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Post-stroke remodeling processes in animal models and humans

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Abstract

After cerebral ischemia, events like neural plasticity and tissue reorganization intervene in lesioned and non-lesioned areas of the brain. These processes are tightly related to functional improvement and successful rehabilitation in patients. Plastic remodeling in the brain is associated with limited spontaneous functional recovery in patients. Improvement depends on the initial deficit, size, nature and localization of the infarction, together with the sex and age of the patient, all of them affecting the favorable outcome of reorganization and repair of damaged areas. A better understanding of cerebral plasticity is pivotal to design effective therapeutic strategies. Experimental models and clinical studies have fueled the current understanding of the cellular and molecular processes responsible for plastic remodeling. In this review, we describe the known mechanisms, in patients and animal models, underlying cerebral reorganization and contributing to functional recovery after ischemic stroke. We also discuss the manipulations and therapies that can stimulate neural plasticity. We finally explore a new topic in the field of ischemic stroke pathophysiology, namely the brain-gut axis.

Keywords

Brain-gut axis, ischemia, microbiota, neuro-inflammation, recovery

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Introduction

Recovery after cerebral ischemia has been attributed to plasticity and reorganization in the brain, with formation of new connections and undertaking of the functions previously performed by the damaged areas by different ipsilateral or contralateral regions. These events are coordinated by cellular and molecular mechanisms. The initial deficit, size, localization and nature of the lesion are crucial elements regulating these processes. Also, sex and age are predictive of motor recovery.[1] Current understanding of the processes underlying cerebral plasticity and reorganization provided the foundation to design therapeutic strategies. Neuronal plasticity is normally associated with axonal sprouting and formation of new synapses, changes in synaptic strength and compensation by the contralateral cortex. Several features of human stroke are identifiable also in animal models. For example, the activation patterns observed in humans by brain imaging have also been identified in pre-clinical models after focal ischemia.[2] In humans, as well as in rat[3] and non-human primate[4] models of stroke, reorganization of the motor areas occurs through the unmasking of existing networks that were initially inhibited, through excessive excitability, strengthening of existing connections, dendritic and synaptic reinforcements, neurogenesis and synaptogenesis triggered by vacant synaptic sites.[5,6] The time course of these mechanisms

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is spread over several months,[7] and includes four major overlapping phases: 1/the inflammatory processes and resolution of the injury, 2/diaschisis and secondary remote lesions, 3/tissue repair, 4/compensation processes (with expansion phenomenon first, and then focalization of activity).[8]

In this review, we provide an extensive overview of the mechanisms underlying remodeling processes after ischemic stroke (IS) in the brain, in animal models and humans. Insights in current therapeutics, including behavioral manipulation and strategies to stimulate neural plasticity and functional recovery, are also covered. Finally, we present a detailed analysis on the emerging role of the bidirectional brain-gut axis in IS onset and outcomes.

Functional remodeling in stroke

Unmasking networks, synaptogenesis

In the context of homeostatic plasticity in IS, disinhibition is considered one of the mechanisms that contribute to brain reorganization, through the unmasking of latent connections.[9] This perilesional reorganization was observed in rat[3] and non-human primate models,[4] and in patients[10] (Figure 1). Reactivation of latent circuits has been demonstrated at the spinal level for the phrenic nerves innervating the diaphragm.[11] After the unmasking, an increase in synaptogenesis is observed. McNeal et al. showed the reorganization of spinal projections of secondary motor cortex (M2) in Rhesus macaque (equivalent to supplementary motor area (SMA) in humans) with partial lesion of the area of the arm in the dorsolateral primary motor cortex (M1)/premotor cortex (PMC).[12] An increase in spinal terminal projections from ipsilesional M2 occurred in regions containing interneurons, flexor motor neurons, and hand muscles in laminae VII and IX.[12] Unmasking networks and synaptogenesis period are coherent with the optimal plasticity period after stroke (Figure 2). In the first four weeks after injury, the processes promoting growth are maximal, as shown in rat models of lesion to the sensory cortex, for example, with a very large turn-over of dendritic spines in perilesional areas and synaptogenesis, hyperexcitability and lack of sensory specificity. Between 4 and 8 weeks, the synaptic connections are more specific and hindpaw neurons sprout and connect to innervate the forepaw.[13] The first month after stroke is therefore a particularly plastic and critical period (Figure 2).

Diaschisis and secondary degenerations

In stroke, diaschisis is defined as the loss of functionality in brain regions distant from the primary lesion, which is caused by the de-afferentiation in these

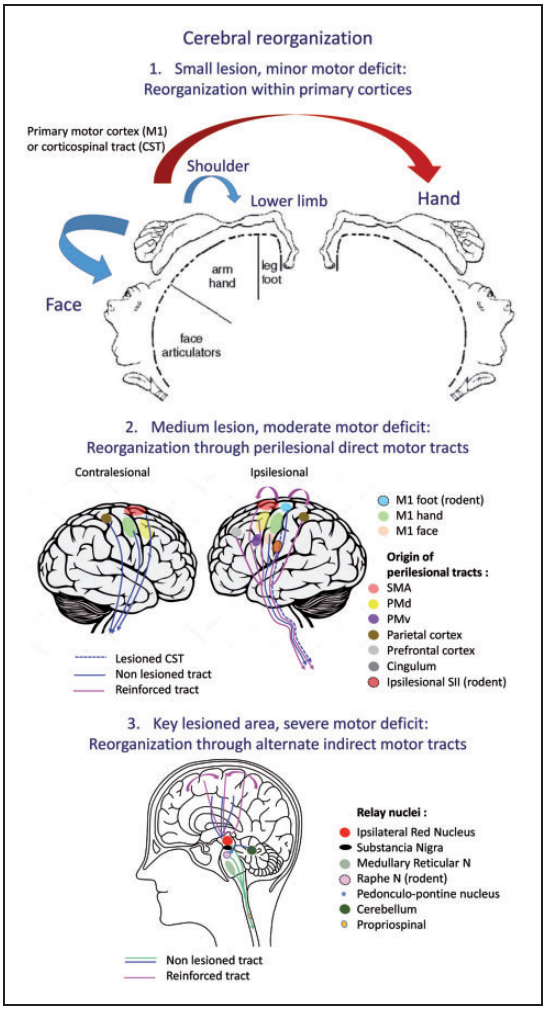

Figure 1. Three schematic patterns of cerebral reorganization depending on lesion size or localization. Case n°1: Small brain lesions are associated with minor motor deficits, caused by injury to the primary motor cortex (M1) or corticospinal tract (CST). Cortical reorganization occurs within ipsilesional (blue) or contralesional (red) primary motor cortices. Case n°2: Medium lesions affect larger areas of the brain, thus causing moderate deficits. Reorganization relies either on perilesional direct motor tracts that may be reinforced (pink) or contralesional areas. Cortical origin of perilesional tracts possibly involved is indicated. Some pathways have been evidenced in rodents. Case n°3: Large lesions or lesions to specific brain areas, though not large in size, may cause severe deficits because of their key localization. Tracts from cortical areas that may be reinforced make relay onto motor nuclei forming alternate motor tracts that are therefore indirect. In each case, depending on severity and structural reserve, mechanisms may involve redundancy or vicariance: unmasking of existing redundant fibers, or short-distance and long-distance dendritic sprouting at cortical, sub-cortical, pontine or spinal levels, and takeover of novel functionalities by non-lesioned motor areas. Assuming that recovery depends on a large neuronal network, lesion size affects structural reserve, and being larger and larger, decreases the number of relevant potential connections, forces reorganization to take place in more distant cortical areas, thus affecting level of recovery. CST: corticospinal tract; M1: primary motor cortex; SMA: supplementary motor area; PMd: dorsal premotor cortex; PMv: ventral premotor cortex.

