemia

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Abstract

The protective effect of therapeutic hypothermia in cerebral ischemia is well accepted in experimental models, and some clinical studies show that there is benefit in humans as well. Long-term observations in animal and clinical studies have documented recovery of neurological function following hypothermia treatment. Diminished damage by hypothermic protection should contribute to the recovery in many ways, but hypothermia appears to enhance regeneration of brain tissue as well. Since regeneration of the brain after damage initiates within hours and is active days and weeks after stroke, prolonged hypothermia might affect regenerative processes which have been documented to occur in these time frames. As there is a lack of data at the basic and clinical levels, the mechanism of neuroregeneration by hypothermia is unclear. Yet, we speculate that hypothermia enhances regeneration by positively influencing neurogenesis, angiogenesis, gliogenesis and synapse/circuit formation after stroke. In this chapter, we will provide up-to-date data from experimental studies and clinical reports on the effect of therapeutic hypothermia on neuroregeneration, with perspectives on future research.

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Recent studies have demonstrated that neurogenesis is evident following injury in the adult brain within the germinal niches in the subventricular zone of the lateral ventricle and the subgranular zone as well as the neocortex, spinal cord, tegmentum, substantia nigra, amygdala, brain stem and regions adjacent to the injury [1]. Neurogenesis is thought to be stimulated by cytokines, chemokines, neurotransmitters, and reactive oxygen/nitrogen species released by dying neurons and activated macrophages, microglia, and astrocytes [2]. Complete neurogenesis requires multiple steps including proliferation, migration, differentiation, survival, and integration of new neurons into the existing circuitry of the brain. However, while neurogenesis signals

appear after injury, successful replacement or regeneration of the damaged brain is scarcely observed. Thus, attempts at successful neurogenesis often fail.

Abrupt changes after stroke disrupt brain homeostasis and shift the environmental condition from maintenance of mature brain cells to resurrection of dormant stem cells. The initial environment after stroke is considered as proregenerative. But with the progression of brain injury, the environment becomes antiregenerative again. Thus, therapies should be explored that can promote successful regeneration of injured brain by preventing or reducing this antiregenerative environment. Therapeutic hypothermia is not only known to prevent serious damage by inhibiting cell death and suppressing the damaging effects of inflammation, but it also stimulates stem cells by modulating a wide spectrum of biological events [3, 4]. We expect that these features of hypothermia lead to an overall net benefit to brain regeneration. Especially when maintained for longer durations, therapeutic hypothermia would be expected to modulate many steps of the regenerative processes. In this chapter, we will provide up-to-date data from experimental studies including stroke, traumatic brain injury and other acute brain injury models, and clinical reports on hypothermic neuroregeneration and suggest areas in need of further investigation.

Hypothermia and Regeneration in Experimental Models

In contrast to neuroprotection, which targets the salvage of dying cells, neuroregeneration strategies attempt to enhance signaling pathways involved in the regeneration and remodeling of damaged tissue. Neurotrophic factors in the brain control synaptic function and plasticity and sustain neuronal cell survival, morphology, and differentiation. In studies in which hypothermia had neuroprotective effects against ischemic brain insults, brain levels of brain-derived neurotrophic factor [5, 6], glial-derived neurotrophic factor [7] and neurotrophin [8] were all increased. The scope of regeneration will be discussed as it pertains to neurogenesis, neuronal connectivity, angiogenesis, and gliogenesis.

Neurogenesis

While neurogenesis in the uninjured aged brain is markedly reduced, some rodent studies have shown that acute brain insults initiate the proliferation of neural stem cells in the subventricular zone and the hippocampal subgranular zone [9]. After stroke, ischemic neurons also lose synaptic connectivity and undergo cell death. It is becoming increasingly recognized that endogenous recovery processes are also activated after stroke, leading to neurogenesis and synaptogenesis. However, these regenerative processes are probably not successful or are incomplete, as evidenced by the permanent disability in most stroke patients. Furthermore, rodent studies indicate that neurogenesis is reduced in aged brains, and stroke seen more frequently in the elderly [10].

Strategies to improve regenerative processes should attempt to enhance proliferation of neuronal precursor cells, migration of precursor cells to the injury area, differentiation of these precursor cells into mature neurons and reconnection between neurons. A few experimental studies have demonstrated the beneficial effects of hypothermia on neurogenesis [3, 11–13]. Cooling has been shown to differentially affect neurogenesis in uninjured animals. In one study that examined neurogenesis in the developing brain, reduction of brain temperature to 30°C for 21 h decreased the number of proliferating cells in the subgranular zone of the hippocampus, but not the periventricular zone [14]. However, under conditions of hypoxia-ischemia in the developing brain, hypothermia to 33°C enhanced the maturation of neural progenitor cells in the striatum and inhibited apoptosis of proliferating neural stem cells that were already increased by ischemic stimuli [13].

In addition to it, obvious beneficial properties could also be used as a model to understand the underlying mechanisms of how to promote endogenous recovery of the injured brain. The mechanism of how cooling may enhance regenerative properties could be explained, in part, by its effect on reducing apoptosis. For example, enhanced neural stem cell survival seems to be linked to the cooling-induced upregulation of the antiapoptotic protein Bcl-2 [13]. In a study of cultured neural stem cells, mild hypothermia also inhibited apoptosis, increased the number of nestin-positive cells and inhibited stem cell differentiation into astrocytes [15]. Adult rodents exposed to forebrain ischemia and subjected to mild hypothermia had increased numbers of newborn neurons in the dentate gyrus compared to animals exposed to ischemia without cooling [3]. By contrast, another study in adult rats with forebrain ischemia showed that hypothermia had no effect on neurogenesis [16]; however, the duration of hypothermia in this study was rather short (33°C for 45 min) and occurred relatively early, either during the ischemic period or during the immediate reperfusion phase. Therefore, it is possible that hypothermia may not have any effect on neurogenesis if it is not applied during a critical time window (or windows), which has yet to be clearly defined. More research in this area is needed, in particular to determine the optimal conditions under which cooling might be expected to positively influence neurogenesis and whether cooling may improve neurogenesis in aged brains exposed to ischemia and related insults.

Neuronal Connectivity

In addition to stem cell genesis, repair of neuronal connectivity is crucial to functional recovery after stroke. To repair the loss of neuronal connectivity, neurite outgrowth and formation of new synapses are essential. A few studies have examined the role of hypothermia on neuronal circuit repair. At the morphological level, neurite and axonal outgrowth were enhanced by applying deep hypothermia (17°C) in organotypic brain slices [17]. A genomic analysis study in a rat model of traumatic brain injury demonstrated that mild hypothermia had a significant effect on gene expression. An analysis of hippocampal gene expression profiles from rats exposed to hypothermia

following traumatic brain injury revealed statistically significant differences in 133 transcripts compared to injured normothermic rats. Of these, 57 transcripts were up-regulated and 76 were downregulated after injury. Those genes involved in synapse organization and biogenesis were especially upregulated in hypothermic animals compared to the normothermic group [18]. Although the scientific literature is still scant, current data suggest that overall, hypothermia supports regenerative processes by enhancing synapse formation and reorganization. The precise mechanisms explaining the hypothermic effects are not clear. We assume inflammation is one of the key players since hypothermia influences the inflammatory response after brain injury and inflammatory cytokines play a major role in modulating neurite outgrowth and regeneration [17, 19].

