Abstract

Recent research has shed new light on facilitator pharmacological and non-pharmacological interventions on neuroplasticity mechanisms, subtending functional and structural post-stroke neural remodelling and consecutive improved clinical outcome. Cell-based and neurotrophic therapies, multimodal pleiotropic molecules, functional interventions to promote learning-related rehabilitation at early stages, represent new challenges and promising avenues of therapeutic strategies to enhance brain recovery after stroke. From molecular mechanisms at neuron level to large-scale neural network reorganization and consequent clinical impact in stroke patients, the present chapter aims to provide an overview of new therapeutically tools in the management of ischemic stroke, promoting neuro-recovery and neuroprotection, beyond the thrombolytic time-limited approach.

Keywords

Neuroplasticity; Ischemic Stroke; Early Rehabilitation; Brain Stimulation

Introduction

Stroke represents the leading cause of long-term disability and the leading preventable cause of disability in adult population worldwide [1]. After ischemic heart disease, stroke is the most common vascular pathology, killing worldwide an estimated 5.7 million people annually [2,3]. In industrialized countries it represents the third
Ischemic Stroke

Ischemic Stroke is the most common cause of death and a major burden with increasing clinical, economic and social impact. Stroke mortality differ from Eastern to Western European countries with higher mortality rates in East Europe whilst low and decreasing rates are reported from most West countries [4,5]. Although mortality has decreased significantly in the past decades, it is the disability that plays an important role in the future social outcome. Many patients have severe disability after stroke and only few gain full recovery and independence. The level of disability can vary from weakness, paralysis to cognitive impairment, including vascular dementia. Stroke leaves worldwide 5 million people permanently disabled [6].

Definition of Ischemic Stroke

In spite of its global impact, the term “stroke” is not defined in a consistent matter in clinical practice, clinical research, or in public healthcare. Having this into consideration, advances in basic science, neuropathology and neuroimaging have improved the understanding of ischemia, infarction, and haemorrhage in the central nervous system [7].

Stroke is characterized as a neurological deficit secondary to an acute focal injury of the central nervous system determined by a vascular cause, including cerebral infarction, intracerebral haemorrhage and subarachnoid haemorrhage [7].

Central nervous system infarction refers to cell death at different levels, such as brain, spinal cord, or retinal, attributable to ischemia, based on pathological imaging (such as positive results on diffusion-weighted MRI), leading to an episode of neurological dysfunction, or other objective evidence of cerebral, spinal cord, or retinal focal ischemic injury in a defined vascular distribution with permanent alteration; otherwise, the clinical evidence of cerebral, spinal cord, or retinal focal ischemic injury based on symptoms that persist more than 24 hours or until death, having other aetiologies excluded.

Secondary to the arrival of thrombolysis and other acute treatments, the importance of stroke and transient ischemic attack (TIA) redefinition became acute, all the more current guidelines differentiate treatment strategies for these two entities.

Early definitions of stroke and TIA focused on the duration of symptoms and signs. More recent studies, using clinical observation and modern brain imaging, have shown that the duration and reversibility of brain ischemia are variable.

Classification of Ischemic Strokes

It is fairly important to realise a precise subtyping of stroke patients, not only for therapeutic decision-making in day to day practice, but also in order to organise patients in different epidemiological studies, clinical trials or even genetically phenotyping.

Stroke is a complex disease with more than 150 known causes. Therefore, determining the subtype might in some cases present some difficulty. In order to determine the
most probable aetiology influencing specific therapy and individual secondary prevention, clinical history and presentation and different diagnostic tests have to be combined [8].

**TOAST Classification**

Since 1993, most clinical researchers use the classification proposed by the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) investigators which classified 11 categories of stroke, subdivided into five groups (see Table 1). Only these five groups were used in further clinical research. However, the downside of TOAST classification is that nearly 40% ischaemic stroke patients presented “undetermined/unclassified” stroke aetiology and evolution was mainly CT-based [6].

**Oxfordshire Community Stroke Project (OCSP) Subtype Classification**

Oxfordshire Community Stroke Project (OCSP) subtype classification was proposed to emphasis the population-based epidemiological study. Transient ischaemic attacks and strokes had been diagnosed by clinical and cranial CT scan, but no evaluation of extra and intracranial arteries was available (see Table 2) [6].

### Table 1: TOAST classification.

<table>
<thead>
<tr>
<th>Stroke subtypes</th>
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<tbody>
<tr>
<td><strong>1. Ischaemic</strong></td>
<td><strong>1.1 Large vessel disease</strong></td>
<td><strong>1.1.1 Extracranial</strong></td>
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<td></td>
<td><strong>1.1.2 Intracranial</strong></td>
<td><strong>1.2 Small vessel disease</strong></td>
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<td><strong>1.3 Cardiac emboli</strong></td>
<td><strong>1.4 Other causes</strong></td>
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<td></td>
<td><strong>1.4.2 Rare large, medium sized artery disease</strong></td>
<td><strong>1.4.3 Rare or hereditary small vessel disease</strong></td>
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<td></td>
<td><strong>1.4.4 Congaloopathy</strong></td>
<td><strong>1.4.5 Metabolic disease with arteriopathy</strong></td>
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<td></td>
<td><strong>1.4.6 Vasculitis</strong></td>
<td><strong>1.4.7 Other rare entities</strong></td>
</tr>
<tr>
<td><strong>1.5 Co-existing causes</strong></td>
<td></td>
<td><strong>1.6 Unknown</strong></td>
</tr>
<tr>
<td><strong>1.7 Unclassifiable</strong></td>
<td></td>
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<tr>
<td><strong>2. Haemorrhagic</strong></td>
<td><strong>2.1 Hypertension related small vessel disease</strong></td>
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<td></td>
<td><strong>2.2 Cerebral amyloid angiopathy</strong></td>
<td><strong>2.2.1 Sporadic</strong></td>
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<td></td>
<td><strong>2.2.2 Hereditary</strong></td>
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<td></td>
<td><strong>2.3 Bleeding diathesis</strong></td>
<td><strong>2.3.1 Drugs induced</strong></td>
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<td></td>
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<td><strong>2.3.2 Other haemostatic disorders</strong></td>
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<td></td>
<td><strong>2.4 Vascular malformation</strong></td>
<td><strong>2.4.1 Arteriovenous malformation</strong></td>
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<td><strong>2.4.2 Dural fistula</strong></td>
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<td><strong>2.4.3 Rupted aneurysm</strong></td>
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<td><strong>2.4.4 Cavernoma</strong></td>
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<td></td>
<td><strong>2.5 Other causes</strong></td>
<td><strong>2.5.1 Tumor related</strong></td>
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<td><strong>2.5.2 Toxic</strong></td>
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<td></td>
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<td><strong>2.5.3 Trauma</strong></td>
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<tr>
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<td></td>
<td><strong>2.5.4 Arteritis, infections</strong></td>
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<td></td>
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<td><strong>2.5.5 Rare entities</strong></td>
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<td></td>
<td><strong>2.6 Co-existing cause</strong></td>
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<td><strong>2.7 Unknown</strong></td>
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<td><strong>2.8 Unclassifiable</strong></td>
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<tr>
<td><strong>3. Subarachnoid haemorrhage</strong></td>
<td><strong>3.1 With aneurysm</strong></td>
<td></td>
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<td></td>
<td><strong>3.2 With dissection</strong></td>
<td><strong>3.3 Traumatic</strong></td>
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<td></td>
<td><strong>3.4 Neoplastic (melanoma)</strong></td>
<td><strong>3.5 Unknown</strong></td>
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<tr>
<td></td>
<td><strong>3.6 Unknown</strong></td>
<td></td>
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<tr>
<td><strong>4. Cerebral venous thrombosis</strong></td>
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<tr>
<td><strong>5. Spinal cord stroke</strong></td>
<td><strong>5.1 Ischaemic</strong></td>
<td><strong>5.2 Haemorrhagic</strong></td>
</tr>
</tbody>
</table>
Table 2: Oxfordshire Community Stroke Project (OCSP) subtype classification.