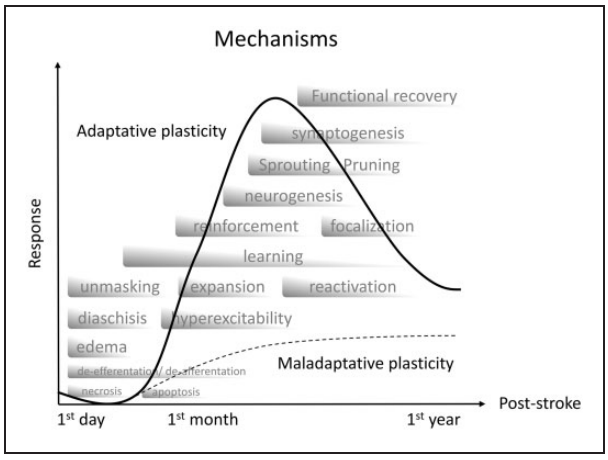

Figure 2. Pathophysiological events and mechanisms of plasticity after stroke. Adaptive plasticity leading to good recovery is major during the first month post-onset. Maladaptive plasticity, when present and if untreated, may increase with time. Y-axis indicates the response, in terms of functional recovery, associated with the level of plasticity (adaptive or maladaptive) after stroke. The mechanisms underlying adaptive or maladaptive plasticity are indicated in the gray bars.

regions, with consecutive hypostimulation. Additionally, a substantial decrease in cellular metabolism, which can be transient or persistent, in the latter case causing secondary lesions, is present in these regions.[11] In animal models of stroke, a lesion in the sensorimotor cortex causes cell death resulting in pyknotic neurons in the ventrolateral nuclei of the thalamus[14] or in the nuclei of basal ganglia in the contralesional hemisphere.[15] Nevertheless, thalamic neurodegeneration has not been confirmed in non-human primate models (marmoset).[16] In humans, subcortical motor stroke causes reductions in gray matter density distant to the lesion in the cerebral peduncles, substantia nigra, pons, cerebellum, thalamus, ipsilesional SMA, and PMC.[17–19] Non-motor areas, such as the contralesional temporal-occipital region, medial frontal gyrus, and superior temporal gyrus, as identified by magnetic resonance imaging (MRI) (voxel based morphometry (VBM) analyses)[19] may also be affected.

Brain activation, and also contralesional premotor activation, depends on the integrity of the corticospinal tract (CST).[20] Histological analyses also showed the presence of secondary demyelination of transcallosal fibers in stroke patients after CST lesion,[21] which correlate with alteration of diffusion tensor imaging (DTI) parameters and with less lateralized activation. A study combining tractography and VBM shows secondary degeneration following subcortical stroke in patients with pure motor deficit. In these patients, structural fiber integrity assessed by fractional anisotropy (FA) was significantly decreased in the ipsilesional brainstem and correlated with the Fugl-Meyer score of the hand/wrist. Atrophy of the substantia nigra, medial frontal gyrus, upper temporal gyrus, SMA, and contralesional post-central gyrus has also been reported.[19]

The efficiency of rehabilitating therapies after IS seems to depend also on the integrity of distant white or gray matter regions. Indeed, secondary degenerations of gray matter in areas distant from the lesion (cerebellum, SMA, PMC, and contralesional occipito-temporal region) have been associated with reduced efficacy of the constraint-induced movement therapy (CIMT).[22]

Interhemispheric balance

Several studies on the relationships occurring between the two M1 in IS have shown excessive excitability of the unaffected hemisphere. As explained above, after IS the non-paretic limb is predominantly used, and this may in turn inhibit the affected hemisphere and worsen motor deficits. Indeed, the transcallosal connections coupling the two hemispheres are mainly inhibitory. In IS, the interhemispheric balance is disrupted. In animal models, this balance is considered more adaptive, but this feature is less clear in humans. A number of neuromodulation protocols have aimed to restore the interhemispheric balance. This can be achieved by developing techniques that inhibit the unaffected cortex[23] or, on the opposite, stimulate the damaged cortex.[24] However, this approach is too schematic, often restricted to patients with subcortical lesion, and owns important limits, as highlighted by a number of negative clinical trials.[25] Di Pino et al. have alternatively proposed "the bimodal balance-recovery model" that includes a new parameter, the "structural reserve," by meaning of the neural pathways and connections spared by the lesion and contributing to recovery after IS.[26] This reserve seems to depend on the integrity of the ipsilesional hemisphere and especially of the motor areas and the CST, and can be evaluated by the Predict Recovery Potential (PREP) algorithm of Stinear.[27] If the structural reserve is high, the prognosis for recovery can be better predicted by the interhemispheric balance model and the contralesional hemisphere would be inhibited. Conversely, in the case of a low ipsilesional structural reserve, recovery would depend more on a vicariance model and the contralesional hemisphere would not be inhibited. This could help to better guide the choice of neuromodulation protocols for each patient (personalized therapy) (Figure 1).

For correctness, the time course of the cerebral reorganization where contralesional M1 is transiently hyperexcited before normalizing must be added to this model. Inhibition in the subacute phase can then

be beneficial.[21] Thus, non-invasive stimulation by bilateral transcranial direct current stimulation (tDCS: stimulate ipsilesional M1 and inhibit contralesional M1) may be beneficial for re-learning in chronic patients.[28] To summarize, the contralesional hemisphere has a bivalent role depending on the level of structural reserve, either contributing[21] or being deleterious to the recovery after IS.[29]

Key fiber tracts

Motor tracts. The integrity of the M1 cortex is essential for the recovery of functions such as hand dexterity, a complex movement that requires a bi-hemispheric working network, which instead results interrupted in IS. Total destruction leads to chronic deficits in dexterity, as observed in rodents.[14] On the contrary, regarding strength, animal models have shown that the recovery after stroke is possible, albeit slow.[14] The PREP algorithm evaluates the prognosis for upper limb motor recovery 12 weeks after an IS using clinical data collected 72 h after injury.[27] This method includes the measurement of shoulder abduction strength and finger extension, electrophysiological data (i.e. presence of a motor evoked potential (MEP) of the extensor carpi radialis muscle on the paretic side two weeks after IS), and the FA within the posterior limb of the internal capsule, measured by DTI. In addition, two meta-analyses showed that the FA of the CST and the 24–72 h fiber ratio are both good predictors for motor recovery. The first analysis was based on 15 studies ($n = 414$ stroke patients)[30] and the second on $n = 117$ patients.[31] Recent studies have also evaluated the FA of alternative motor fibers, such as the cortico-rubrospinal[32] and the corticoreticulo-spinal[33] tracts, which may act in synergy. Importantly, it seems that adding other parameters like the volume of the caudate nucleus gray matter, would improve prediction of stroke recovery.[34] So far, parameters obtained from DTI assessing fiber remodeling remain potentially interesting but yet less investigated in this context.

Language tracts. Voxel-based analyses have helped to establish the localization of the structural damage responsible for aphasic symptoms, dissociating semantic and phonological processes and recognition versus production in IS patients.[35] The lesion load of the arcuate fasciculus influences speech production, naming and fluency, and predicts the severity of outcome after injury. The Predicting Language Outcome and Recovery After Stroke (PLORAS) study, conducted on 270 stroke patients, identified the regions and the main tracts that are fundamental to language production and recovery.[36,37] Corbetta et al., using a multivariate approach taking into account behavioral and structural variability, confirmed the location of language areas in stroke patients displaying deficits with different modalities.[38]

Homeostatic plasticity and Hebbian plasticity

The rules of post-lesion learning or re-learning can be divided into two conceptual categories: 1/homeostatic plasticity mechanisms, where neurons receive an appropriate amount of synaptic inputs and regulate their activity relative to the network and 2/Hebbian plasticity mechanisms, where synaptic strength is redistributed to promote the connection of neurons or synchronous networks,[5] or conversely to diminish non-functional aberrant circuits (Figure 2). Based on these two mechanisms, two phenomena have been described in animal models of stroke: the balance between peri-lesional hypoexcitability and disinhibition phenomena, and the changes in synaptic efficiency.