Angiogenesis

Mild hypothermia has been shown to enhance angiogenesis in focal cerebral ischemia [20], spinal cord injury [21] and traumatic brain injury models [22]. Although these angiogenic effects by hypothermia are presumably beneficial to repair processes, their clinical significance is still uncertain. In fact, a few studies suggest that angiogenesis may actually be detrimental to brain repair. For example, one study of acute stroke patients showed that an early dominance of proangiogenic factors, including platelet-derived growth factors, vascular endothelial growth factors and their receptors, stromal cell-derived factor 1 and hepatocyte growth factor, was associated with mild short-term neurological deficits, but that an acute antiangiogenic status (as defined by elevated plasma endostatin levels) also predicted a worse long-term functional outcome [23]. Furthermore, pharmacologic stimulation of angiogenesis using high-dose vascular endothelial growth factor impeded recovery of neurological function in a rat model of global cerebral ischemia and caused neuronal damage in uninjured control brains [24]. However, neuroblasts which will further differentiate into fully functional neurons were identified in close proximity around the immature newly created vascular network after stroke [25]. In another study, hypothermia reduced secretion of vascular endothelial growth factor by cultured retinal pigment epithelial cells [26], which suggests that the effect of hypothermia on angiogenesis might be diverse depending on the tissue.

Gliogenesis

Oligodendrocytes succumb to brain insults and undergo cell death with a susceptibility that is similar to neurons, and hypothermia attenuates oligodendrocyte death, demyelination and circuit dysfunction [27]. Hypothermia (32°C) increased the number of oligodendrocyte precursor cells in a primary culture taken from embryonic mouse brains [28]. As a result, greater numbers of oligodendrocyte precursor cells that undergo cell cycle progression were maintained in a less well-differentiated state. However, an *in vivo* study using a hypoxia model in preterm fetal sheep demonstrated that hypothermia (30°C) was associated with an overall reduction in hy-

poxia-induced loss of immature oligodendrocytes, but did not prevent the hypoxia-induced reduced proliferation of oligodendrocytes within the periventricular white matter [29].

Reports of the effects of hypothermia on endogenous cell genesis in the injured and uninjured brains are somewhat conflicting. Some reports [14, 29] indicate that hypothermia suppresses stem cell proliferation, whereas many reports indicate the opposite [3, 15, 28], and some even suggest that cooling promotes progenitor cell differentiation towards neurogenesis over gliogenesis [13, 15]. Hypothermia to temperatures lower than 30°C seems to suppress cell proliferation and phase-specific and nonspecific cell cycle arrest as a result of reduced energy supply [14]. However, small temperature decreases seem to protect against progenitor cell death [15, 29]. Thus, we speculate that mild hypothermia enables the differentiation of precursor cells while preventing apoptosis, and that cooling to lower temperatures seems detrimental to cells and blocks their proliferation.

Astrocytes comprise the largest population of cells in the ischemic core during the subacute to chronic period after stroke [30], and reactive astrocytes are the main component of the glial scar. However, glial scar formation in the brain can obstruct neurite outgrowth and regeneration [31], and blocking astrocyte activation and related reactions can exacerbate inflammation and increase injury responses [31]. Thus, enhancement of gliogenesis may do some harm. How hypothermia affects gliogenesis has not yet been studied in any depth.

Conclusion and Future Perspectives

Although the effect of therapeutic hypothermia in brain regeneration after stroke is far from clear, under specific conditions it seems to have beneficial roles in survival, proliferation, differentiation and migration of stem/progenitor cells, and reconstruction of neural circuitry. Clearly, more research is needed in this area. To date, most of the studies addressing this topic applied cooling relatively early on. Yet, its effects on regeneration were observed days to months later. Thus, identifying the key events linking early cooling and its downstream effects on regenerative processes need to be identified. It is also conceivable that the beneficial effects of therapeutic cooling may not require early intervention, and would have obvious implications at the clinical level where intervention may potentially be initiated days to weeks and even months later.

Although there is no specific treatment to enhance or promote neuroregeneration at present, there is substantial ongoing research in this area, such as small-molecule, growth factor and cell-based therapies. When such therapies become available, combination therapy with therapeutic cooling and pharmacological interventions should certainly be explored. Therapeutic hypothermia has the potential to enhance the brain's endogenous restorative mechanisms, possibly with the aid of pharmacological or cell-based treatments. In case of drug combination therapy, very little is currently

known about the effects of hypothermia on the pharmacokinetics and pharmacodynamics of drugs and this will be another field of future interest. As neuroregeneration strategies develop, there will be increasing needs for neurological or biological markers to predict outcomes in patients as we are facing the need in case of the patients resuscitated after cardiac arrest [32, 33]. These prognostic biomarkers will be crucial in informing treatment decisions. Neuroregeneration is still a field in its infancy, and there are still many questions that remain as to whether and how therapeutic cooling may play a role.

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High Voltage Electric Potentials to Enhance Brain-Derived Neurotrophic Factor Levels in the Brain

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Abstract

Development of a safe method to increase brain-derived neurotrophic factor (BDNF) levels in the brain is expected to enhance learning and memory, induce tolerance to cerebral infarction or tolerance to depressive state, improve glucose metabolism, and suppress appetite and body weight. We have shown that repetitive applications of high-voltage electric potential (HELP) to the body increase BDNF levels in the brain, improving learning and memory in mice. Here, we investigated the effects of HELP treatment for a chronic period on the BDNF levels in the mouse brain, and on body weight in mice and humans. Adult mice were exposed to 3.1 or 5.4 kV HELP (on the body), 5 h a day for 24 weeks, and BDNF levels in the brain and alterations in body weight were analyzed. Humans [age, 53.2 ± 15.5 years old; BMI, 27.8 ± 5.6 (mean ± SD, n = 6)] were exposed to 3.9 kV HELP (on the body) for 1 h a day, continuing for 33 months (2.8 years) under the monitor of body weight. In mice, the HELP application elevated BDNF levels in the brain at least temporarily, affecting body weight in a voltage- and time-dependent manner. In humans, the HELP treatment reduced body weight compared to the pretreated initial values without any aversive effects (p < 0.002, one-way ANOVA with the post hoc Holm-Sidak test). The results in mice indicated that 3.1 kV HELP was considered insufficient for a continuous elevation of intracerebral BDNF, and 5.4 kV HELP was considered as excessive. HELP with an appropriate voltage can be utilized to increase BDNF levels in the brain for a prolonged period. We anticipate further investigations to clarify the effect of the optimal-leveled HELP therapy on memory disturbances, neurological deficits after stroke, depression, diabetes, obesity and metabolic syndrome.

Brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain [1], is known to promote neuronal differentiation/maturation in the developing central nervous system [2, 3], participates in multiple forms of learning and memory by promoting dendritic outgrowth and synaptic formation [4], and protects neurons from lethal ischemic stresses by inducing tolerance to cerebral infarction (infarct tolerance) [5].