<table>
<thead>
<tr>
<th>Oxfordshire Community Stroke Project (OCSP) subtype classification</th>
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<tbody>
<tr>
<td>1. Cerebral infarct</td>
<td>CT diagnosis within 28 days</td>
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<tr>
<td>2. Lacunar Infarct</td>
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<tr>
<td>3. Total anterior circulation infarcts</td>
<td>Ipsilateral motor and sensory deficit of 2 areas (face, arm or leg) and higher cerebral dysfunction</td>
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<tr>
<td>4. Partial anterior circulation infarcts</td>
<td>Only 2 of the 3 components of the total anterior circulation infarcts</td>
</tr>
<tr>
<td>5. Posterior circulation infarcts</td>
<td>Defined posterior circulation syndrome</td>
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ASCO Phenotypic Classification

ASCO phenotypic classification is a novel variant of stroke subtyping that revolves on stroke aetiology and the existence of an underlying disease, judged by grade of likelihood as source of individual stroke. It differs from classifications in the emphasis on the most likely cause of stroke. Every patient is characterized by ASCO (A – atherosclerosis, S small vessel disease, C cardiac source, O other cause) and each of the 4 phenotypes is graded 1 to 3 points. This can be applied in treatment strategy [5].

Pathophysiology of Ischemic Stroke

Ischemic strokes occur as a result of an obstruction within a blood vessel supplying a local region of the brain, except when there is general circulatory failure due to cardiac arrest or systemic hypotension of various reasons (cardiac insufficiency, sepsis, etc.) involving diminished perfusion in a vascular territory, potentate by vascular stenosis.

Ischemic strokes are due to brutal decreases in blood supply and glucose to the brain parenchyma, involving either embolic obstruction of a brain vessel (cardiac, artery-to-artery embolic source) or initiation of the coagulation cascade at the level of ulcerated atherosclerotic plaques (thrombotic mechanism), the underlying condition for this type of obstruction is the development of fatty deposits on the vessel walls.

The brain uses glucose as a primary source of energy, but does not store glucose. Or, the lack of circulation blood in a certain brain area deprives neurons of necessary substrates [9]. Mechanisms of neuronal injury at the cellular level are caused by hypoxia or anoxia. Ischemia-induced activation of vasoactive enzymes released by endothelium, leucocytes, platelets and other neuronal cells and has a destructive effect [10,11]. At molecular level, the development of hypoxic-ischemic neuronal injury generates the so called “excitotoxicity” by extracellular over-expression of certain activator neurotransmitters, such as glutamate and aspartate. The increased concentration of these molecules, especially glutamate further results in changes of the neuronal calcium channels state, involving N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxanole propionate (AMPA) receptors and which determines membrane depolarization and activates the N-methyl-D-aspartate (NMDA) receptor and rapid translocation of calcium ion (Ca$^{2+}$) from extracellular to intracellular spaces in cerebral tissues. As known, Ca$^{2+}$ is an intracellular messenger during and immediately after
an ischemic period, and influences the cascade of events that lead to subsequent neuronal injury, via enzymes such as proteases, lipases, and endonucleases and cytokine release [12-14].

Leukocyte recruitment to the ischemic areas occurs within minutes after ischemia and besides mechanical obstruction of the microcirculation, promotes vasoactive agents, such as oxygen free radicals, cytokines and nitric acid. The effects of these mediators include vasodilatation, vasoconstriction, increased vascular permeability, increased platelets aggregation and molecular adherence to the endothelial wall [15-17]. In the same line of evidence, circulating endothelial cells in the peripheral blood probably represent the most direct evidence of endothelial cell damage in acute ischaemic stroke, as they respond promptly to hypoxia and cause multilevel effects (cellular, biochemical and immunological) [18].

The neurons in the injured brain area suffer a concerted attack by oedema, necrosis of the cellular metabolites, resulting in expansion of the necrotic area and neurologic deficits. The ischemic penumbra surrounding the focal ischemic area provides a potential “window” to reverse or improve neurological deficits by reperfusion [19,20].

The ischaemic penumbra is a dynamic process, existing even in the centre of the infarct for a short period of time before irreversible necrosis sets in and propagates to the neighbouring tissues over time, and may persist for more than 12 h after the onset of stroke symptoms [20].

Cellular integrity and function are preserved in this area of limited ischemia for variable periods of time, within hours [21]. The ischemic penumbra initially defined as a region of “misery perfusion” is a critical interface between infarcted core and viable surrounding tissue. Data shows that cell death mechanisms characterising penumbra consists in apoptotic-like processes which progress more slowly than necrosis. Therefore, if normal perfusion could be restored rapidly, there can be a better outcome regarding the surrounding tissue [21,22].

The effect of the reduced perfusion is what defines the evolution the penumbra and it is influenced by other events and comorbidities usually encountered in stroke (hypoglycaemia, temperature, blood pressure). Therapeutic windows in acute stroke management are highly focused in saving the ischemic penumbra, which becomes a target for intervention, neuroprotection and neuro-repair. Penumbra is a subject to various deleterious metabolic processes including excitotoxicity, oxidative stress and inflammatory response, implying the evolution of the concept of ischemic penumbra, which becomes not only a diagnostic issue, but also, a key player in brain plasticity after stroke and recovery.

Protein synthesis and apoptosis are consequences of ischemia. From biochemical point of view, penumbra is distinct to the ischemic core and healthy tissue which is well illustrated by the term peri-infarct tissue. It is interesting to know that reperfusion therapies may not be useful to save the viable penumbra (recanalization methods),
but may trigger brain plasticity mechanism. Studies have shown that neurogenesis may be helpful in penumbra recovery: administration of vascular endothelial growth factor has prompt neurogenesis in subventricular zone and angiogenesis in ischemic penumbra [23,24].

New research is needed to address specifically penumbra involvement in the eventual evolution from injury to repair. The concept of ischemic penumbra has been evolving over the last 30 years with consequent practical implications in patient’s recovery immediately after stroke. Therefore, it encompasses not only the hemodynamic dimension, but also a constellation of molecular events that may be a valuable window for therapeutic intervention and neurorepair in stroke [25]. It is well known that time window after stroke is crucial for effective therapeutically intervention, therefore rehabilitation protocols should critically address besides well-documented and individualized interventions, the timing issue, in order to promote recovery.

Brain Plasticity after Stroke and Potential Early Modulatory Interventions in Stroke Recovery

Despite significant improvements in acute stroke management and development of multidisciplinary platforms of care such as stroke units, at present, only early recanalization is regarded as an effective treatment attenuating the severity of brain damage in the management of acute ischemic strokes. Recanalization can be obtained either by the administration of intravenous tissue plasminogen activator (IV-tPA) [26] or by endovascular interventions including intra-arterial thrombolysis [27], mechanical thrombectomy [28], or mechaniaspiration [29].

“Time is brain” paradigm has gained sustainable clinical evidence, as robust data demonstrates that intravenous administration of recombinant tissue-type plasminogen activator within a limited time window (most studies quote 4.5 h) has a beneficial impact on survival rates post stroke and functional impairment [30,31]. The time window within which this pharmacological manoeuver is therapeutic has not been definitively established yet [32,33]. It is generally accepted that after 4.5-6h, intravenous thrombolysis is no more efficient, hence the time-dependent limitations and necessity of prompt interventions. The Third European Co-operative Acute Stroke Study (ECASS III) was a randomized controlled trial of IV alteplase versus placebo administered 3-4.5 h after the onset of stroke [34-36].