With respect to homeostatic plasticity, it has been observed that at the periphery of the injury in a 0.2 mm thick crown, activated astrocytes induce a decrease in the uptake of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and this leads to an increase in the GABAergic signaling and a hypoexcitability of the pyramidal neurons adjacent to the lesion.[39,40] A therapeutic strategy aimed at counteracting this inhibition seems promising and a Phase IIb clinical trial with inverse agonists (NAMs, negative allosteric modulators) targeting the alpha5 subunit of the $GABA_A$ receptor is ongoing.[41] In addition, the loss of lateral connections after brain injury leads to hyperexcitability of the surviving neurons, which can also cause spontaneous transient synchronous activity (0.1–1 Hz). This state of disinhibition creates a permissive environment for synaptic sprouting, as shown in rat models.[41] The sprouting process in the brain is mainly regulated by microglia. Homeostasis mechanisms participate in the formation of new synapses to restore the initial level of connections. The molecular determinants of this plasticity are partially unraveled and involve pro-inflammatory cytokines, chemokines, signaling molecules, growth proteins, and growth factors[40,42] (see Figure 3 for detailed factors). Neurotransmitters and brain-derived neurotrophic factor (BDNF) are, furthermore, involved in the overexpression of glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that increase synaptic efficacy.[5] Genetic polymorphism of BDNF seems to alter motor function in stroke patients,[5,43] however, findings are discordant. At the molecular level, learning and memory storage paradigms are associated with changes in the expression of stathmin, Rb3, growth-associated protein 43

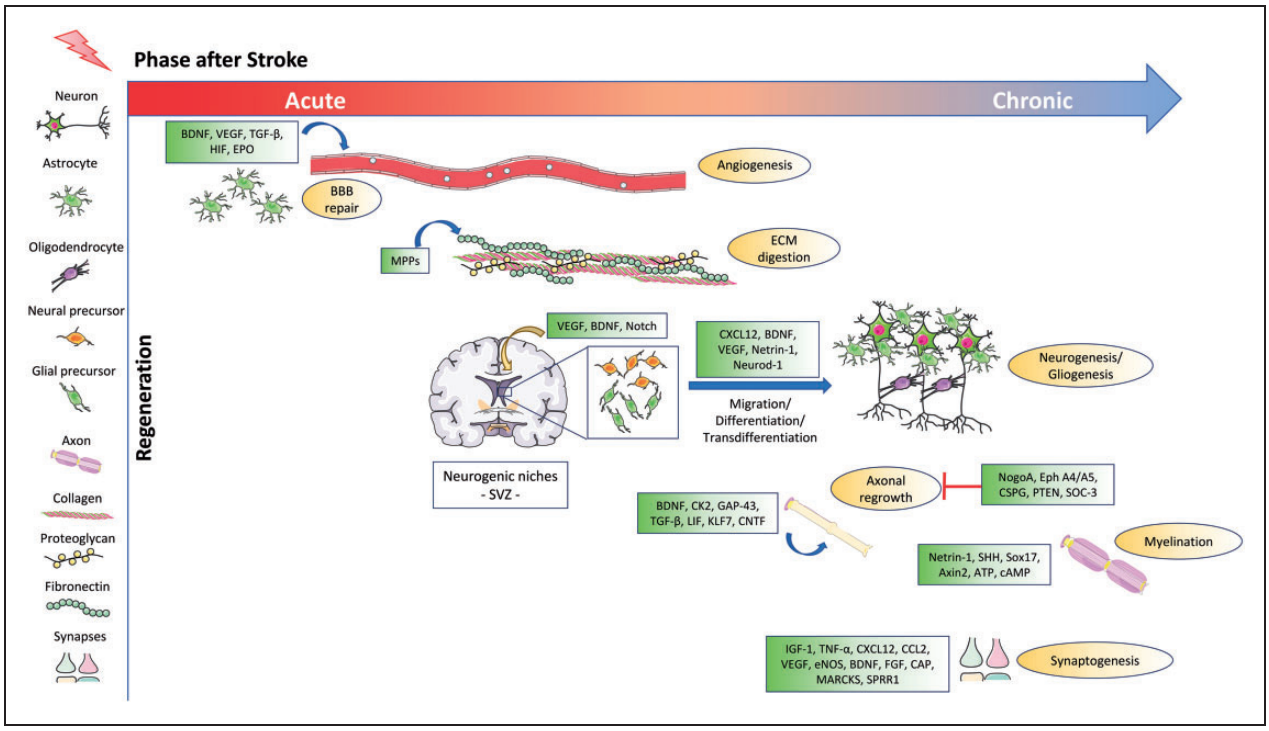

Figure 3. Mechanisms of regeneration after stroke. During the acute and the chronic phases after stroke, angiogenesis, neuro- and gliogenesis need to be reestablished in the brain. Crucial mediators for angiogenesis are BDNF, VEGF, TGF-β, HIF and EPO. The excess of ECM has to be digested by MMPs. VEGF and BDNF also participate in neurogenesis and gliogenesis starting from neuronal and glia precursors in the neurogenic niches (i.e. the SVZ). Cell migration, differentiation and (trans)differentiation is triggered by CXCL12, Nestrin-1 and Neurod-1, in addition to VEGF and BDNF. To complete the regeneration process, factors like CK2, GAP-43, LIF, KLF7, CNTF favor axonal regrowth. Netrin-1, SHH, Sox17, Axin2, ATP and cAMP are involved in myelination. Finally, synaptogenesis is stimulated by IGF-1, TNF-α, CXCL12, CCL2, VEGF, eNOS, BDNF, FGF, CAP, MARCKS, SPRR1. Control mechanisms to avoid aberrant axonal growth that may inhibit regrowth include NogoA, Eph A4/A5, CSPG, PTEN, SOC-3. BBB: blood–brain barrier; BDNF: brain-derived neurotrophic factor; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor-beta; HIF: hypoxia-inducible factor; EPO: erythropoietin; ECM: extracellular matrix; MMPs: metalloproteinases; SVZ: subventricular zone; CXCL12: C-X-C motif chemokine ligand 12; GAP-43: growth associated protein 43; LIF: leukemia inhibitory factor; KLF7: Kruppel-like factor 7; CNTF: ciliary neurotrophic factor; SHH: sonic hedgehog signaling; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; IGF-1: insulin-like growth factor 1; TNF-α: tumor necrosis factor-alpha; CCL2: C-C motif chemokine ligand 2; eNOS: endothelial nitric oxide synthase; FGF: fibroblast growth factor; CAP: cortical cytoskeleton-associated protein; MARCKS: myristoylated alanine-rich C-kinase substrate; SPRR1: small proline repeat rich protein 1; CSPG: chondroitin sulfate proteoglycans; PTEN: phosphatase and tensin homolog; SOC-3: suppressor of cytokine signaling 3.

(GAP-43), and the Nogo signaling system. All pathways are involved in post-stroke recovery.

The principle of Hebb leads to learning by repetition (temporal synaptic facilitation) or convergence (spatial synaptic facilitation) contributing to strengthening (or, conversely, weakening) of synaptic connections, also called "spike-timing-dependent potentiation tLTP (long-term potentiation) and depression tLTD (long-term depression)."[44] This type of plasticity has been demonstrated in humans by applying paired transcranial magnetic stimulations (TMS) of the posterior SMA 6 ms before that of M1. This results in an increase of the corticospinal excitability for 30 min whereas a delay of 15 ms between the two stimulations decreased the excitability.[45] TMS stimulation and more particularly using PAS protocols (Paired Associated Stimulation: cortical stimulation coupled with peripheral electric stimulation), imply processes similar to tLTP. The redundancy mechanism involving the strengthening of existing connections and synaptic enhancement is regulated by synaptic efficiency changes.[5] Post-stroke, activity-dependent Hebbian-like synapse-based learning rules that could reinforce new circuits are set up.[46] Models of neural networks operating on homeostatic and Hebbian plasticity and receiving kinematic data signals from six muscular spindles of the arm as inputs show a return of the discharge level into perilesional cells after virtual injury. This is a sign of good recovery, whereas network recovery was unsuccessful in the absence of homeoplasticity.[47]

Drugs such as selective serotonin reuptake inhibitors (SSRI) shown to enhance motor recovery[48–50] induce

M1 facilitations,[51] LTP-like facilitations after PAS stimulations,[52] and reduce the expression of inhibitory interneurons in the PMC.[53] Given the post-lesional increase in spinal projections of the raphe nucleus,[54] a spinal contribution is likely in the improvement observed after SSRI. Changes in plasticity may explain the abnormalities in connectivity observed by imaging in stroke patients. For example, a noradrenergic drug increases the connectivity between M1 and SMA in post-stroke patients.[55]