In addition to the role in memory and infarct tolerance, BDNF in the brain plays a critical role in energy balance, glucose metabolism, and in reward centers of the brain impacting on feeding behavior and body weight [6–8]. Genetic impairment of BDNF synthesis caused hyperphagia and aggressiveness [9], obesity, hyperglycemia, hyperinsulinemia, hyper-low-density-lipoproteinemia, and a short life span in mice [10]. In contrast, increased BDNF levels in the brain by a direct infusion of exogenous BDNF suppressed appetite, decreased blood glucose levels by increasing insulin sensitivity, and caused body weight loss in a dose-dependent manner without any metabolic toxicity in normal rats [11]. Furthermore, genetic BDNF insufficiency was linked to severe obesity in humans [12]. Therefore, increased BDNF levels, if achieved by a safe method, should suppress normal or excessive appetite and reduce body weight.

Specific Types of Electric Stimulation

A brief period of direct current electrification (3 VDC, 100 μ s, 20 Hz for 1 h) [13]; epileptiform discharges [14], chronic electroconvulsive therapy for the treatment of major depression [15], and repetitive spreading depression [16] have been shown to elevate BDNF levels in the brain. However, none of these types of stimulation is applicable to healthy individuals to increase BDNF levels in the brain, improve cerebral functions or glucose metabolism, and to increase cerebral viability.

Recently, we have demonstrated that the treatment with high-voltage electric potential (HELP), characterized by a selected voltage on the body, increased BDNF levels in the mouse brain [17]. Animals with increased BDNF levels by the HELP treatment showed improved learning and memory, and achieved infarct tolerance [17]. In addition, HELP treatment suppressed age-dependent body weight gain at least for 12 weeks in mice [17].

The High-Voltage Electric Field Therapy

Repetitive exposure of the body to various levels of electric field (EF) for 1 to several hours a day, by direct contact with a working electrode (≤ 30 kV RMS, 50 or 60 Hz), under the condition that the body is insulated from surrounding electrotransformable materials (metals), making indirect (air-) contact with the ground electrode (= 0 V), was approved in 1963 by the Ministry of Health, Labor and Welfare, Japan as a safe alternative therapy to be utilized for improving headache, shoulder/neck stiffness, insomnia, and chronic constipation. Although a selected EF level generated by 7.0 kV (50 or 60 Hz) at the generator suppressed a restrained stress-induced increase in plasma lactate levels in rats [18], the underlying biological mechanisms by which EF acts on the body, the optimum protocols, the maximum length of improving period, and the optimal EF level for the improvement of these symptoms have been unclear.

In this study, we investigated the effect of HELP treatments with either of 2 selected (high or low) voltages at the body for a prolonged period (24 weeks) on the BDNF levels in the mouse brain, or the effect of a HELP treatment with a specific voltage on the body, considered as optimal, for 33 months on human body weight. Fundamentally, the voltage at the output level generated at the transformer diminishes in the electric cord connecting to the working electrode (HELP supplier), affected by the resistance between surfaces of the working electrode and the body (due to attachment area, clothes, skin or hair conditions), the body weight (the size of impedance), and by the surrounding structures of the HELP application system (the extent of leakage of electric potential from the insulated elevated zone to the outer zero level). Therefore, it was essential to measure and regulate electric potential (= voltage level) on the body, not at the output level of the transformer, or essentially variable EF levels in the air around the body.

Experimental Procedure and Treatment Protocol

The experimental protocols were approved by the NCVV animal research committee. All efforts were made to minimize suffering and the number of animals used. In the clinical trial, participants agreed to set and use the medical apparatus, a HELP applier, in their house. The specific HELP level designed in this study was generated by a medical apparatus (an EF generator ≤ 9.0 kV) approved by the government for the treatment of headache; chronic constipation; neck/shoulder stiffness, and/or insomnia. Informed consent to use the HELP method (with a definition/regulation; 3.9 kV on the body) in individual homes, and to monthly monitoring of body weight with or without treatment was obtained from every participant.

Experiment 1 (Experimental Procedure in Mice)

Male adult C57BL/6J mice (8–9 weeks old, 22–24 g, Clear Japan Inc., Osaka, Japan) were placed in a temperature-controlled room under a regular light/dark cycle and had free access to food and water ad libitum throughout the experiment. Each mouse cage, 17 × 23 × 12 cm in size, composed of 2-mm-thick acryl boards, housed 5–6 mice. A mattress type HELP supplier, 30 × 50 cm in size, connected to the HELP generator (an AC transformer; 60 Hz) made originally for humans, was placed under the cage. The counter electrode, 50 × 150 cm in size and grounded (= 0 V), was located below the HELP supplier (>20 cm from the animal). The elevated electric potential area, consisting of the HELP supplier, the cage, and the mice, was electrically insulated from the surroundings. Mice in the cage received HELP at a single voltage, making indirect contact with 0 V (ground) via air.

In the setting, the HELP generator elevated the electric potential at the surface of the body to 0 V (control), 3.1 or 5.4 kV, for 5 h a day over consecutive 3, 6, 12, or 24 weeks. The EF level generated by the HELP supplier in the cage was approximately 14–15 V/m, or 24–25 kV/m, respectively, in a vertical direction in the absence of animals.

After the 3.1- or 5.4-kV HELP treatment over 3, 6, 12 or 24 weeks, BDNF levels in the forebrain were measured using ELISA (n = 24–25 per group), and the escape latencies were monitored for the analysis of spatial learning, comprising cognition/orientation, working memory/consolidation, and recall/navigation (n = 24–25 per group), using the Morris water maze test arranged for mice, 4 sessions per a day for consecutive 5 days with a cutoff time set at 300 s, as described in detail elsewhere [17].

Experiment 2 (Treatment Protocol in Humans)

Male or female volunteers [body mass index (BMI) >22, normal, overweight, or obese] were enrolled. The distribution of age was from 33 to 71 years old (53.2 ± 15.5 , mean \pm SD; n = 6, male: 4, female: 2). The BMI in the HELP-treated group was from 22.1 to 36.8 (27.8 ± 5.6). An EF generator, i.e. a medical apparatus originally made for humans (K-7 or K-9, Hakuju, Co. Ltd., Tokyo) for the usage in the clinic or at home, with a multistep function to freely adjust the output levels (AC 60 Hz, ≤ 7.0 or 9.0 kV at the transformer), was set in individual homes as a HELP applicator. The safety of this system for humans was established by the Japanese government in 1963.

A mattress type HELP supplier set on the bed, 30 \times 50 cm in size, was connected to the generator. The ground under the floor in the room (= 0 kV, >20 cm away from the body surface) was utilized as a counter electrode. The zone of elevated electric potential, consisting of the HELP supplier and the body, was electrically insulated from the surroundings by means of an insulating (rubber) mattress, so that the HELP-exposed human body makes indirect contact with the ground via air.