Immediately after stroke, brain dynamics both at local (lesion site) and global (remote areas) is altered. It is largely known nowadays that after initial nervous tissue injury, endogenous mechanisms in terms of molecular and larger-scale brain networks dynamics supporting brain repair are initiated, defining neural plasticity. This complex phenomena initiated after brain injury (vascular, traumatic, etc.) is based on the same fundamental mecha-
isms characterizing learning processes and development in healthy brains, and has been intensively studied by different approaches, from clinical to neuro-molecular level [37-41].

It has been shown that prognosis after stroke largely depends on a number of factors such as size and localization of the lesion, age of the patient, co-morbidities like atrial fibrillation, hypertension, diabetes, etc. [42]. Therefore, post-stroke management is currently still based on secondary prevention of cerebro-vascular risk factors and physical therapeutic approach of deficits. Usually, rehabilitation strategies are initiated after the subacute phase. In a clinical trial by Ronning and Guldvog [43], the authors reported that initiation of rehabilitation program in subacute phase post stroke facilitates recovery, patients with moderate and severe lesions showing the best functional outcome. Yet, there is an increasing need to better understand pathological mechanisms underlying early recovery post stroke and promotion of more targeted therapeutically approaches, both in terms of mechanisms and time-related efficacy following vascular injury.

Brain activity post stroke is based on its intrinsic capacity to initiate new neural patterns of activity in a self-organized manner. The challenge to improve stroke recovery is to understand how to these patterns of activity may be internally or externally modulated in order to provide optimal recovery. A large body of data support the fundamental role of neural plasticity in functional recovery [44]. Therefore, progression of recovery itself can be thought of a process of both reinstatement and relearning of lost functions, as well as adaptation and compensation of spared, residual function.

The term “neural plasticity” globally refers to both “functional” and “structural” changes in the context of brain reorganization, in order to achieve better post-injury recovery [45]. From molecular to macroscopic levels, the brain is engaged in a complex and time-dependent process of regeneration such as gene expression, protein synthesis, new synaptic pathways, reorganized anatomical connections within brain regions surrounding the altered tissue or remotely, are re-creating the contextual biological environment for progressive re-establishment of impaired functions [46-48]. Therefore, a better understanding of these mechanisms subtending brain plasticity would generate new and fundamental cues about better therapeutic strategies to enhance brain recovery.

It is known that functional recovery largely depends on the extension of the focal lesion and localisation. Yet, the tissue necrosis after stroke it seems not be the unique contributor to the extent of the deficit, but also the dysfunction of the tissue surrounding the focal lesions (penumbra—pathological characteristics have been discussed in the first part of this chapter).

Penumbra (glial and neuronal cells) may be also early targeted by neuroprotective agents, contributing to the
blocking of the deleterious ischemic cascade [49]. Not only the areas adjacent to the lesion are undergoing complex functional and structural changes post-injury, but also interconnected remote areas—part of the large scale functional networks.

The diaschisis phenomena meaning dysfunction of remote areas secondary to a localized lesion is a non-negligible factor in post-stroke brain dynamics, demodulation [50,51].

Therefore, recovery after stroke implies dynamic mechanisms and brain inter-related processes in order to generate compensatory background for lost functions. Wieloch and Nikolich (2006) in an excellent review, postulate three major phases in brain recovery after stroke: 1) reversal of diaschisis and cell repair initiation; 2) functional remodelling in terms of reorganization of existing synapses and neuronal pathways; 3) structural changes driven by “experience” (rehabilitation) and prompting new neural connections [41]. Studies in animals and humans have already proven the reorganization of neural representations at different levels, after focal injury [52-56].

The neurobiological basis of spontaneous recovery immediately after the focal vascular lesion has been extensively studied both at microscopic and macroscopic level using animal models.

At cellular level, recovery of function following cortical injury implies plasticity genes activation, growth-promoting factors for enhanced anabolic processes and growth-inhibitory proteins to modulate axonal aberrant outgrowth (see below for the description of neurotrophic factors involved in stroke recovery mechanisms). Dynamic reorganization at synaptic level on a time scale ranging from milliseconds to days and months, promotes reshaping of neural networks and functional recovery after stroke [57].

Cellular stress, brain oedema and local inflammation cause aberrant neurotransmission and morphological changes which affect the infarcted tissue [48]. Yet, repair and plasticity genes are promptly activated immediately after stroke, initiating cell genesis, axonal sprouting and synaptogenesis which subtend neuroplasticity after injury [44].

It is known that stroke mechanisms stimulate axonal growth and growth-promoting molecules (such as phosphoproteins and synaptophysin, and more recently growth and differentiation factor—GDF 10) which imply formation of new connections in peri-infarct cortex and connected areas, therefore contributing to the remapping of the anatomical connections and emergence of new patterns of neural activity contributing to functional recovery [58,59]. Therefore, neural growth promotors and neuro-activators specifically targeting these early post-lesion processes may impact considerably the formation of new neural representations.

Another mechanism prompting recovery post stroke is represented by ischemic long-term potentiation. It is
known that consolidation of new patterns of activity driven by experience and learning is driven by fast synaptic changes leading to rapid, yet enduring synaptic strengthening and long-term depression (LTD, weakening of synaptic strength) [60]. The LTD mechanism based on the rule of “use it or lose it”, by weakening unused connections, may promote the consolidation of patterns of intensely used specific neural connections, which could contribute to the post stroke recovery. Exposures to a “rich” environment or certain learning paradigms have been shown to determine an increase in synapses number [61]. It is well documented both short-term and long-term modifications at synaptic level are based on molecular and intracellular changes driven by activity (e.g. use of the limb in the context of rehabilitation programs) which further support consequent remodelling of functional neural circuits at larger scales in the brain [60]. In animal studies, enriched and complex environments result in a greater number of synapses and improvement in sensorimotor function [44].

The ischemic LTP (iLTP) represents a form of ischemia – induced plasticity of excitatory synapses, implying N-methyl-D-aspartate (NMDAR) receptors, calcium/calmodulin - dependent proteins, calcium – dependent protein kinases, proteases (calpain), which globally increase Ca^{2+} overload with consequences on neurons response capacity to excitatory stimuli [62]. This implies not only beneficial (increased synaptic strength immediately after lesion occurrence), but also negative effects on neural local dynamics post-stroke, in terms of excitotoxicity, cell hyper-excitability [63]. It is proposed that inhibition of iLTP blocking may be associated with consequent NMDAR inhibition which is known to enhance recovery after brain injury [64].

Interestingly, recent data has shown that thrombin—a serine protease modulating coagulation processes—may be a central player in the dynamics of ischemia-induced synaptic plasticity [65]. Currently, thrombin inhibitors are used in clinical practice as a pharmacological agent for prevention of stroke [66]. Yet, it is still to be clearly established if thrombin and blood-brain barrier contribute essentially to the tuning of neuroplasticity mechanisms post-stroke [67].

At systems level, Nudo and colleagues demonstrated that use-dependent neural functional reorganization takes place in non-human primates following stroke by showing that motor learning results in changes to the motor cortex, which are specific to the area of cortex involved [52]. Yet, cortical plasticity depends both on the damaged area and areas connected to the damaged territory. It has been shown that recovery after stroke primary engages ipsilateral brain areas, followed by contralateral brain areas involvement, especially in severe lesions [50].

Van Meer and collaborators (2012) stated four major issues about brain plasticity related changes at systemic level following stroke: (1) improvement of sensorimotor function correlates with restoration of interhemispheric connectivity and neural patterns of activity in the bilateral sensorimotor cortex; (2) recovery of sensori-
motor function after an extended stroke is correlated with increased structural integrity of the ipsilesional cortico-spinal tract; (3) restoration of inter-hemispheric functional connectivity between bilateral sensorimotor cortices is associated with unilateral improvement of structural integrity of the ipsilesional corticospinal tract; (4) improved functional outcome is associated with repaired structural integrity within the ipsilesional corticospinal tract [68].