Structural remodeling in stroke

Axonal plasticity and regeneration, dendritic and synaptic sprouting

In the adult central nervous system, the lesion of an axonal end is rarely transformed into a growth cone, rather it forms a retracted bulb.[56] To survive an axotomy, an injured axon must quickly repair its ruptured membrane. Rearrangements of the local cytoskeleton often occur in the axonal end and sometimes form a growth cone that may sprout. However, in order to obtain substantial axonal regeneration, the cell body must reactivate an axonal growth program that will ensure the synthesis of the necessary materials, the transport and assembly of the various components along the axon and at the nerve ending. Axonal and dendritic sprouting has been observed in animal models at the cortical level in the perilesional and contralesional hemisphere,[57,58] in the contralesional subcortical regions,[15] in the brainstem,[54,59] and spinal marrow.[11,12,54,60–64] A study in macaque monkeys reported ipsilesional projections that are either direct or indirect because they produce new synapses with red nucleus neurons, reticular formation, tecto- and vestibulospinal tracts to enhance flexor control.[65–67] Dexterity may also rely on another indirect pathways such as the propriospinal neurons[68,69] (Figure 1). This mechanism corresponds to a reorientation of a circuit with the formation of an axonal detour.[6] The ipsilesional motor projections are increased after stroke but this is not always correlated with a better motor recovery.[6] On the other hand, adjacent cortical areas may be able to take over the missing function.[70] In a rat model of stroke, after M1 lesion in the area of the forepaw, Starkey et al.[63] demonstrated dendritic sprouting and synaptogenesis of corticospinal neurons from the hindpaw area at the cervical level, capable of restoring the connection with the motor neurons of the frontpaw. This new wiring was correlated with the level of motor recovery of dexterity in animals. Another detour was demonstrated in a study on ventral PMC neurons of monkeys that formed connections with perilesional M1 neurons.[71] The axonal detour has also been observed in rat models[11] and in patients[72] at the cerebral (transcallosal fibers, red nucleus), pons, and spinal level and may correspond to a "double-crossing" with the take-over of motor functions by the contralesional hemisphere.

Numerous imaging studies have shown the involvement of the primary contralesional M1[10] (Figure 1). Since uncrossed direct spinal projections contribute marginally to dexterity, it is likely that the contribution of the contralesional hemisphere and of M1 in particular, passes through interhemispheric connections in the corpus callosum. Nishimura et al.[73] showed in the Rhesus macaque that the pharmacological inactivation of the contralesional M1 one week after the injury of the CST prevented the motor recovery of the paretic hand. Synaptic sprouting between the perilesional and contralesional areas is triggered by synchronous neural activities at very low frequencies of activation (0.1–0.4 Hz).[41] The set of re-connections revealed in rodents, non-human primates, and patients is summarized in Figure 1.[8]

After IS, intracellular machinery, gene expression, and molecular signaling need to be reactivated. The microenvironment of the lesion and perilesional areas are preponderant, with the release by glial cells of growth factors such as BDNF and vascular endothelial growth factor (VEGF) necessary for axonal regrowth, synaptogenesis and revascularization of the tissue (Figure 3).[74]

Numerous molecular mechanisms and a transcriptome of neural regeneration have been described for white matter lesions, particularly in the CST.[11,75] Very recently, casein kinase (CK) 2 has been reported to be involved in cell injury in white matter during IS,[76] suggesting the use of CK2 inhibitors, which are currently in phase I–II clinical trials for cancer therapy, also in IS patients.[77] Some factors, such as HIF, are related to white matter angiogenesis. Others, such as GAP43, transforming growth factor beta (TGF-β), and transcription factor KLF7 (Kruppel-like factor), are required for axonal survival and sprouting (Figure 3).

In patients, axonal sprouting has been demonstrated by its marker, GAP43, identified in peri-lesional tissue after stroke.[78] Nevertheless, this immunohistochemical staining may lack specificity. It has been shown that the CST, retinal ganglion cells, or dorsal root ganglion neurons have sometimes different regulators and signaling molecules. Thus, depending on the lesion, specific and targeted treatments need to be taken into consideration.[11,61]

Regrowth and axonal connections are limited by the expression of glial growth inhibitory molecules (Figure 3).[40,79,80] These are possible therapeutic targets to enhance brain plasticity after IS. For example,

chondroitinase ABC (ChABC) has been proposed for its ability to digest chondroitin sulfate proteoglycans (CSPG) and has been demonstrated to be effective for motor skills recovery in a rat model of IS.[81] Also EphrinA4 plays an important role in the inhibition of axonal outgrowth: blocking its downstream target Rho-associated kinase (ROK) improved functional recovery in a mouse model of stroke.[82] Other noxious mechanisms for axonal regrowth need to be inhibited to improve functional recovery after stroke (Figure 3).[40] Zones that are permissive or inhibitory for sprouting are spatially distinct. The glial scar contains growth-promoting and growth-inhibitory molecules, which are both upregulated, and is surrounded by the growth-permissive zone.[40]

Other mediators participate in the recruitment, maturation, and differentiation of oligodendrocyte progenitors. Stem cells and progenitor cells migrating from the neurogenic niches secrete matrix metalloproteinases to digest the components on their way to the target tissue (Figure 3).[83]

Since some mechanisms are common to ontogenesis, some authors propose that regeneration in the brain recapitulates development. However, the conditions of the adult brain, especially after stroke, are different from the developing tissue.[40] The capability of axonal regeneration following developmental mechanisms decreases with age.[61] Similarly, some mechanisms are common to cancer; however, it is difficult to argue that regeneration recapitulates cancer.[40] For example, tissue regeneration does not happen in uncontrolled manner, does not unbalance homeostasis, and most of newly-formed cells die by apoptosis or senescence.

Unfortunately, in IS, the number of regenerated fibers is very small and they elude detection by current behavioral, imaging or electrophysiology tests. Different therapies, factors and treatments have increased axonal plasticity and synaptic sprouting such as inosine, VEGF, erythropoietin (EPO), anti-NOGO-antibody,[6,84] electrical cortical or spinal stimulation,[85,86] repetitive TMS or tDCS in stroke patients[87] coupled with physical therapy[88,89] or, simply, physical exercise.[90] The evidence of axonal sprouting is reminiscent of that reported after theta burst stimulation ("LTP-like" plasticity). A supranormal volume of gray matter in the ipsilesional pre-central gyrus predicts better recovery after cortical epidural stimulation in stroke patients.[91] Stimulations applied during the walking on a treadmill can help strengthen adaptive relay circuits related to motor activity. The time window is important since intensive training too early is harmful.[62,92] Conversely, plasticity of the contralesional cortex can allow effective compensation with the non-paretic hand but may aggravate the phenomenon of "learned non-use" of the paretic limb.[93]

Stem cells, angiogenesis, neurogenesis, gliogenesis, myelination

At the end of the 90 s, adult neurogenesis in the brain was discovered in animals and then humans.[94] It occurs mainly in the subventricular zone (SVZ), in the dentate gyrus of the hippocampus and in other circumventricular areas as observed in humans.[95] SVZ stem cells proliferate, then migrate through vessels and radial glia towards the olfactory bulb and the ventromedial prefrontal cortex, and differentiate into neuroblasts and neurons (Figure 3).[96,97] In animals and humans, after brain injury, neurogenesis is stimulated.[95,98,99] Tissue regeneration concerns lost neurons but also structural and feeder cells, namely glial cells and vessels. Neurogenesis is tightly coupled with angiogenesis for the proliferation and migration stages leading to the concept of the "Neurovascular niche." Immature neurons migrate along vessels to the lesion to differentiate. The main migration regulatory factor is CXCL12 that attracts neuroblasts expressing the CXCR4 receptor to the vessels. Endothelial BDNF also attracts neuroblasts and netrin-1 is required for oligodendroglial progenitor migration to the corpus callosum (Figure 3).[100] Conditioned media from vascular cell cultures promote neuronal differentiation, as does the basic helix-loop-helix proneural transcription factor Neurod1. Post-stroke angiogenesis has been observed in patients[101,102] and mechanisms similar to how axonal growth cones explore their surroundings have been described with tip cells sensing guidance cues.[103] If BDNF stimulates neurogenesis and plasticity, its therapeutic use is restricted by its pharmacokinetic properties. BDNF is known to bind the tropomyosin-related kinase B (TrkB) and p75 neurotrophin receptors (p75NTRs).[104] Thus, targeting TrkB has been suggested as effective therapeutic option to promote angiogenesis and neurogenesis, by using specific ligands administered in a specific time window to favor functional recovery instead of BDNF.[105]