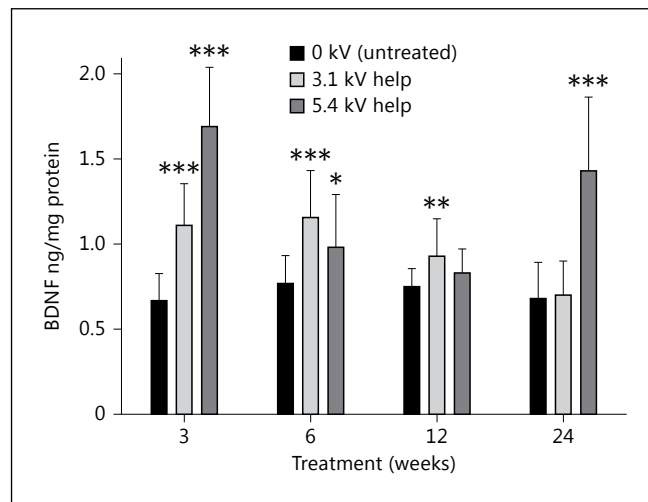
A specific HELP, adjusted to 3.9 kV on the body compared to the ground level (0 kV), was applied via the HELP generator. The treatment was performed for 1 h a day over consecutive 33 months at night while sleeping by setting a timer.

The selection of the voltage was based on the findings that various ranges of HELP differently increase BDNF levels in the mouse brain for a temporary or a prolonged period in mice, differently improving learning and memory function, and reducing or increasing body weight at a chronic phase. Family members of the enrolled persons, who were supposed to have the same diet menu in the same house during the study period, served as references. The distribution of age in the reference group was from 22 to 65 years (46.0 ± 16.9 , mean \pm SD; n = 6, male: 4, female: 2), and the BMI range was from 23.6 to 29.8 (27.7 ± 2.8 , mean \pm SD). There was no significant difference in physical conditions between the treated and the reference groups.

Statistical Analysis

Alterations in body weight before and after the HELP treatment were analyzed using one-way ANOVA with the post hoc Holm-Sidak test. A value of $p < 0.05$ was considered significant, and the results were presented as mean \pm SD. Intention-to-treat analysis was used in the assessment of the human study that accepts all data obtained from every participant.

Fig. 1. Alterations in BDNF levels in the mouse brain. The treatment with HELP (3.1 and 5.4 kV) for 12 weeks significantly reduced body weight compared with the corresponding untreated controls. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. $n = 24\text{--}25$; error bars indicate SD.



Results

Alterations in Brain-Derived Neurotrophic Factor Levels in the Mouse Brain (Experiment 1)

After the HELP treatment, the BDNF levels in the brain increased in a mono- (3.1 kV) or biphasic manner (5.4 kV), compared with the corresponding 0-volt-treated controls during the same period (fig. 1). There were significant increases in the BDNF level after 3, 6, and 24 weeks in the 5.4-kV HELP-treated group ($p < 0.05$), or after 3, 6, and 12 weeks in the 3.1-kV HELP-treated group ($p < 0.05$).

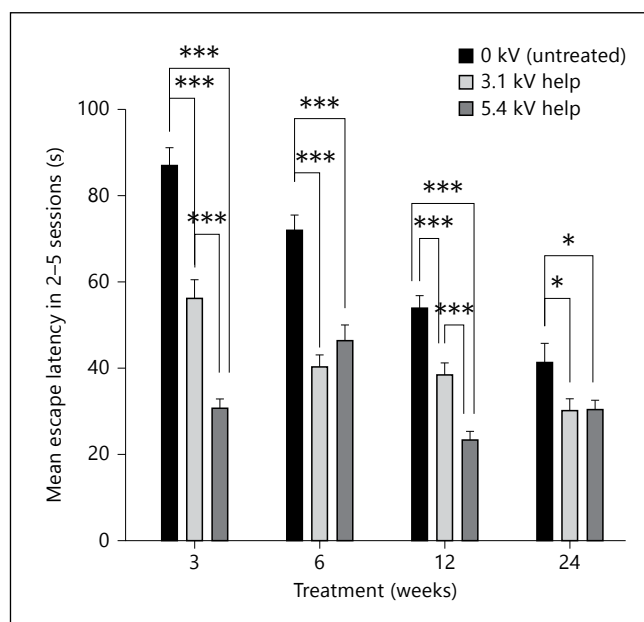
Alterations in Learning and Memory in Mice (Experiment 1)

After the 3.1- or 5.4-kV HELP treatment, the time needed to escape to the platform (escape latency) decreased significantly, compared with the corresponding 0-volt-treated controls (fig. 2). Among the treated and control groups, the greatest improvement in the escape latency score was 6 weeks after 3.1 kV HELP ($p < 0.001$), and 3 or 12 weeks after 5.4 kV HELP ($p < 0.001$). It was found that 0-kV-treated control animals showed a steady and significant improvement in spatial learning from young adulthood to middle-aged time.

Alterations in the Mouse Body Weight (Experiment 1)

After HELP treatment for 6 weeks, the body weight was significantly reduced, compared with the corresponding 0-kV-treated controls ($p < 0.05$) (fig. 3). In the 3.1-kV HELP-treated group, the significant reduction at 12 weeks disappeared at 24 weeks. Although improvement was observed in the Morris water maze test, a significant (paradoxical) increase in body weight was observed 24 weeks after consecutive 5.4-kV HELP treatment.

Fig. 2. Alterations in learning and memory function in the Morris water maze test. After HELP treatment with either voltage, spatial learning significantly improved in the Morris water maze test compared to the corresponding untreated controls. * $p < 0.05$; *** $p < 0.001$. $n = 24$ – 25 ; error bars indicate SEM.



Alterations in Body Weight in Humans (Experiment 2)

The body weight was reduced gradually by the 3.9-kV HELP treatment (fig. 4). After the induction of HELP, the reduction pattern of the body weight was biphasic, achieving the first reduction compared to the pretreated control value at 12 months, which disappeared but appeared again from 26 to 33 months ($p < 0.001$, $t = 3.16$ – 4.70) (fig. 4). There was no significant side effect in subjective or objective aspects, during or after the treatment.

The BMI at the end of the observation period was -4.8 ± 1.4 compared to the pretreated initial value in the treated group, and $+3.5 \pm 2.0$ compared to the initial value in the reference group ($p < 0.001$, t-test).

As regards the compliance with the protocol, every participant in the treated group reported that they followed the protocol throughout the period except for some days (<30 days in a year) when they were away from home. A person in the reference group (initial BMI: 29.8) had lost more than 10 kg during the initial 12 months, but encountered a rebound phenomenon, ending up with 3 kg more compared to the initial value.