Functional MRI studies in rats have shown that early after stroke, brain activation during stimulation of affected paw is mainly in the contralesional cortex; later after stroke onset, activity shifts towards the ipsilesional cortex [69]. In other words, there is a dynamic involvement of local and global processes to compensate for lost functions at the brain level.

Wahl et al. showed in rat that large lesions may be compatible with favourable outcome when a growth-promoting immunotherapy against growth inhibitory protein applied to boost the sprouting of new fibres [70]. In humans, Nhan and colleagues (2012) showed by using perfusion-weighted imaging and functional magnetic resonance imaging during a finger movement task in stroke patients, that improved clinical outcome was related to increased activation within sensory cortices of both brain sides, including bilateral secondary somatosensory areas [71]. The authors stated that after stroke, early cortical activation that will later increase in parallel with recovery is often already identifiable and it can be remote from the vascular territory of the infarct. Callauti et al. used PET technique and a simple motor task to show that a highly significant activation of motor-related areas is necessary both in early and late stages of stroke recovery to perform the task [72]. In the same line of evidence, Marshall et al. studied patients in the acute (1 week) and chronic (3 to 6 months) stages post-stroke, using a complex self-paced motor task and showed early contralesional primary sensorimotor cortex hyperactivity and late ipsilesional hyperactivity [73].

Wahl et al. showed in rat that large lesions may be compatible with favourable outcome when a growth-promoting immunotherapy against growth inhibitory protein applied to boost the sprouting of new fibres [74]. Both animal and human data show clearly that earlier initiation of rehabilitation therapy is required for a better functional outcome. However, Humm and collaborators (1998) found that for a period of time after unilateral brain injury, surviving neural tissue surrounding the lesion may be vulnerable to extremely high behavioural demand [75]. The researchers showed that forced overuse of the impaired forelimb during the first 7 days postlesion, caused expansion of neuronal injury and exacerbated neurological function in a rat brain injury model, indicating that excessive rehabilitation at an early stage of injury can interfere with the restoration of function.

Yamashita and Abe (2012) discuss two possible strategies for recovery after ischemic stroke. The first one
concerns modalities to compensate brain damage and enhance brain reorganization and rewiring, subtending neuroplasticity. The second one is neuronal regeneration therapy, centred on repair of disrupted neuronal networks [76].

In recent years, there is an increasing interest in developing multitarget therapeutically strategies to modulate pathophysiological complex processes post stroke [77]. Neuroprotective agents such as Cerebrolysin, administered early in the stroke evolution, have been shown to modulate neural growth and cell survival mechanisms and promote neuronal integrity and neurogenesis, while inhibiting excitotoxicity, apoptosis and neuro-inflammation processes after stroke [78-80].

Edaravone is another pleiotropic agent with antioxidant properties and modulatory properties upon signalling pathways post stroke, which may potentially be useful in early phases after brain injury as a protector against vascular damage and intracerebral haemorrhage [81]. Like Edaravane, curcumin is another antioxidant which may repair damaged brain circuits by mediating endogenous brain-derived neurotrophic factor and signalling mechanisms (BDNF), with impact on stroke recovery [49,82]. Also curcumin may mediate cell death and mitochondrial processes, with enhanced blood-brain barrier penetration could attenuate cell death mediated by a variety of insults and maintain mitochondrial function [49].

Minocycline is another neuroprotective agent which interferes in the ischemic cascade, possessing anti-inflammatory properties and anti-apoptotic activity and is a neuroprotective agent with beneficial aspects in stroke lesions, via inhibitory mechanisms of nitric oxide synthase (iNOS) and mitogen-activated protein (MAP) kinase, inhibition of glutamate toxicity, and inhibition of microglial activation. These pleiotropic drugs may represent valuable therapeutically alternatives in early phases of ischemic stroke, yet additional translational studies are needed in order to prove their place in the neuroprotection clinical strategies in stroke patients [82,83].

In another line of evidence, regeneration therapy allowing for the repair of disrupted neuronal networks with newly supplied neurons is also a possible strategy for treating post stroke patients in early phases. Activation of intrinsic neural stem cells or transplantation of extrinsic neural stem cells or neural cells derived from stem cells such as embryonic stem cells and induced pluripotent stem may be future alternatives to “correct” vascular damage [84].

Regeneration therapy allowing for the repair of disrupted neuronal networks with newly supplied neurons is also a possible strategy for treating post stroke patients. Activation of intrinsic neural stem cells or transplantation of extrinsic neural stem cells or neural cells derived from stem cells such as embryonic stem cells and induced pluripotent stem may be future alternatives to “correct” vascular damage [55].
Despite the large progress in understanding brain reorganization after stroke and promotion of new pharmacological and non-pharmacological strategies to target multi-level “healing” processes, the precise intervention of these approaches is still to be clarified, especially in terms of time- and experience-related modulation of neuronal circuitry and interrelated mechanisms that underlie post-stroke recovery.

**Growth Factors**

In recent years, attempts to rehabilitate stroke patients have encouraged the use of growth factors because they have important roles in the development and functions of the nervous system. The best known growth factors are neurotrophic factors, especially brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), erythropoietin (EPO), granulocyte-colony stimulating factor (G-CSF), and vascular endothelial growth factor (VEGF). Increased expression of these growth factors after brain injury is associated with neuronal repair.

**Neurotrophins**

Neurotrophins promote proliferation, differentiation and survival of neuronal cells. Moreover they ensure synaptic functions, neurotransmitter release and neuroplasticity [85]. Decreased levels of neurotrophins were found in neurodegenerative disorder such as Alzheimer’s disease, Parkinson’s disease, Huntington disease and also in psychiatric diseases (depression, drug abuse).

They are represented by nerve grow factor (NGF), brain-derived neurotrophic factor (BDNF), Glial cell line-Derived Neurotrophic Factor (GDNF) and neurotrophins 3 to 7 which bind selectively to Trk receptor tyrosine kinase and p75 pan-neurotrophin receptor (p75NTR). NGF binds to TrkA, BDNF and neurotrophin-4 to TrkB, and neurotrophin -3 to TrkC [85]. p75NTR can bind to each neurotrophin. The neurotrophins are synthesized as pro-neurotrophins. In brains injury BDNF can be synthesized by nerves or by mononuclear cell and T and B lymphocytes [86].

NGF, first identified neurotrophic factor, acts on sensory, sympathetic and cholinergic neurons. Neurotrophins do not cross the blood-brain barrier and for this reason, the intra-cerebro-ventricular (ICV) delivery was used in recent studies. Other strategies such as NGF-releasing implants, NGF-releasing microspheres of size less than 100 μm injected into the brain using the stereotaxic technique, recombinant human NGF (rhNGF)-releasing microspheres injection into the brain with fimbria-fornix transection, and nasal delivery of recombinant forms of NGF are under development [87].

BDNF is a neurotrophic factor that plays an important role in controlling intercellular and intracellular signalling pathways that ensure plasticity and cell survival [88]. The greatest amounts of BDNF are found in the hippocampus and in the associative cortex. Higher BDNF levels are associated with a slower rate of cognitive decline.
and decreased levels of BDNF or pro-BDNF were found in brains of patients diagnosed with cognitive impairment [89,90]. BDNF increase presynaptic activity, particularly in glutamatergic synapses, and also stimulate postsynaptic glutamate receptors and activated the BDNF/PI3K/AKT pathway [91,92].

BDNF was shown to have anti-apoptotic action after stroke, to reduce infarct size and neuronal cell death [93]. Low levels of BDNF were observed in patients with risk factors for stroke, such as coronary artery disease, acute coronary syndrome, and diabetes mellitus [94].

BDNF levels were reported to remain constant during first week after stroke [95], but BDNF levels were found lower in patients with post-stroke depression [96]. Antidepressant drugs acting through monoamine G-protein-coupled receptors may increase expression of neurotrophins and its receptors [97].