After IS, neurogenesis may arise also from astrocytes and pericytes[106] (for review see Zhu et al.[107]). Specifically, astrocytes in the striatum, after activation, may generate neuroblasts, immature (after one week) and mature (after two weeks) neurons in mouse models of IS: this neurogenic program is regulated by Notch signaling and boosted by VEGF.[108,109] Similarly, brain pericytes may contribute to neurogenesis after ischemia/reperfusion injury in mice, as shown by the expression of nestine (for neural stem cells) and doublecortin (for immature neurons).[110]

In rats, it has been shown that only 0.2% of newly generated neurons survive.[111] In addition, they remain confined to the edge of the lesion and are ineffective in replacing extensive neuronal tissue. One explanation is

that the intralesional microenvironment is not conducive to cell life. Nevertheless, other territories targeted by neurogenesis, such as perilesional regions, could influence functional recovery. Re-innervation of the substantia nigra after secondary lesions has been shown after intrastriatal grafts of neuronal cells.[112] Various factors promoting or, conversely, decreasing myelination have been highlighted: exercise, enriched environment or social isolation, and stress. In parallel, an increase in gray matter demonstrated by MRI in the hippocampus and bilateral sensorimotor cortex was correlated with the improvement of the paretic limb 10 days after CIMT in chronic stroke patients.[22] Finally, variations in genotype can influence regeneration capacities (*Val*/*Met* polymorphism, apolipoprotein (Apo) E4), the expression of growth factors and the response to different rehabilitating therapies.[43]

In conclusion, the regeneration of the injured tissue is mainly a spontaneous process (Figure 2) and though effective in repairing small lesions, it is not sufficient to fill major lesions or to recover tissue integrity and lost function.

Adaptive and maladaptive mechanisms

Maladaptive plasticity in the brain is defined as the reorganization that limits recovery after IS. This phenomenon is responsible for compensatory movements, coordination deficits, syncinesis, spasticity, pain, dystonia, and even epilepsy. When untreated, it gradually worsens over time (Figure 2).[8,93,113] Between 15 and 40% of stroke patients suffer from disabling spasticity.[114] Increased muscle tone and reflexes can contribute to disability, limit recovery and may affect the patient's daily mobility. In addition, chronic spasticity leads to contractures and pain. Maladaptive plasticity has been demonstrated in patients and in non-human primate models of IS.[93,113,115] Although commonly associated with the contralesional hemisphere, it can also involve aberrant connections in the ipsilesional hemisphere. On the other hand, adaptive recovery after IS may depend on the direct non-crossed CST in the contralesional hemisphere, which is physiologically responsible for proximal movements and trunk mobility, but also for the posture of the hand.

In the case of severe deficits after IS, the compensatory movements of the shoulder and trunk make it possible to regain a degree of functionality. However, although compensation allows the accomplishment of the task in the short term, it may be associated with reductions in movement ranges and pain sensation in the long term. In addition, the use of the healthy upper limb for motor substitution can cause the underuse of the paretic limb, a phenomenon called "learning of non-use," with consequent reduction of the ability to recover motor skills. In order to limit non-use, CIMT can be offered to patients with moderate deficits. From the molecular point of view, in rat models of IS, training of the non-paretic limb leads to a reduction in neural transcription factor synthesis, contrarily to what happens when training of the paretic limb is performed.

Inflammation and neuro-inflammation

The response after focal injury can be divided into three major phases that overlap in time: 1/cell death and inflammation, 2/cell proliferation for tissue replacement and 3/tissue reconstitution.[116] Cerebral ischemia is followed by an inflammatory reaction in the impacted tissue for several days and even months. Inflammation is a process also involved in the expansion of the lesion and neurological damage.[117] It is still necessary in guarded proportions to clean the cell debris and fight against the ongoing aggression. Protected by the blood–brain barrier (BBB), the brain has its own defense system and innate immunity. There are two major phases of inflammation. The first one mainly involves activated microglia, the brain's macrophages that derive from the yolk sac. Microglia is one of the four major types of cells in the brain with neurons, astrocytes, and oligodendrocytes, the latter two representing the macroglia. After injury, resident microglia are immediately activated by increased extracellular adenosine triphosphate (ATP) and loss of neuronal contacts (Figure 4). Historically, two main phenotypes of microglia were described: M1 and M2 microglia. However, this mere classification is nowadays disused and it is clear that during brain injury, including IS, microglia may acquire diverse phenotypes with overlapped gene expression and function, and exert pro- and anti-inflammatory actions.[118] In the acute phase of IS (24 h), activated microglia in the core of the lesion express markers as CD11b, CD45, and CD68 and release pro-inflammatory mediators (Figure 4). After this phase, microglia become activated in the penumbral zone.[119] Recently, using a rat model of middle cerebral artery occlusion (MCAo), Boddaert et al. have elucidated part of the mechanisms involved in the pathways of microglia polarization after IS.[120] The transcript analysis to study the expression of typical signal receptors affecting microglia phenotype in the perilesional area, identified CD8 signaling as an important mechanism in IS, as confirmed also by in vitro microglia stimulation.[120] Another newly described factor is Sphingosine 1-phosphate receptor subtype 3 (S1P3), which seems specifically associated with microglia activation and polarization through nuclear factor-kappa B (NF-κB) signaling in IS.[121] The activation of microglia corresponds to transcriptional activation of