Discussion

Increased BDNF levels in the brain act as a suppressant of appetite and body weight gain [11, 19]. In the present study, a chronic HELP treatment with a selected voltage on the body significantly suppressed body weight of normal (by 5.8% per 33 months, i.e. 2.1% per year) or overweight healthy volunteers (by 6.0% per 33 months, i.e. 2.2%

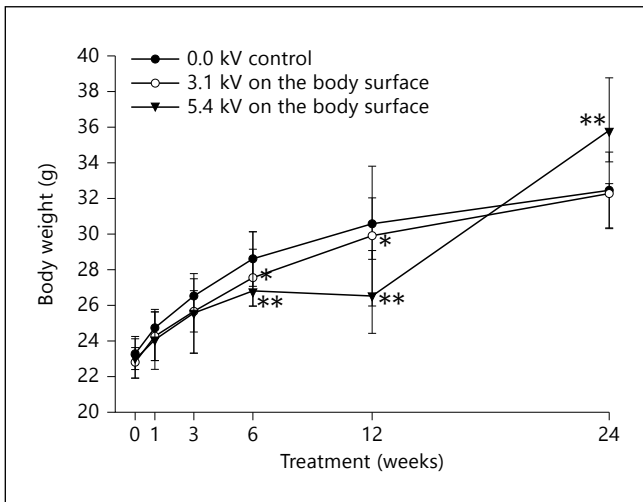


Fig. 3. Alterations in body weight in mice. Twelve weeks after HELP treatment with either voltage, body weight was significantly reduced compared to controls. Twenty-four weeks after HELP, there was no significant reduction in the 3.1-kV-treated group, but a significant increase (considered as a rebound phenomenon) was observed in the 5.4-kV-treated group. Alteration in body weight followed weeks after the alterations in BDNF levels in the brain (fig. 1). * $p < 0.05$; ** $p < 0.01$, compared with the corresponding controls. $n = 24-25$; error bars indicate SD.

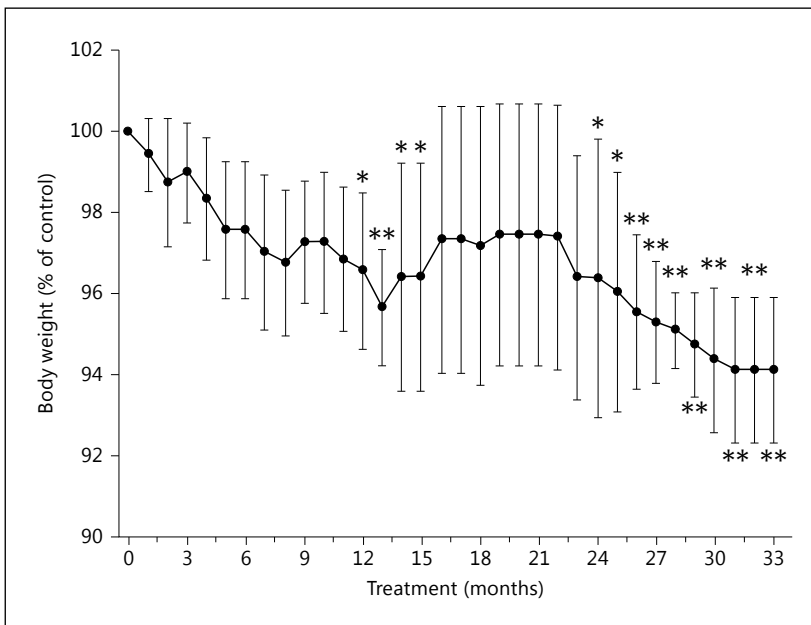


Fig. 4. Alterations in body weight after the HELP treatment in humans. There were significant, biphasic reductions in body weight after the exposure to 3.9 kV HELP, performed for 1 hour per day, for 33 months. * $p < 0.01$; ** $p < 0.001$. $n = 6$; error bars indicate SD.

per year). Statistical differences between values before and after the treatment were detected in a biphasic manner. The characteristic alterations in the human body weight was consistent with the observation that 5.4 kV HELP, i.e. a relatively higher voltage than 3.9 kV, induced biphasic increases in BDNF levels in the mouse brain (fig. 1). Although BDNF levels in the human brain before and after the HELP treatment were not analyzed in the present study, consistent and stable reduction in the body weight after the HELP application may indicate that the BDNF levels in the human brain were increased for a prolonged period.

Mice engineered to overexpress BDNF demonstrated enhanced performance in spatial learning [20]. Significant improvements in spatial learning were observed after a HELP therapy, at the time point when BDNF levels in the brain increased, especially in the cortex [21], which was in accordance with the evidence that increased BDNF levels in the cortex enhance spatial learning and memory [22].

Restoration of decreased BDNF levels in the brain has been an aim of antidepressant treatment [23]. Interestingly, the 4 symptoms that are improved by the EF therapy are major symptoms of masked depression. Increased BDNF levels after the EF therapy using various EF levels may improve depressive mood disorder, which may be resulting in improvements of the 4 symptoms, at least for a temporary period.

In our previous study, intracerebral infusion of recombinant BDNF (8 μ g total) continuing for 1 or 2 weeks (prolonged and slow injection), but not for 3 days (short and rapid injection), was associated with the development of infarct tolerance in rats [5]. Repetitive spreading depression stimulations that increase BDNF levels in the brain [24] induced infarct tolerance, 2 weeks after repetitive spreading depression in rats and 1 week after spreading depression in mice [25, 26]. Importantly, the rate of passage of biological time is inversely correlated to $[\text{body weight}]^2$, as represented by longevity and the heart/respiration rate among animal species [27]. The biological time of 1 week in mice is considered to be several months in humans.

Thus, the development of infarct tolerance appears to require prior and prolonged elevation of intracerebral BDNF levels. However, BDNF levels in the brain do not necessarily last for a long time after various HELP therapies but fluctuate and sometimes decline, depending on its voltage level and the treatment period (fig. 1). Because there was a rebound-like phenomenon of the body weight in mice (fig. 3), possibly caused by a rapid and excessive increase and the following unresponsive, silent period (fig. 1, the decline at 12 weeks in the 5.4-kV group), the selection of voltage in HELP treatment is extremely important to elevate intracerebral BDNF levels for a prolonged period. In the present study, we selected 3.9 kV on the body for the human therapy, a mathematically simulated value that can elevate BDNF levels for a prolonged time, taken from data obtained in our experimental studies using mice, which ranged between 3.1 kV (insufficient) and 5.4 kV (excessive).

As regards safe and practical methods to increase BDNF levels, physical exercise [28] and chronic dietary restriction [29] have been shown to upregulate BDNF in the brain. In addition to these safe methods that are not necessarily applicable to many

people, HELP with an appropriate voltage for 1 h per day may have the potential to increase BDNF levels in the brain for a prolonged period. Increased BDNF theoretically improves neurological, emotional, and metabolic disorders associated with reduced or subnormal BDNF levels in the brain [30]. Additional studies focusing on the effect of HELP with an appropriate voltage on the body surface (not unstable, excessive or insufficient) on impaired cognitive/learning function, depressive mood disorders, and other neurological, mental and metabolic disorders are required to better understand the significance of HELP therapy. A sophisticated HELP therapy with an optimal voltage on the body, rather than the traditional EF therapy with various electric powers, has the potential as an innovative, macrobiotic new intervention to recover or improve human health.