Recently it has been shown that stroke patients with the BDNF Val66Met polymorphism can have affected BDNF functions, and as a result of these they have reduced capacity for use-dependent plasticity in the motor cortex and have impaired motor learning [98-100]. Also it has been suggested that those with BDNF-66Met allele may have difficulty to recovery after stroke [101]. Non-carriers of the polymorphism were seen to have greater excitability changes post stroke and those with BDNF-Val66Met were seen to have a greater imbalance in interhemispheric cortical excitability [102,103].

Physical exercise, omega-3 fatty acids have been found to normalize BDNF. It was suggested that higher aerobic fitness level is associated with larger hippocampal volume and improved neuronal health [104]. On the other hand acute stress and cortisol administration can lead to reduced BDNF levels [105].

The recombinant form of the tissue plasminogen activator (rt-PA), besides its fibrinolytic function, is involved in neuronal migration, synaptic outgrowth and plasticity, in neurotransmission, and cognitive function [106]. It has been showed that t-PA can cleavage pro-BDNF and that exogenous t-PA increases mature BDNF expression in the hippocampus through N-methyl-D-aspartate (NMDA) receptor activation [107,108].

It is reported that rTMS increase blood BDNF levels in patients with depression [109,110]. Daily 5-Hz rTMS for 5 days significantly increased serum levels of BDNF in healthy human subjects, resulting in activation of BDNF-TrkB signalling [111]. Moreover, Val66Met polymorphism of the BDNF gene negatively influences the effect of rTMS on post-stroke upper limb hemiparesis [112]. These findings suggest that rTMS-induced modulation of BDNF-TrkB signalling in the brain [111].

BDNF and pro-BDNF have opposite effects via TrkB and p75NTR, respectively [113,114]. BDNF could be involved in increased brain excitability, while pro-BDNF seems to play a role in reducing brain excitability [114]. Low-frequency rTMS plus physical therapy increased lev-
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Levels of mature BDNF and MMP-9 in post stroke patients and that was correlated with improvement of upper limb motor function [115]. BDNF, pro- BDNF, and MMP-9 could be potentially useful biomarkers of the response to therapy in post stroke patients with upper limb motor deficit [115]. Increased concentrations of BDNF have been reported to limit the extent of ischemic lesions in the white matter of the brain in subjects with risk factors for cardiovascular diseases [116]. BDNF promotes vasodilation by production of prostaglandin I2 (PGI2) which activates pro-survival signalling by activation of peroxisome proliferator-activated receptor delta (PPAR), and this in turn may increase the resistance of cerebral circulation against injury [117].

Using neurotrophins in ischemic stroke is encumbered by various obstacles such as the difficulty of passing through the BBB, the short biological half-lives, and the short temporal window in which such treatments are effective (therapies must be instituted within 1–6 h after event) [118]. In recent years science made remarkable progress in developing neurotrophin receptor (Trk) agonists with longer biological half-lives and BBB permeability [119]. Small molecule BDNF mimetics activate TrkB signalling and prevent neuronal degeneration in hippocampal neurons [120] and also promote increase in the number of neurons adjacent to stroke site [121].

Using intraventricular injection of a viral vector encoding BDNF, glial-derived neurotrophic factor (GDNF), NGF, insulin-like growth factor-1 (IGF-1), or vascular endothelial growth factor (VEGF) 30 min after ischemic insult was observed that hippocampal CA1 pyramidal neurons were rescued [122]. Intranasal administration of BDNF reduces the number of apoptotic neurons, enhanced microglial activation, suppressed tumour necrosis factor-α expression and enhanced expression of the anti-inflammatory cytokine interleukin-10 [123].

Candesartan, the inhibitor of rennin-angiotensin system, was found to reduce stroke volume and to promote TrkB expression [124]. Natural compounds from plants such as flavonoids baicalin and jasminoidin, gastrodia elata blume can promote BDNF expression and functional recovery after stroke [125,126]. Also Niacin may increase BDNF and TrkB expression in neurons and reduces infarct volume in experimental stroke [127].

**Insulin Growth Factor 1**

Insulin and insulin growth factor 1 (IGF-1) adjusts energy metabolism, cognition, and neuronal survival. Insulin acts as a metabolic regulatory hormone, while IGFs maintain the role of mitogen growth factors. Insulin and its receptors are abundant in the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala and septum [128].

IGF is synthesized in the liver under the action of growth hormone (GH) and subsequently may pass the BBB and can influence local synthesis of IGF-1. Synthe-
sis of IGF-1 locally in the brain is not regulated by GH. IGF-1 binds his receptor, the IGF-1R, and can activate different pathways including the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) pathways [129,130]. The IGF receptors are present in higher numbers in amygdala, cerebellum, and entorhinal cortex [131]. Insulin can bind to the IGF1 receptor (and less to the IGF2r), the reverse does not happen, as IGFs bind less well to insulin receptors.

IGF-1 seems to be involved in neuroplasticity processes, dendritic growth and stabilization, formation of neuronal circuitry and promotes neuronal survival under oxygen and glucose deprivation [129]. Impairments in brain insulin and IGF signalling contribute to neuronal loss, synaptic disconnection, tau hyper-phosphorylation, amyloid beta accumulation, and metabolic disturbances. Low levels of serum IGF-1 were associated with cognitive dysfunction [132].

By stimulating neurogenesis, angiogenesis and myelination, IGF-1 can play important roles in recovery after stroke [133]. Recent studies showed that IGF-1 may stimulate neurogenesis and migration of new neurons from the SVZ in affected striatum in a mouse model of stroke [134] and its administration via intranasal route or viral delivery, increase the number of neurons in the area affected by ischemia [135,136]. Also can inhibits apoptosis induced by hypoxia and excitotoxicity and promote neuronal survival [137,138]. IGF-1 stimulates angiogenesis and may enhance new blood vessel formation in experimental stroke [139].

After cerebral ischemia IGF-1 level is increased suggesting that IGF-1 may mediate protection in the ischemic lesion [140], and low levels of IGF-1 are associated with poor outcome in patients with stroke [141]. Exogenous administration of IGF-1 in the acute phase of stroke can reduces the neuronal damage and neurological deficits, especially intramuscular administration can prevent muscle atrophy and improve motor function scores [142,143]. The natural way to stimulate the production of IGF-1 is regular physical training [144].

**Erythropoietin**

Erythropoietin (EPO) is produced in the kidneys and liver in adults. EPO play a key role in erythropoiesis, but has also been found to have a number of other important functions in brain by mediating anti-inflammatory, anti-oxidant, anti-apoptotic and neurotrophic effects.

In cerebral ischemia EPO proved neuroprotective and neuro-regenerative effects [145]. EPO binds by EPOR and activates the Janus family tyrosine protein kinase 2 (JAK-2) and MAP kinase pathways, generating activation of ERK1/2, PI3K/AKT, NFK-B, and STAT-5 [146]. Also EPO increases the Bcl/Bax ratio in microglia and inhibits caspase-3 and -9 [146].

EPO administration in three doses, the first immediately after stroke and other remote one week or adminis-
tered daily for three days after stroke revealed that EPO reduces infarct volume and improved cognitive functions [147,148]. Even three days after ischemic injury EPO when administered improved white matter repair [149].

The German Multicentre EPO Stroke Trial, a Phase II/III trial failed to show any benefit. Stroke patients received EPO three doses at 6, 24, and 48 h after the onset of stroke had increased intracerebral haemorrhage, cerebral edema, thromboembolic events and mortality [150]. The investigators concluded that the increased death rate was due to the interaction of EPO with recombinant tissue plasminogen activator (rtPA). Another study concluded that administration of EPO in combination of tissue plasminogen activator exacerbates brain haemorrhage when treatment is initiated 6 h after stroke [151]. These studies suggested that patients treated with thrombolytic drugs should not receive EPO.