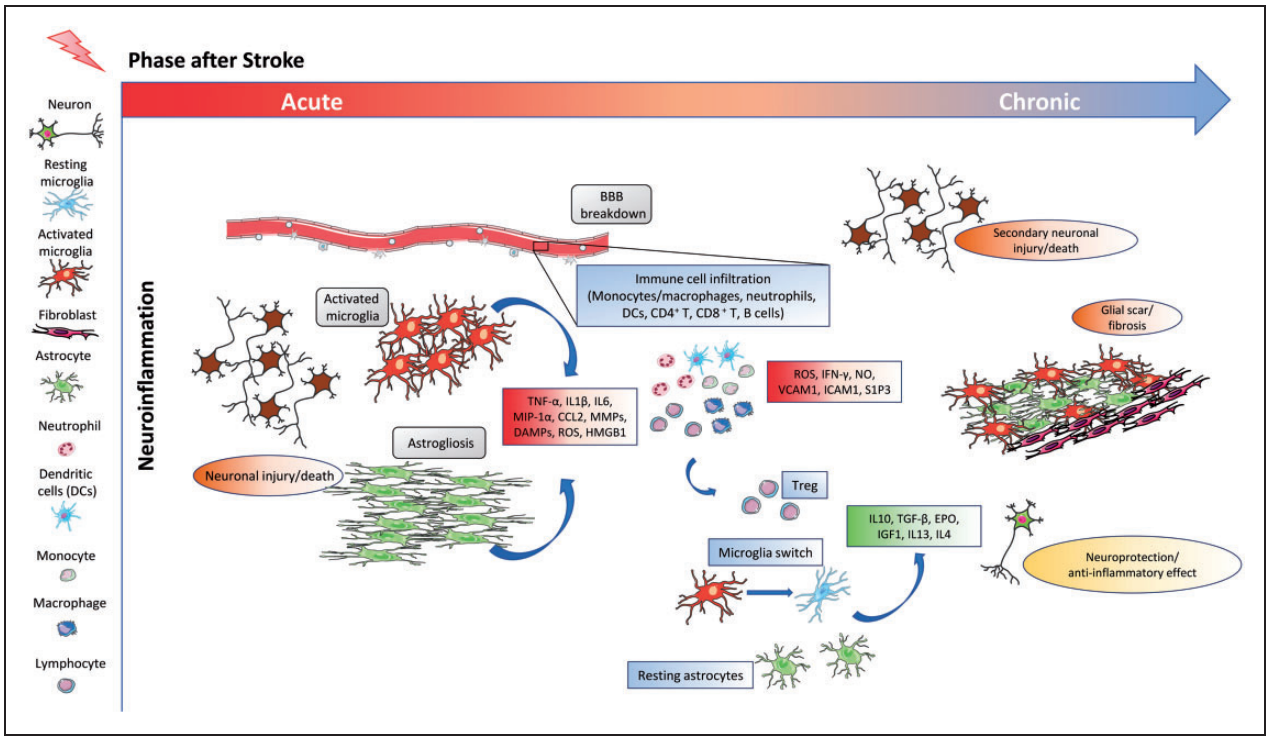

Figure 4. Mechanisms of neuroinflammation after stroke. The first event happening is the BBB breakdown, together with neuronal injury/death and gliosis consequent to hypoxia. Gliosis includes activation of astrocytes (astrogliosis) and of microglia, with a switch from the anti-inflammatory to the pro-inflammatory phenotype. Gliosis is then characterized by the release of pro-inflammatory molecules, such as TNF-α, IL1β, IL6, CCL2, MIP-1α, MMPs, DAMPs, ROS, HMGB1. Additionally, activated astrocytes and microglia, together with fibroblasts and pericytes migrating from the meninges and the blood vessels, form a physical barrier called "*glial scar*" that contains inflammation. BBB rupture leads to the infiltration of immune cells, namely monocytes, leukocytes and DCs, which also release pro-inflammatory mediators (ROS, IFN-γ, NO, VCAM1, ICAM1, S1P3). The amplified inflammatory scenario causes secondary neurotoxic effects. In a later phase, Treg cells counteract $CD4^+$ T-cell cytotoxic effects, initiating the protective phase. This phase is also characterized by the switch of microglia to the non-inflammatory phenotype and the release of TGF-β, which together with IL10, EPO, IGF1, IL13, IL4 favors neuroprotection. BBB: blood–brain barrier; TNF-α: tumor necrosis factor-alpha; IL1β: interleukin-1beta; IL6: interleukin-6; CCL2: C-C motif chemokine ligand 2; MIP-1α: macrophage inflammatory protein-1 alpha; MMPs: metalloproteinases; DAMPs: damage-associated molecular patterns; DCs: dendritic cells; ROS: reactive oxygen species; HMGB1: high mobility group box 1; IFN-γ: interferon gamma; NO: Nitric oxide; VCAM1: vascular cell adhesion protein 1; ICAM1: intercellular adhesion molecule 1; S1P3: sphingosine 1-phosphate receptor subtype 3; TGF-β: transforming growth factor-beta; IL10: interleukin-10; EPO: erythropoietin; IGF1: insulin-like growth factor-1; IL13: interleukin-13; IL4: interleukin-4.

pro-inflammatory genes and synthesis of pro-inflammatory cytokines (Figure 4). However, microglia do exert a biphasic function, being beneficial by releasing neuroprotective factors such as TGF-β.[122] Astrocytes are also involved in the second phase of inflammation mediated by anti-inflammatory microglia, by releasing neuroprotective factors such as EPO or TGF-β (Figure 4).[123] Activated microglia damage blood vessels and participate in the destruction of the BBB. Secreted pro-inflammatory cytokines induce the expression of adhesion molecules intercellular adhesion molecule-1 (ICAM1), vascular cell adhesion protein-1 (VCAM1), P- and E-selectin allowing the interaction between the endothelium and the hematopoietic immune cells, particularly leucocytes.[124] The latter infiltrate the ischemic zone, mainly in peri-lesional area and produce pro-inflammatory cytokines, thus aggravating inflammation (Figure 4). This is the second type of inflammation, in which also astrocytes are involved.[123] Microglia-derived cytokines and chemokines induce also the recruitment of T cells that cross the BBB to reach the site of injury.[125] The detrimental role of T cells, more than B cells,[126,127] in the acute phase of stroke has been described earlier by different authors,[128,129] and widely reviewed.[130,131] T cell subsets have distinctive effects, detrimental versus beneficial, during IS. In preclinical models, the data on the dual mechanism of T cells obtained in deficient or impaired models show that Th2 and Th1 responses oppositely affect infarct size, aggravating or inhibiting it, respectively, targeting inflammatory pathways.[132] This has been directly correlated with neuronal death.[132] Based on the evidence

that, in patients, T cell invasion has been shown to persist for years after IS,[133,134] experimental models have been used to evaluate chronic T cell invasion in the ischemic brain and its significance. A recent study has found a prolonged (at one month) activation of $CD4^+$ and $CD8^+$ T cells in a transient MCAo model.[135] Proliferating T cells were found in the peri-infarct area, close to reactive astrocytes, indicating that they may play a role in the neural repair process after IS. Focusing on remodeling after IS, more important is the role of the T-reg subset, whose recruitment has been associated with late, but not acute, phase at one week, and has been identified as a cerebroprotective mechanism (Figure 4).[136,137] Importantly, during the sub-acute phase of IS, regulatory T-regs are essential to counteract cytotoxic T-cell effects, which cause neuronal death in the penumbra via interferon-gamma (IFN-γ) release (for review see Gauberti et al.[130] and Drieu et al.[131]). Not only T-regs regulate neural recovery; a recent study has shown their role in glial scar formation during the chronic phase of stroke, via the negative regulation of the interleukin-6 and STAT3 pathway in microglia and astrocytes.[137] The beneficial effect of T-regs is also present during exogenous delivery of bone marrow-derived stem cells (BMSCs), which is neuroprotective after IS (for review see Vahidy et al.[138]). Specifically, a minority population of T-regs existing within the BMSCs serves as robust mediators of the immunomodulatory and neuroprotective effect provided by BMSC transplantation after IS.[139]

Inflammation after IS also involves neutrophils and dendritic cells (DCs) that are not present in the healthy brain (Figure 4). However, they can be found following the rupture of the BBB and express surface molecules indicating their active state. In general, neutrophils, together with microglia/macrophages, account for the phagocytosis of cellular debris and DCs initiate an adaptive immune response ensuring specific protection against the injury.[140–142]

Inflammatory cell infiltration and astrocytosis last about one month in rodents and 3 months or more in IS patients. Lipid mediators such as lipoxins, marexins, protectins, resolvins have been recently demonstrated to be protective against inflammation at this stage.[143]

Post-stroke inflammation plays a role in the induction of axonal regeneration processes, mainly via cytokines ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) (Figures 3 and 4). Compared to the peripheral nervous system, the privileged immune status of the brain and the absence of endogenous antibodies cause a delay in the elimination of myelin and axonal regeneration after injury.

Remote inflammation and additional amyloidosis. Imaging with ^{11}C-PK11195 positron-emission tomography (PET) radiotracer in stroke patients has shown that remote inflammation and lesions are observed in the de-afferented CST, and are not associated with recovery.[144] Specifically, the study combined PET and DTI-MRI to demonstrate differential temporal dynamics of local and remote activated microglia, which were differentially related to anterograde fiber tract damage. This evidenced a relationship between microglial activity and fiber tract integrity in human subcortical stroke, with different repercussions on clinical outcomes. By evaluating the results from experimental and clinical studies,[145] the same study explored the possible interaction between neurodegenerative inflammation and vascular processes in determining cognitive decline after IS. The results propose that both vascular (presence of amyloid deposits) and inflammatory (microglia activation) events should be jointly assessed as predictors of cognitive recovery, since they may differently impact on patients' outcome.[145]

Glial scar. In IS, in addition to the microglial reaction, the astrocytes will also be activated and respond to this aggression by forming, around the lesion, a physical and chemical barrier called "*glial scar*" (Figure 4).[116] A small fraction of glial fibrillary acidic protein (GFAP)-positive cells are however BrdU-negative, indicating that the increase in the number of GFAP positive cells is due to cell division and not to migration.[146] Villapol et al. showed that, three days after injury, reactive astrocytes acquire a hypertrophic morphology.[147] They also found the presence of astrogliosis extending from seven days to two months after injury.