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Prevention of Post-Stroke Disuse Muscle Atrophy with a Free Radical Scavenger

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Abstract

In spite of appropriate treatment in the acute phase of stroke, quite a few patients with hemiparetic stroke become disabled and stay in a wheelchair or bedridden state in the chronic phase. In stroke patients, gait dysfunction results mainly from severe hemiparesis due to ischemic damage to the motor neuron tract and partly from disuse muscle atrophy in paretic and nonparetic legs. Disuse muscle atrophy occurs even in healthy subjects as early as 4 days after bed rest immobilization and progresses further correlating with the duration of immobilization. Although detailed mechanisms of disuse muscle atrophy remain unclear, free radical scavengers are known to play an important role in the development of disuse muscle atrophy. One of the neuroprotective agents, edaravone, a free radical scavenger, succeeded in proving clinical usefulness in a phase III clinical trial in Japan. In this trial, stroke patients were administered edaravone for 14 days consecutively. The results of the edaravone trial are taken to indicate that long-term administration of a free radical scavenger may prevent disuse atrophy thereby improving functional outcome. We performed a randomized pilot study in hemiparetic stroke patients to test the validity of this view. Acute stroke patients were randomly allocated to two groups, one receiving edaravone for 3 days (short-term group) and the other for 10–14 days (long-term group). At 3 months after stroke, the grade of femoral muscle atrophy was significantly milder and the maximum walking speed was significantly faster in the long-term group than in the short-term group. The study suggests that long-term administration of a free radical scavenger may prevent the development of leg disuse atrophy thereby ameliorating locomotor function. Attention should be paid to myoprotective drug therapy in acute stroke, since it may be easier and clinically more effective than neuroprotective therapy from the viewpoint of functional prognosis.

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Motor weakness is one of the most common symptoms of stroke which largely disturbs activities of daily living in stroke patients. Even with optimal therapy, 15–30% of stroke patients are permanently disabled and 20% require institutional care at 3 months after onset [1]. Such a long-term functional disability is caused mainly by damage to the motor neuron tract at the frontal cortex, the corona radiata, the internal capsule or the pons which leads to hemiparetic weakness of the contralateral upper and lower limbs. The impaired function of the motor tract may be restored afterwards by the compen-

satory development of the motor neuron tract as discussed by several authors in this book. Yet, even after the compensatory development of the motor neuron tract, motor dysfunction may remain unaltered, if disuse muscle atrophy is developed in the paretic limb associated with muscle weakness and/or articular contracture during the acute and subacute phases. Disuse muscle atrophy occurs most likely in the paretic lower limb of elderly stroke patients who are bedridden during the acute phase of stroke. The antigravity muscles of the lower limbs, such as the soleus, gastrocnemius, and vastus lateralis, are most commonly affected by immobilization [2, 3]. Stroke is primarily a central nervous system disease. However, we should be reminded that secondary muscle changes play an important role in the persistence of lower-limb disability in the chronic phase. The muscle changes may partly be reversible and may be treatable with rehabilitation. Yet, delayed treatment of muscle changes likely fails to restore normal muscle structure and muscle strength eventually leading to articular contracture. Therefore, myoprotective drug therapy should be initiated in the early phase of stroke. Myoprotection is considered easier and clinically more effective as compared with neuroprotection. Unfortunately, however, little attention has been paid to myoprotective drug therapy in the past history of acute stroke management.

Muscle Changes with Ageing

During normal ageing, muscle tissue gradually changes in its distribution with advancing age; fast-twitch muscle fibers (MHC type IIa and IIb) decrease and mitochondria-rich slow-twitch muscle fibers (MHC type I) increase. This change in fiber type distribution results from motor unit denervation and subsequent reinnervation from adjacent intact muscle fibers. The changes result in reduction of muscle strength. According to the study of Kostka [4], muscle strength decreases by 18% between 30 and 60 years of age, and further decreases by another 20% between 60 and 90 years of age. Thus, elderly subjects are in a preparatory condition for motor weakness even prior to stroke. Once stroke occurs in elderly subjects, it likely causes severe hemiparesis which forces elderly patients to stay bedridden for 1–2 weeks or longer. Immobilization due to the bedridden state likely causes disuse muscle atrophy in the paretic leg as well as the nonparetic leg (fig. 1).

Immobilization and Disuse Muscle Atrophy

Long-term muscle disuse due to immobilization or chronic bed rest is known to induce muscle atrophy and muscle strength reduction particularly in legs [5, 6]. Disuse atrophy of lower-limb muscles was confirmed to occur following 35 days of bed rest in healthy young subjects with a mean age of 24 years [7]. Disuse atrophy occurs more rapidly in older subjects. In the study of Kortebein et al. [8], lower-limb disuse atrophy was observed following 10 days of bed rest in healthy subjects with a mean age of

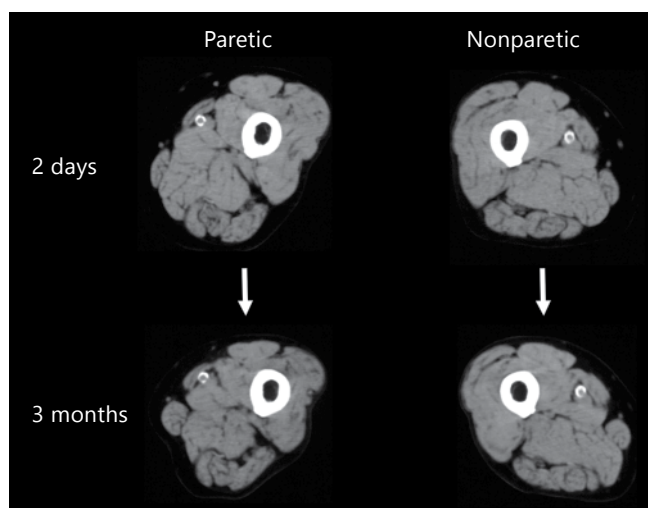


Fig. 1. Femoral muscle volume changes in a 64-year-old stroke patient with severe hemiparesis. Femoral CT shows bilateral muscle atrophy at 3 months after stroke.

67 years. In the study of Paddon-Jones [9], elderly patients showed the same amount of lean leg muscle mass after 3 days of hospitalization as healthy older subjects experienced after 10 days of inactivity, and a 3-fold greater loss of lean leg muscle mass than a younger cohort confined to bed for 28 days. Thus, elderly subjects may develop disuse muscle atrophy even a few days after bed rest immobilization. Early development of disuse atrophy was confirmed to occur in rats with hind limb unloading [10]. The soleus muscle mass began to decrease at 4 days after hind limb immobilization.

Mechanisms of Disuse Atrophy

Recent advances in cellular and molecular biology have provided quite a few pieces of evidence for understanding the pathophysiology of disuse atrophy. Disuse atrophy of skeletal muscle occurs due to both a decrease in muscle protein synthesis and an increase in the rate of proteolysis [11, 12]. It has not yet been fully clarified how a decrease in muscle protein synthesis and an increase in proteolysis are initiated. However, experimental studies indicated a close relationship between oxidative stress and disuse muscle atrophy [13–15]. More specifically, atrophic muscles of rats showed elevated levels of thiobarbituric acid reactive substances and oxidized glutathione, which are both markers of oxidative stress. Furthermore, an increase in the formation of superoxide radical anions (O_2^-) was indicated by elevated Cu/Zn-containing superoxide dismutase activity in the cytoplasm of atrophic muscle cells, and 12 days of disuse atrophy showed a 2.2-fold rise in xanthine oxidase levels relative to controls. Histochemical studies using transmission electron microscopy have revealed elevated levels of reactive oxygen species such as H_2O_2 in atrophic muscles. Metal compounds such as iron have also been shown to increase oxidative stress in atrophic muscles, and the administration of the iron-chelating agent,

deferoxamine, suppressed the increase in thiobarbituric acid reactive substances and oxidized glutathione in the atrophic muscles of rats [16]. In more recent years, the role of iron in oxidative stress has attracted close attention in the field of physiology [17, 18].