Granulocyte-colony stimulating factor (G-CSF) has been introduced in clinical trials for stroke patients and was demonstrated that five days of G-CSF treatment improved neurological score and metabolic activity in the area surrounding the infarction [152]. G-CSF in chronic stroke patients was administrated with no adverse events [153]. Both administrations of EPO and G-CSF in patients with brain injury may have synergistic effects on tissue plasticity and in promoting angiogenesis, neurogenesis and functional recovery [154,155]. Associations of EPO and G-CSF were well tolerated by stroke patients and no side effects were observed. These association increased erythropoietin, CD34+ hematopoietic stem cells, white blood cells and neutrophils in the peripheral blood of stroke patients, but despite that no thromboembolic accidents or other vascular events was found [156]. Regarding functional improvement after EPO+G-CSF therapy there was an improvement in the grip power of the dominant hand 6 months later [156].

Vascular Endothelial Growth Factor

In the regenerative process an important role has angiogenesis which involves formation of new vessels in the brain injury area. Increased density of blood capillaries in the peri-infarcted areas was found in patients who died by stroke. For this reason therapeutic strategies for recovery must regulate the process of angiogenesis and through it will be able to stimulate neurogenesis, neuronal and synaptic plasticity.

Vascular endothelial growth factor (VEGF) is a key factor in angiogenesis and a stimulator for pro-angiogenic factors such as insulin-like growth factor-1, platelet-derived growth factor, etc. [157,158]. Today we know four types of VEGF (A-D) and three types of VEGF receptors (VEGFR-1, VEGFR-2, VEGFR-3). In the brain the most frequent VEGF factor is VEGF-A and its receptor VEGFR-2 [159].

VEGF stimulates phosphoinositide 3’ kinase/AKT/endothelial nitric oxide synthase pathway, and suppresses
formation of the reactive oxygen species [160]. Also can stimulate neurogenesis thought its neurotrophic effects [161].

After cerebral ischemia within minutes for weeks angiogenetic processes is stimulated, but it is unclear whether this process is beneficial for brain recovery [162]. Various studies have shown that the process of neovascularization can have adverse effects [163,164].

After stroke VEGF-A levels were found increased suggesting his possible involvement in reducing infarct volume [165]. The brain cells, after ischemic event, secrete angiogenic peptides in order to generate new collateral channels and to ensure neuroprotection, regeneration, and removal of necrotic debris (“the clean-up hypothesis”) [166]. This process was revealed only in the affected hemisphere, where were found increased micro-vessel density and increased numbers of macrophages [166]. The pro-angiogenic state created by VEGF and BDNF can generate a “neurovascular niche” in which neural stem cells are generated [167]. The high VEGF expression was observed in affected hemisphere in less than 3 hours after stroke, and the reactivity keep greatly for 3 to 7 days [168,169].

But increased levels of VEGF can have unintended consequences like endothelial barrier disruption and increased vascular permeability, which may cause cerebral edema and infarct expansion [170,171]. Therefore it is not recommended early administration of VEGF, while his late administration proved to decrease infarct volume, increase vessels in the injury site, increase neurogenesis and improve neurological deficit [172,173].

Today there are studies that come to demonstrate that the proangiogenic effect of VEGF may not be accompanied by increased vascular permeability suggesting that the protective effects predominate [174,175]. In particular VEGF-B isoform promotes cell survival without the predominant vascular permeability, while VEGF-A isoform promoted angiogenetic effects [176]. Also administration of VEGFR-1 and VEGFR-2 agonist agents may have beneficial effects in the treatment of patients with ischemic stroke who survived more than 72 hours [177].

Neo vascularization may be both adaptive and maladaptive, so a better understanding of the angiogenetic process is necessary before clinical treatments can be fully developed. However VEGF is a key factor for neurovascularization and neurogenesis and has potential utility as therapy in stroke.

Cell-Based Therapy

In recent years cell-based therapy became a promising approach, beside “neurotrophic” therapy in stroke rehabilitation. Bone marrow stromal cells (BMSCs) treatment of stroke has been shown to enhance neural repair by regulating neurogenesis, angiogenesis, oligodendrogenesis, axonal sprouting, and synaptogenesis [178-180]. The major mechanism of exogenous BMSCs transplantations in
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Experimental stroke is not by direct cell replacement but by neurotrophic and immunomodulatory effects [181].

Mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs) are subsets of BMSCs [181]. BMSCs can be differentiated into mesenchymal lineages, neurons, and glial cells [182].

It has been observed that BMSCs can secrete BDNF, NGF, VEGF, and hepatocyte growth factor (HGF) [183]. Further it has been shown that sub-populations of MSCs may promote neuronal cell survival and neurogenesis [184]. After transplantation of BMSCs in experimental ischemic stroke was noticed enhanced NPC proliferation in the SVZ and SGZ and has been proven NPC migration to the affected area [185]. BMSCs can also differentiate into endothelial cells in ischemic brain [186] and also can stimulate these cells to express VEGF and Ang1 [187].

They are still discussing whether MSCs can pass through BBB, some claiming they cannot pass BBB [188] and other who argue that they may be able to cross the BBB in sufficient quantity to exercise their functions [189]. Using pluripotent stem cells derived neurons, BMSCs or embryonic stem cells derived MSC, administered either intracerebral or intra-arterial, were tried direct replacement of neurons destroyed by stroke [190-192].

Intracerebral administration has been encumbered by undesirable side effects such as fever and meningeal signs [193]. Intravascular administration has advantages including easy injection and a widespread BMSCs distribution, but also burdened by unwound effects like micro-emboli which may cause pulmonary emboli and micro-strokes [194].

Until now 12 patients, in a phase 1, and 18 patients, in a phase 2 randomized trials, with basal ganglia infarct of 6 months to 6 year duration received human neuronal cells [195,196]. In other studies, 5 five patients with basal ganglia infarcts were transplanted intracerebral with fetal porcine cells, and other 5 patients were treated with intravenous autologous MSC [197,198]. Only in 6 patients how received human neuronal cells were found increased fluorodeoxyglucose at the transplant site during PET-scan at 6 months later and improvement in the European Stroke Scale [195]. In the second trial there was no improvement in the primary outcome [196]. In transplantation with fetal porcine cells only two patients showed improvement that persisted at 4 years, two patients had secondary effects such as neurological worsening and seizure [197]. In the last trial with intravenous autologous MSC there was improvement in the Barthel index, modified Rankin scale, and imagistic explorations revealed less atrophy in the per-infarct area and less ventricular dilation in the MSC group [198].

In recent years, adipose derived stem cells (ADSCs) have been widely studied because represent an important source of multipotent cells than bone marrow tissue [199]. ADSCs are abundant in fat tissue and they can be easily
obtained through suction-assisted lipoaspiration [200]. They are important for regenerative medicine since recent studies have shown that ADSCs may have neuroprotective effects by secreting BDNF, NGF, IGF, and FGF factors, and also by stimulating angiogenesis [201]. Unlike bone marrow MSC, ADSCs produce significantly higher amounts of growth factors like VEGF-A, angiopoietin-1 and BDNF [202-204]. Under hypoxic conditions ADSCs secrete increased levels of VEGF and adopt a proangiogenic phenotype [205].

Stem cell therapy holds great promise in medicine, but there are many obstacles to be overcome before these treatments can be approved as safe and also beneficial. Beside difficulties regarding cell production, purification, differentiation, another problem is immunological incompatibility between transplanted cell derivatives and potential recipients. In the future, using allogeneic cells will solve the problem of immunity, knowing that these cells may not require any immunosuppressive drugs to be used.

**Spontaneous and External Stimulation-Based Post Stroke Recovery**

The recovery of altered brain functions post stroke involves complex mechanisms, time-dependent including penumbra reorganization, differential connectivity of ipsilateral or contralesional areas across interhemispheric interactions [206-209]. Rehabilitation strategies modulate one or more of these mechanisms promoting selection and stabilization of newly formed functional connections subtending recovery.