Other cell types come into play in the formation of the peri-lesional scar. Perivascular or meningeal fibroblasts invade the core of the lesion and secrete type I or IV collagen and components of the extracellular matrix, thus causing the formation of a fibrotic scar (Figure 4).[148] From the first three to five days and therefore in parallel with this process, the reactive astrocytes will come around the lesion but will not go to the heart of the lesion.[149] The scar is also composed of newly generated and elongated astrocytes.[150] This dense area does not exceed half millimeter around the edge of the lesion and no neuronal cell type is detected within it. Glial scar formation is associated with the overexpression of extracellular matrix inhibitors such as proteoglycans (Figure 4), the concentration of which increases with the closeness to the lesion core. This intertwining between astrocytes and the secretion of these molecules forms a barrier impervious to any exchange. However, the glial scar is more elastic than the parenchyma.[151] Expression levels of glial intermediate filaments (GFAP, vimentin) and extracellular matrix components (laminin, collagen IV) correlate

with tissue softening. This, therefore, creates an environment that does not promote axonal growth and cellular regeneration.[152] All of these processes have disadvantages in preventing some of the tissue regeneration but this disadvantage may be an advantage as it prevents the increase in lesion size, the expansion of inflammation and the exaggerated demyelination of perilesional axons.[153]

The last category of glial cells, *oligodendrocytes*, seem also to react in case of ischemic injury[154] but their action is not yet well established because they are more difficult to study.

Brain-gut axis in stroke

Bidirectional signaling occurs between the gut and the brain in health and disease, the so called "*brain-gut axis*" (for review see Mayer et al.[155] and Aziz and Thompson[156]). Anatomically, this communication involves the CNS, the autonomic nervous system, the enteric nervous system (ENS) and the hypothalamic pituitary adrenal axis, and neuro-immuno-endocrine mediators.[157] The signaling involves serotonin, acetylcholine, glutamate, GABA, short- and long-chain fatty acids, histamine, catecholamines, hormones, cytokines, glucocorticoids and nutrients. In neurological diseases, the brain-gut axis appears altered, with consequences on the onset, severity and outcomes of several disorders.[158–162] During the last two decades, unexpected but existing brain-gut axis has been described, and in part characterized, also in stroke. The first evidence appeared when observing the gastrointestinal (GI) alterations occurring in stroke patients.[163] Beside the top-down communication, a bottom-up signaling gut-to-brain also exists and is mainly orchestrated by gut commensal bacteria, the *microbiota*, and immune system interactions, transmitted to the brain. Microbiota-immune system interactions may affect predisposition to and outcomes of IS.

In the following part of this review, we report the current knowledge on the interaction between the brain and components of the gut, microbiota, immune and ENSs, in IS pathophysiology (Figure 5).

Gut microbiota and stroke

The attention of stroke researchers to the brain-gut axis increased exponentially when it became clear that the gut microbiota plays an important role in brain development, physiology and pathology. During IS, commensal bacteria undergo microbial imbalance, a phenomenon known as "dysbiosis." Dysbiosis has been described, for example, in a large Chinese cohort of stroke patients, after analysis of fecal samples which were processed to extract bacterial DNA.[164] From the technical point of view, advances in this field have become possible thanks to methodological improvements in gene amplicon sequencing and bioinformatics analyses. This study identified the depletion of three main commensal bacteria in stroke patients: *Bacteroitedes*, *Firmicutes*, and *Proteobacteria*, and enrichment in opportunistic bacteria, such as *Enterobacter*, *Megasphaera*, and *Desulfovibrio*. The findings were confirmed in animal models of stroke, in which brain ischemia, produced by MCAo, induced changes in the intestinal microbiota composition,[165] with a different microbial composition 72 h after the lesion, compared to sham mice, and a marked decrease in the level of *Prevotellaceae* and increase in *Peptococcaceae*. From these papers, it is evident that the microbiota composition in stroke patients and animal models is altered; however, the bacterial species identified were different. This discrepancy highlights one of the limits of research in this topic, the "man versus mouse" question, which cannot be underestimated.

In the study by Houlden and coll., the change in microbial profiling was associated with an increase in noradrenaline level, but not in other neurotransmitters, in the caecum of MCAo mice.[165] This could be explained by the observed increase in sympathetic innervation in the caecum of stroke animals, confirming an alteration of the brain-gut axis. The link between increased sympathetic innervation and dysbiosis in stroke was identified in intestinal goblet cells, a specialized cell population which indirectly influences gut microbiota by releasing noradrenaline. This finding highlights the existence of a brain-to-microbiota axis via the autonomic nervous system.

The role of commensal bacteria in stroke pathophysiology became clear when researchers explored the reasons of infections in patients, and surprisingly found that stroke causes bacterial proliferation and translocation of bacteria from the intestine to the blood, spleen, liver, and lung, consequent to intestinal mucosal damage.[166] However, the mechanism of bacterial translocation is largely unknown and available techniques cannot provide definitive answers. Possibly, aberrant neural-immune cross-talk is a contributing factor (see paragraphs below). Mucosal damage in the intestine may be responsible for increased exposure to and translocation of bacteria and endotoxins observed in stroke patients. Brain injury and the consequent alteration of neuro-endocrine signaling play very important roles in the regulation of intestinal barrier. For example, in rat models of stroke induced by MCAo, it has been shown that ischemic stress provokes intestinal barrier disturbance, by means of edema, thickening and shortening of the villi, and motility changes, probably due to altered ghrelin signaling in the gut.[167]