Another factor that may be involved in the development of muscle atrophy is elevation of the intracellular calcium ion (Ca^{2+}) levels during muscle inactivity [13–15]. Ca^{2+} levels in the cytoplasm of atrophic muscle cells were approximately 4-fold higher than levels in normal muscle cells. This accumulation of intracellular Ca^{2+} during muscular disuse may occur due to ionic disturbances of the cell membrane, which retards cellular removal of Ca^{2+} . This resulting elevation of cytosolic Ca^{2+} may lead to the activation of calcium-dependent proteases that mediate, thus leading to the breakdown of muscle tissue.

In addition to inducing muscle atrophy during disuse, oxidative stress was shown to be enhanced following remobilization in rats [19]. Oxidative stress markers increased rapidly when rabbits with leg immobilization began to move the immobilized leg. This suggests that the production of free radicals occurs continuously in patients who start moving paretic limbs. In order to prevent disuse muscle atrophy, therefore, free radicals need to be suppressed continuously for a considerably long duration.

Long-Term Free Radical Scavenger Improves Functional Outcome of Stroke

Edaravone, a free radical scavenger, was shown to have neuroprotective action and to ameliorate functional outcome of acute stroke patients in a placebo-controlled double-blind study performed in Japan [20]. Edaravone is now widely used clinically for the treatment of acute ischemic stroke in Japan. This is the only drug in the world which has been approved as a neuroprotective drug and permitted for clinical use in the management of acute stroke. During the last 2 decades, more than 30 substances with neuroprotective action were subjected to phase III clinical trials to confirm the efficacy in improving the clinical outcome of acute stroke patients [21–25]. However, all of them including free radical scavengers, except for edaravone, failed to show clinical effectiveness. Questions are raised here. Why could only edaravone prove clinical usefulness? Are free radical scavengers superior to the other types of neuroprotective agents, such as NMDA receptor antagonists or Ca^{2+} channel blockers? If free radical scavengers are superior to the other types of neuroprotective drugs, then why did the other free radical scavengers, such as tirilazad [21] or NXY-059 [22], fail to show clinical effectiveness? In order to find the answers to these questions, we should pay attention to the design of the phase III clinical trial of edaravone which quite differs from that of all the other neuroprotective drugs. In the edaravone trial, ischemic stroke patients admitted within 72 h after onset were enrolled into the study. They were randomly allocated to two groups. In one group, edaravone was intravenously administered twice daily for 14 days, and in the other group, placebo was given in a similar manner for 14 days. Thus, the time limit of initial drug administration and the duration of drug administration are enormous-

ly longer in the edaravone trial as compared with the other phase III trials of neuroprotective drugs. In almost all the phase III trials of neuroprotectants, the drugs were initially administered within 6–12 h after stroke onset and were continued for 1–3 days. For instance, in the clinical trials testing the efficacy of a free radical scavenger, tirilazad, the drug was first given within 4–12 h after onset and was continued for 3–4 days [21]. Likewise in the SAINT II study for evaluating the efficacy of a free radical scavenger, NXY-059, the drug was initially administered within 6 h after onset and was continued for 3 days [22]. Such an early and short-term administration of drugs is reasonable for testing the clinical usefulness of neuroprotective drugs, since irreversible changes likely occur within 6 h in the majority of ischemic neuronal cells and the process of neuronal cell death may be completed within several days after onset. In most phase III clinical trials of other types of neuroprotective agents, the drugs were given in a similar manner as in the SAINT II study or tirilazad studies. The results in these studies were all unsuccessful. In the edaravone phase III trial, the initial administration of the drug was considerably late; the average time for the first drug administration was 37.3 h after onset. Thus, the initiation of edaravone administration was unfavorably late for testing the clinical efficacy of neuroprotective drugs. Presumably, in the edaravone trial, neuronal cells in the majority of ischemic areas may have had irreversible changes prior to edaravone administration. Edaravone was given consecutively for 14 days in all the patients. Such a long-term administration of a free radical scavenger may be meaningless from the viewpoint of neuronal protection, since the process of neuronal cell death is likely completed within several days after onset. On the other hand, delayed and long-term administration of a free radical scavenger for 14 days is meaningful, if the target is myoprotection, since the process of disuse muscle atrophy begins at several days after immobilization and is enforced at the time of remobilization. The results of the phase III clinical trial of edaravone suggest that the beneficial effects of the drug may be attributable to myoprotective effects rather than to neuroprotective effects.

Prevention of Muscle Atrophy with a Free Radical Scavenger

In order to test the hypothesis that long-term administration of a free radical scavenger may prevent disuse muscle atrophy of lower limbs and improve locomotor function in stroke patients, we performed a pilot study (Muscle Atrophy Restraint with Vigilant Edaravone Long-Term Use after Stroke: MARVELOUS) in patients with ischemic stroke [26]. The study was an open-label, multicentered, randomized, controlled pilot study performed in Japan. Written consent was obtained from all patients prior to registration. The study protocol was permitted by the local ethics committees of all the institutes that participated. Acute ischemic stroke patients with hemiparesis were randomly allocated to receive edaravone for 3 days (short-term group) or 10–14 days (long-term group). These two durations were determined on the basis of the assumption that 3-day administration likely exerts neuroprotective effects and 10- to 14-day administra-

tion may provide both neuro- and myoprotective effects. Inclusion criteria were: (1) age 20–79 years, (2) definitive paresis or paralysis of the leg of the diseased side, (3) admission within 24 h after onset of stroke, (4) no neurological deficits prior to stroke, (5) no thrombolytic therapy and (6) well-maintained consciousness level. Edaravone (30 mg, twice daily) was administered intravenously during the first 3 days of admission in the short-term group and during the first 10–14 days of admission in the long-term group. All the patients were admitted to an acute stroke center and underwent bedside rehabilitation therapy followed by out-of-bed rehabilitation therapy. In order to evaluate changes in femoral muscle volume, the circumference of the thigh was measured at 15, 10 and 5 cm above the upper end of the patella bilaterally in a position with legs stretched on the floor. The circumference of the lower leg was also measured at its largest part bilaterally in the same manner to evaluate crural muscle volume. The first measurement of the leg circumference was performed within 4 days after admission, and the values were regarded to represent the baseline leg muscle volume. The second and third measurements were performed at 3 weeks and 3 months after stroke onset, respectively. The reduction of leg circumference at 3 weeks and 3 months after stroke onset as compared with the baseline value was expressed as percentage reduction and was cited to represent the degree of muscle atrophy. The primary endpoints of the study included the degree of leg muscle atrophy and the severity of leg locomotor dysfunction at 3 months after stroke onset. The severity of locomotor dysfunction was evaluated with maximum walking speed (MWS) over a distance of 10 m. Data are expressed as percentages for categorical variables or means for continuous variables. A Pearson χ^2 or Fisher exact test was used for the statistical comparison of qualitative or categorical variables. An unpaired Student t test was also used to compare differences in the degree of disuse muscle atrophy and MWS between the two groups. A p value of <0.05 was considered to be statistically significant.