New insights into the role of experience-related motor recovery fostered task-oriented learning therapies [210]. It is well known that Hebbian mechanisms underlie activity related neural reshaping. Both spontaneous and intervention-mediated rehabilitation imply time-dependent processes in order to obtain maximum efficacy in brain reorganization both at synaptic and large-scale networks [211].

One of the fundamental principles of neurorehabilitation in stroke is that repetitive and specific learning programs may promote mechanisms of neural plasticity underlying improved function. It is well established that following different brain destructive events, such as stroke or trauma, a cascade of regenerative events is initiated, lasting from weeks to months.

It has been proven so far that behavioural experience is the most potent modulator of brain plasticity [206]. Functional and structural brain imaging studies (MRI) showed structural plasticity co-localized with areas exhibiting functional remodelling in the human brain after stroke [212]. Brain connections can be reshaped and reorganized by training strategies, implying repetition and temporal coherence.

Studies in animals, but also clinical observations in humans proved that perseverant motor programs may en-
train specific cortical areas previously damaged by vascular insult and provide gradually improved performance. The reorganized neural activity driven by „experience” (for instance, at the sensorimotor cortical level) underlies „functional plasticity” [52,213]. Concomitantly, there are also morphological changes at the level of brain areas affected by stroke, named “structural plasticity” [169,214,215].

One of the most prominent illustrations of “functional therapies” following stroke is given by Traub et al. (1997) who proposed the constraint-induced movement therapy to treat upper limb hemiparesis after stroke [216]. Based on behavioural data in animals, this modality of neurorehabilitation is based on the restriction of movements of the unaffected limb, which would foster the gradual use of the affected limb (initially not used). Therefore, intensive physical training would enhance the “use” modality of the motor programs for the paretic limb, by modulation of the underlying cortical patterns.

Mirror therapy is another therapy which has been developed by Ramachandran and Roger-Ramachandran to control abnormal sensation in phantom limb syndrome [217] and today is used in stroke rehabilitation [218]. The technique consists of placing a mirror between the two upper limbs and patients with the unaffected upper limb performing hand movements facing the reflective surface. The patient has the illusion that the healthy limb he sees moving in the mirror is the affected one and associates correct movement with his intention. The mirror illusion increases activity in the precuneus and the posterior cin-
Transcranial Magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a novel technique that is used in various diseases including depression, attention deficit, obsessive–compulsive disorder, bipolar disorder, migraine and stroke [224-226]. As described by many authors, this complementary and non-invasive method externally modulates cortical excitability, by inducing brief and pulsed magnetic field and causing localized depolarization of superficial cortical and subcortical neurons, located between 1.5 and 2 cm below the cranial bone [225].

TMS can be applied as a single-pulse, paired-pulse or in trains (repetitive TMS). Single-pulse TMS is used to analyse different aspects of sensorimotor cortex and pyramidal tract function by measuring the motor threshold (MT) and motor evoked potential parameters [225]. Paired-pulse TMS, which used two magnetic pulses separated by a variable inter-stimulus interval, can evaluate the short and long interval intra-cortical inhibition and intra-cortical facilitation [227]. Repetitive TMS can use stimulation at 1 Hertz or less (slow rTMS) or stimulation at a frequency higher than 1 Hz (fast rTMS). Studies have shown that rTMS at low frequency produces long-lasting inhibition, which is called as long-term depression, whereas repeated high-frequency stimulation can produce excitation through long-term potentiation [228-230].

Using TMS after stroke, it has been shown that excitability of the peri-infarct cortex is reduced in the vicinity of the vascular injury [231]. It is already well-known that peri-infarct areas play a role in neurological recovery via plasticity mechanisms [232]. Therefore, throughout several weeks of motor rehabilitation, cortical representations of the affected muscles undergo a dynamic process of remodelling and enlargement [233].

Recent data postulated that stroke may affect the balance of transcallosal inhibitory pathways between motor primary 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.

Low-frequency stimulation (≤1 Hz) delivered to the unaffected hemisphere may diminish hyperactivity of the intact brain, while high-frequency rTMS (>1 Hz) delivered to the affected cortex promotes recovery mechanisms [234]. For instance, it has been shown that facilitators stimulation of ipsilesional primary motor cortex (M1) may enhance functional connectivity between M1 and ipsilesional supplementary motor area, while inhibitory stimulation of contralesional M1 decreases contralateral connectivity, but strengthens ipsilesional connectivity [234,235]. This differential modulation, “upregulating” the lesioned hemisphere or “downregulating” the intact hemisphere, being associated with improved motor performance [236]. Therefore, rTMS could be used as a therapeutically tool to restore the balance of interhemispheric
inhibition after stroke. As stated by Pinter and colleagues (2013), future studies involving large patient populations are needed to address better selection criteria for rTMS benefit in stroke patients, as a function of age, time from stroke onset or stroke severity [237]. Furthermore, structural factors such as corticospinal structural integrity and cortical target location for rTMS may play an important role in the known inter-individual variability of the intervention effect post-stroke [238,239].

Even though it is already known that rTMS modulates cortical and subcortical networks, there is still need to a deeper understanding of the exact mechanisms facilitated by this technique, and thus rendering it more accessible to routine clinical use. Furthermore, after rTMS administration there have been noticed lasting changes in neurotransmitter release, signalling pathways and gene expression, perduing days to weeks [240]. Also was found that high frequency rTMS stimulation of the rat thalamus increased adult neurogenesis and restored experimentally suppressed neurogenesis in the dentate gyrus [241]. Yet, it is important to better observe potential longer-term effects of rTMS over brain activity (over months an even years) of rTMS via longitudinal studies.

Repetitive TMS can have side effects such asseizure induction, effects on cognition, effects on mood, transient effects on hormones and lymphocytes, transient auditory shift, pain and headache, burns from scalp electrodes [227]. There are few absolute contraindications to TMS treatment like pregnant women, children under 6 years, patients with intracranial metallic implants, patients with cardiac pacemakers and spinal cord stimulators [242]. In general a family history of epilepsy is regarded as a contraindication for fast TMS.

In summary TMS might provide a new insight into the pathophysiology of the nervous system and can be used as a therapeutic tool for improving the efficacy of neurorehabilitation after stroke.

Transcranial Direct Current Stimulation (tDCS)

Transcranial direct-current stimulation represents another non-invasive, yet less costly procedure, which implies the application through the scalp of a direct and low-voltage current over a specific region which induces polarisation of ratherdistant brain regions [243]. Nu-doand collaborators (1990) showed in rats that a repeated applicationof very low-intensity electrical stimulation to the motor cortex induced changes in movement representations [206]. Further studies have proven that transcranial current alike rTMS, modulates brain motor and cognitive functions based on plasticity mechanisms and network connectivity [244]. TDCS seems to modulate activation-induced regional cerebral blood flow (rCBF) changes during voluntary movement [245]. Following the first days after a focal vascular lesion, there is generally an exacerbated excitability in the penumbra and also in more distant brain areas [246].
Oxygen deprivation occurring during stroke modifies abruptly the normal neuronal transmembrane ionic balance eventually determining cell death. Local and distant neural remodelling tempts to restore the lost neuronal functions, partly caused by the loss of convergent inputs in the region affected by the infarct, edema and reduced cerebral blood flow. It seems that tDCS interferes with neuronal membrane resting potentials promoting shifted activation of sodium and calcium-dependent channels and N-methyl-D-aspartatereceptors (NMDA-R) which enhance synaptic plasticity via long-term-potentiation (LTP) and long-term-depression (LTD)-like mechanisms [247,248]. It is known that tDCS facilitates the release of brain-derived neurotrophic factor (BDNF), which modulates the induction of NMDAR-dependent LTP. The releases of neural growth factors after stroke seem to facilitate neuronal regeneration in the perilesional cortex [249].