A total of 47 patients were enrolled in the study, and 3-month follow-up was completed in 41 patients (21 in the short-term group and 20 in the long-term group). As shown in table 1, there was no significant difference in age, gender, and severity of neurological symptoms on admission. Edaravone was administered for 3 days in all the patients in the short-term group and for 13.6 ± 1.2 days in the long-term group. Bedside rehabilitation was performed within 3 days of admission in 67% of patients in the short-term group and in 70% of patients in the long-term group. By the end of 3 weeks after stroke onset, rehabilitation therapy was performed for 16 ± 5 days in the short-term group and for 16 ± 7 days in the long-term group. There was no significant difference in the state of rehabilitation between the two groups.

Table 2 shows the state of gait disability and grade of muscle atrophy at 3 weeks and 3 months after stroke onset. Changes in femoral volume measured at 15 cm above the patella are demonstrated as representative markers of leg disuse atrophy. At 3 weeks after onset, 48% of patients in the short-term group and 50% of patients in the long-term group had gait disability either requiring a wheelchair or remaining in a bedridden state. Disuse muscle atrophy was more or less developed bilaterally in almost all the patients

Table 1. Patient characteristics

	Short-term group (n = 21)	Long-term group (n = 20)
Age, mean \pm SD, years	70 \pm 7	69 \pm 8
Male gender, n	13	14
Median NIHSS score on admission	7	9
Severe weakness in paretic leg ¹	11 (52.4%)	10 (50.0%)
Duration of edaravone administration, mean \pm SD, days	3.0 \pm 0.0	13.6 \pm 1.2
Bedside rehabilitation within 3 days	14 (67%)	14 (70%)
Total rehabilitation within 3 weeks, mean \pm SD, days	16 \pm 5	16 \pm 7

NIHSS = National Institutes of Health Stroke Scale.

¹ Severe weakness was defined as an NIHSS motor subscore of 3–4.

Table 2. Grade of leg atrophy and state of walking ability at 3 weeks and 3 months after stroke

	Short-term group (n = 21)	Long-term group (n = 20)
3 weeks after stroke		
Paretic leg atrophy, mean \pm SD, %	5.0 \pm 3.4	4.4 \pm 4.1
Nonparetic leg atrophy, mean \pm SD, %	3.7 \pm 4.4	2.0 \pm 3.8
Gait disability, n (%)	10 (48)	9 (45)
3 months after stroke		
Paretic leg atrophy, mean \pm SD, %	8.3 \pm 5.2	3.6 \pm 5.9**
Nonparetic leg atrophy, mean \pm SD, %	5.7 \pm 6.4	1.5 \pm 6.03*
Maximum walking speed, cm/s	53.6 \pm 54.8	97.9 \pm 67.3*

Gait disability was defined as patients in a wheelchair or bedridden state. Leg atrophy was represented by femoral volume reduction at 15 cm above the patella. *p < 0.05 vs. short-term group; ** p < 0.01 vs. short-term group.

in both groups. The grade of muscle atrophy was 5.0 \pm 3.4% in the paretic leg and 3.7 \pm 4.4% in the nonparetic leg in the short-term group and 4.4 \pm 4.1% in the paretic leg and 2.0 \pm 3.8% in the nonparetic leg in the long-term group. There was no significant difference in the grade of atrophy between the two groups. At 3 months after stroke, muscle atrophy in the short-term group became more remarkable as compared with 3 weeks after stroke, whereas the atrophy in the long-term group became less remarkable as compared with 3 weeks after stroke. The grade of atrophy was 8.3 \pm 5.2% in the paretic leg and 5.7 \pm 6.4% in the nonparetic leg in the short-term group and 3.6 \pm 5.9% in the paretic leg and 1.5 \pm 6.0% in the nonparetic leg in the long-term group. The grade of atrophy in the long-term group was significantly less prominent both for the paretic leg (p < 0.01) and nonparetic leg (p < 0.05) as compared with that in the short-term group. The MWS over the 10-meter distance was 53.6 \pm 54.8 cm/s in the short-term group and

97.9 ± 67.3 cm/s in the long-term group. The MWS was significantly faster in the long-term group than in the short-term group ($p < 0.05$). There was a significant negative correlation between the grade of paretic leg atrophy and MWS ($r = -0.87$, $p < 0.001$).

Thus, the pilot study suggests that a free radical scavenger may prevent the development of leg disuse atrophy and ameliorate locomotor function in hemiparetic stroke patients, if administered for a considerably long time. It remains unclear why anti-atrophic effects were not remarkable at 3 weeks after stroke and became prominent at 3 months after stroke. This may be explained by the delayed involvement of apoptosis during the course of disuse muscle atrophy, which eventually leads to muscular cell shrinkage and/or death [17, 18]. As a result, the clinical manifestation of muscle atrophy may not become apparent until changes in the muscular intracellular environment have taken place, which may occur over a period of several weeks. Edaravone is a drug with neuroprotective action. Therefore, the possibility that the prevention of muscle atrophy is mediated partly by neuroprotective action cannot be excluded completely. It seems, however, obvious that the prevention of disuse muscle atrophy is closely connected with improvement of functional outcome.

Conclusions

Leg motor weakness and resulting locomotor dysfunction in stroke patients are attributable to both ischemic brain damage and disuse muscle atrophy. In the brain, neurons are unable to survive for extended periods of time under ischemic conditions, and the protection of such vulnerable brain tissue with drug therapy is difficult in a clinical setting. In contrast, muscle is a robust tissue, and the process of disuse muscle atrophy takes place gradually following stroke, which may provide a larger therapeutic window of opportunity. Therefore, agents that offer myoprotection may provide an easier and more effective treatment option to ameliorate the functional disability of stroke patients. Based on the results of the MARVELOUS study, the use of antioxidant therapy for as long as possible appears to be warranted to provide the maximal level of both neuroprotection and myoprotection. Larger, randomized controlled clinical trials are required to confirm the beneficial effects of free radical scavengers in the management of stroke.

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29 Following Charcot: A Forgotten History of Neurology and Psychiatry

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After decades of focusing on how to alleviate and prevent recurrence of acute CNS injuries, the emphasis has finally shifted towards repairing such devastating events and rehabilitation. This development has been made possible by substantial progress in understanding the scientific underpinnings of recovery as well as by novel diagnostic tools, and most importantly, by emerging therapies awaiting clinical trials. In this publication, several international experts introduce novel areas of neurological reorganization and repair following CNS damage. Principles and methods to monitor and augment neuroplasticity are explored in depth and supplemented by a critical appraisal of neurological repair mechanisms and possibilities to curtail disability using computer or robotic interfaces. Rather than providing a textbook approach of CNS restoration, the editors selected topics where progress is most imminent in this labyrinthine domain of medicine. Moreover, the varied background and origins of the contributors lend this book a truly global perspective on the current state of affairs in neurological recovery.