Even though easy applicable, tDCS is not currently implemented as a standard therapeutically manoeuver in stroke patients, mostly due to variable effects depending largely on the state of the patients’ brain and the task performed [250]. Yet, it may represent a promising venue of enhancing brain recovery in neurorehabilitation settings.

Epidural Cortical Stimulation (ECS)

Preliminary animal and human studies showed improvement of motor symptoms after stroke with invasive cortical stimulation [251,252]. Further animal studies involved cortical motor stimulation (M1) by subdural electrodes with a favourable motor outcome [253,254]. Stimulation-related structural changes included a larger proportion of neurons in perilesional cortex, enhanced synaptic density and production of neurotrophic factors [255]. In humans, this therapeutically approach is still a matter of controversy, positive results largely depending on the mass of corticospinal tract remained intact after vascular damage [256].

So far, even though theoretically may represent a potent and lesion-focused approach for brain recovery, it still needs further confirmation and risk-benefit proper evaluation individualized for each patient. Development of non-invasive functional methods (such as functional MRI) to assess brain functions may provide more refined support for ECS in well-selected patients.

Functional Electrical Stimulation (FES)

Functional electrical stimulation (FES) is a form of electrical stimulation applied in rehabilitation practices on a nerve pathway or motor point to produce a muscle contraction that has the ability to be assimilated in the normal motor engram. The patient uses the stimulation to execute a functional movement and depending on the severity of the paresis can be used as a functional substitute [257]. Studies have shown that FES is able to improve axonal conduction velocities, axonal growth, and the my-
elimination of peripheral nerves [258]. It has been shown that FES device enhances peripheral nerve activity (efferent activation) and corresponding muscle and joint proprioceptive feedback (afferent activation). The implication of FES in central nervous system plasticity mechanisms requires further clarifications.

**Robot-Assisted Devices**

Medical rehabilitation is focused on restoring the health and functional abilities of people after illness or injury such as stroke. A adequate rehabilitation program can reverse many disabling conditions or can help patient scope with deficits that cannot be reversed by medical care. Rehabilitation address esthe patient’s physical, psychological and environmental needs. It is achieved by restoring the patient’s physical functions and or modifying the patient’s physical and social environment.

Medical devices have increased the lifespan of patients, survival after an stoke episode, serious medical problem or a disease until now considered lethal. Success is also followed by other medical problems that must be carried by the patient, the family and the therapeutic team in terms of rehabilitation of these people in everyday life, with full support from society [259].

A major goals of rehabilitation is to make quantitative and qualitative improvements in daily activities in order to improve quality of independent living.

Robots appear for the first time in medicine in rehabilitation medicine with the scope to support the rehabilitation team in order to accomplish more results in less time. Robots are being developed for an extensive applications within rehabilitation domain, including use as exercise aids, activity of daily life, mobility aids, and remote some devices. Of these potential applications, robotic exercise devices have been best studied in clinical research and appear safe and also beneficial.

Today, robotic devices are used to replace limbs absence, perform surgical procedures to provide therapy neurorehabilitation patients with stroke, to teach children with learning disabilities and perform a growing number of other tasks related to health. According to the Robot Institute of America, a robot is “a reprogrammable, multi-functional manipulator designed to move material, parts, tools, or specialized devices through various programmed motions for the performance of a variety of tasks”. Given this definition, medical robotics includes a number of devices used for surgery, medical training, rehabilitation therapy, prosthetics, and assisting people with disabilities [260]. Therapeutic robots collect data that can be used to quantitatively measure the patient’s progress throughout the recovery process, enabling therapists to optimize treatment techniques. In addition, robot-assisted therapy systems have the potential to provide extended periods of unsupervised therapy, which could increase efficiency and reduce cost.
Robotic rehabilitation therapy is extremely attractive in post-stroke recovery as multifunctional pre-programmed devices can deliver individualized intensive training. One application of robot-assisted therapy is improvement of gait function in patients with stroke [261]. Some studies conducted in patients with chronic stroke reported comparable effects on gait function between the robot-assisted therapy and conventional gait training. These results indicate that the robot-assisted therapy with end-effector-type devices cannot replace conventional therapy in patients with chronic stroke. However, the other five trials, which enrolled patients with subacute stroke, demonstrated that robot-assisted therapy in combination with conventional physiotherapy produced greater improvement in gait function than conventional gait training alone. This means that the addition of robot-assisted therapy with end-effector-type devices to conventional physiotherapy can be recommended for use in patients with subacute stroke [261].

Virtual Reality

Virtual reality is a computer-generated, interactive simulation that maps the real environment by affecting human senses, and shows all activity in real time and with real speed. Virtual reality sends the user a great number of sensory information comparable to authentic experiences.

This modern computer technology emulates learning process in the real world, while allowing the addition of extrinsic feedback and increasing the frequency, duration, and even intensity of an exercise. Virtual environment enables the user to have the opportunity to interact with objects and situations produced by the hardware [264]. A distinctive platform creates well-defined and customized activities, combining factors such as intensity, variation and specificity of the tasks described as significant for increasing plasticity of the brain.

Virtual reality technologies allow creation of a simulated environment so that proper adjustment of exercise intensity and feedback would provide the patient with safe and effective training and rehabilitation. Virtual reality has now emerged as a promising tool in many domains of therapy and rehabilitation. Offers the potential to create systematic human testing, training, and treatment environments that allow for the precise control of complex, immersive, dynamic 3D stimulus presentations, within which sophisticated interaction, behavioral tracking, and performance recording is possible.

Virtual reality was first applied in paretic upper limb rehabilitation after a stroke in a framework designed to
promote motor activity relearning different movements (hand, elbow and shoulder) and functional tasks [265]. Once the script is defined, programmed learning tasks to be performed in the virtual environment are stored in the motion tracking device. Patients are taught to reproduce trajectories set by the virtual trainer to develop the appropriate path in his absence, while upper limb movements were monitored virtual teacher, displayed in real time and recorded motor rehabilitation has been applied in patients with acquired brain injury with some success, but application in the rehabilitation of these subjects’ cognitive deficits remains unsystematic and its potentialities appear high but still undocumented [266].

Virtual reality therapy for stroke victims has infinite possibly and potential. It was proven to help improve motor impairment as mentioned in a 2011 study published by the American Stroke Association found. In the 2011 study found that 11 out of 12 studies previous studies showed a significant benefit from rehabilitation with virtual reality. Another benefit of using Virtual reality therapy in patients post stroke is being able to offer many different treatment options for each patient as no stroke survivor has the same impairment. Stroke physical therapy can be individualized to render the most effective treatment which will optimize rehabilitation and help the patient get close to where they were physically and motor skill wise before suffering from a stroke.

Experimental data showed that the use of virtual reality and interactive video gaming may be beneficial in improving upper limb functions and activities of daily living (ADL) outcome, when compared with the same amount of conventional therapy [267]. Virtual reality may be easily used in hospital settings where global motor activity and gait training may be more difficult to apply on a regular basis.

Up to date, there is too few data on cognitive functions rehabilitation with this approach. Overall, there is need for further studies in this field so far, to allow definitive conclusions about precise impact and place in stroke recovery strategies.

Conclusion and Further Perspectives

Recent research has shed new light on facilitator pharmacological and non-pharmacological interventions on neuroplasticity mechanisms, subtending functional and structural post-stroke neural remodelling and consecutive improved clinical outcome. Cell-based and neurotrophic therapies, multimodal pleiotropic molecules, functional interventions to promote learning-related rehabilitation at early stages, represent new challenges and promising avenues of therapeutic strategies to enhance brain recovery after stroke. Brain stimulation techniques show promising potential for modulatory intervention on plasticity mechanisms subtending rehabilitation programs in stroke.
patients. On the other hand robotic devices and virtual reality have capabilities to continue as technological development and in the near future these methods are expected to become an integral part of rehabilitation like an important accessory to traditional rehabilitation approaches.

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