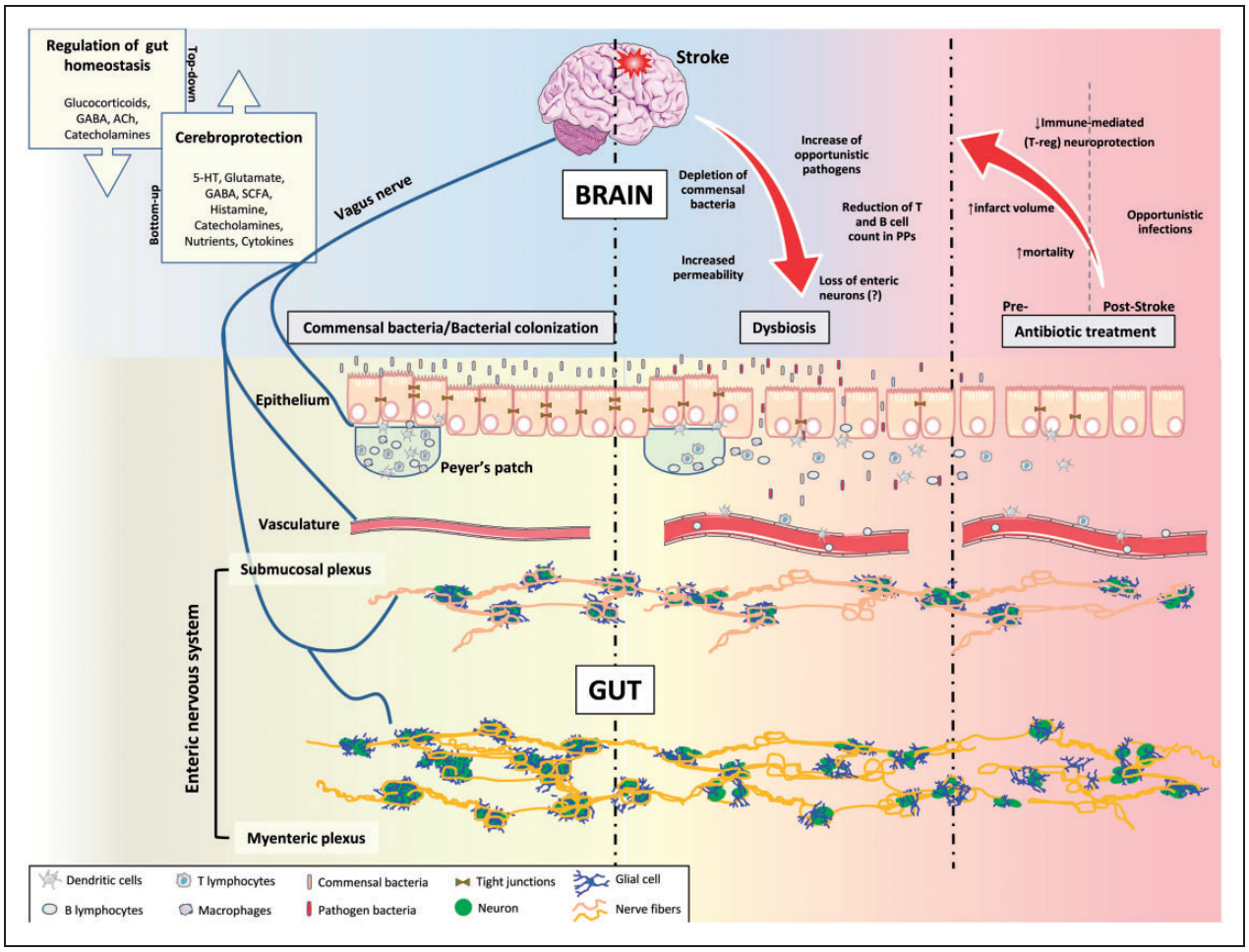

Figure 5. Schematic representation of the brain-gut axis in healthy and stroke conditions. In physiological conditions (left side), the communication between the brain and the gut occurs through the vagus nerve (part of the autonomic nervous system), the enteric nervous system and its cells (glia and neurons), the epithelium and commensal bacteria (microbiota). In this situation, the regulation of intestinal homeostasis and cerebroprotection are assured by bottom-up and top-down signaling, respectively. In stroke (middle and right side), the brain-gut communication is altered, with morphological and functional consequences in both directions. Specifically, stroke causes alteration in microbiota content and composition. This correlates with immune/inflammatory responses in the brain, through the interplay microbiota/immune system. Antibiotic treatment after stroke (post-stroke) causes impairment of the immune-mediated (T-reg) neuroprotective response in the brain and facilitates opportunistic infections. On the other hand, in mice, antibiotic treatment given before stroke induction (pre-stroke) revealed to worsen the outcome of the disease, with increased mortality and infarct volume. GABA: gamma-aminobutyric acid; Ach: acetylcholine; 5-HT: serotonin; SCFA: short-chain fatty acids.

Confirmation of commensal bacterial translocation in post-stroke infections was provided by Stanley.[168] Using germ-free (GF) and specific pathogen free (SPF) animals, the authors proved that post-stroke infections from *Escherichia coli* likely originate from the host gut microbiota and are not acquired from the environment, since GF mice did not show positive cultivable bacteria, compared to SPF mice. The results of the study were corroborated by perspective observations in stroke patients, in which commensal bacteria residing in the intestinal tract were found in large amount (more than 70% of detected bacteria) into peripheral tissues, including the lung, liver, and spleen.[168]

Gut microbiota exerts bidirectional communication with its targets, modulating GI and brain functions by the interplay with the immune, vascular, autonomic, and ENS. In the last decade, pre-clinical studies designed to unravel this communication have been numerous, through the possibility to raise and keep GF animals, a valid strategy to evaluate the impact of gut microbiota on organ functions. However, GF animals harbor confounding factors, somewhat far from the human situation, such as immune deficiencies, and altered brain physiology and anatomy. For this reason, more appropriate studies have been conducted in broad-spectrum antibiotic-treated animals, which,

advantageously, recapitulate the situation in patients undergoing antibiotic therapy to avoid infections consequent to stroke. With this valuable approach, Winek et al. demonstrated that gut microbiota exert protective effects on survival after stroke in the MCAo mouse model, protecting from the excessive mortality observed between days 5 and 7 in antibiotic-pretreated mice.[169] However, the antibiotic pretreatment (during eight weeks) did not affect the volume of ischemic lesion in the brain. Additionally, microbiota-depleted mice developed acute ulcerative and necrotizing colitis and systemic immunodepression when the antibiotic cocktail was stopped 72 h before operation. The two findings seem linked, since the altered systemic immunity after stroke may lead to the breakdown of intestinal mucosal barrier and translocation of bacteria and their products, but direct evidence is still missing. Intriguingly, these effects were reversed by the continuous antibiotic treatment or colonization with the microbiota obtained from SPF animals before MCAo surgery. These findings open interesting clinical questions on the importance of gut microbiota for stroke outcome in antibiotic-treated patients.

Going further in the evaluation of commensal bacteria on stroke outcome and on the possibility to recolonize the intestine with "good" bacteria, Spychala et al.[170] have recently reported an intriguing finding: the "age of microbiota" is crucial in stroke, in the sense that microbiota composition differs in young compared to aged animals. Indeed, by performing MCAo in young versus adult mice, the authors showed that the two groups of animals had different stroke outcome, in terms of mortality and severity. More intriguingly, "youthful" flora seems to be protective when transplanted in aged mice, impacting on behavior and infarct volume in the brain. Additionally, mechanistic evaluation identified short chain fatty acids as possibly responsible for this protective action. This is a complete study revealing "top-down" and "bottom-up" signaling between the brain and the gut in stroke and the first one assessing and correlating behavioral evaluation with microbiota features.

Intestinal immune system and stroke

IS is characterized by immune reaction, with the infiltration of intestinal lymphocytes into the brain, appearing from hours to days, which aggravates tissue injury. Concerning brain-to-gut communication, stroke has differential effects on the cellularity of gut-associated lymphoid tissue (GALT), the immune system of the gut. The first investigation of this topic demonstrated a significant reduction of T and B cell counts in the GALT of mice after MCAo, while no difference in the number of natural killer cells and macrophages was detected.[171] Additionally, no significant changes in intraepithelial and *lamina propria* lymphocytes subsets were observed. More recently, the exploration of stroke's consequences on intestinal immunology and morphology have shown that the number of T lymphocytes in the Peyer's patches increased, activating intestinal immunity, and that the recruitment involved one specific chemokine, CCL19.[172] This mediator is indeed expressed in the intestinal epithelium and involved in B and T lymphocyte recruitment. The link between stroke and intestinal immune system seems to occur via the intermediation of commensal microbiota, suggesting again that it is a key player in regulating and influencing disease processes in the brain. The first evidence of an existing link between altered intestinal flora, immune system and stroke outcome has been provided by Benakis, in a well-designed and clear study where the authors induced intestinal dysbiosis in mice undergoing MCAo, by the administration of antibiotics.[173] A neuroprotective effect was intriguingly found. The use of amoxicillin/clavulanate to reduce the number of opportunistic bacteria in the microbiota in mice before inducing stroke with the MCAo model, showed that infarct volume in the brain was reduced, compared to mice with normal or resistant microbiota. This was accompanied by better preserved sensorimotor functions in the antibiotic-treated mice. Additionally, in showing that intestinal T-regulatory and IL-17-positive gamma-delta T cells (derived from the GALT), are capable of migrating from the gut to the meninges after stroke, the study demonstrated a tangible link between altered flora and stroke outcome. Once in the meninges, these cells secrete IL-17 and IL-10, which exert neuroprotection by controlling the trafficking of monocytes and neutrophils in the brain during stroke. This was the first study revealing the existence of a *gut-to-brain axis* in stroke. The results was confirmed by Singh in the same year.[174]

The proof-of-concept that bacterial colonization impacts on stroke outcome and post-stroke immune-mediated neuroinflammation has been recently provided by experiments performed in SPF, GF, and colonized ex-GF mice.[175] In this study, stroke outcome was different in the three groups of animals, with improved outcomes in ex-GF and SPF compared to GF mice. More interestingly, by investigating the effect of commensal flora in lymphocyte-deficient mice, the authors demonstrated that a physiological gut microbiota is required to generate an adequate lymphocytes-driven immune reaction and to provide consequent brain tissue protection. This finding proves the importance of gut microbiota for cerebroprotection in stroke.

ENS and stroke

The brain is not the only nerve tissue in the body. The so called "second brain" or "little brain," which runs along the intestine and is named ENS, is responsible for the control of GI function, independently from the brain.[176] After stroke, patients may suffer from intestinal complications.[163] This leads to hypothesize that stroke evokes intestinal dysfunction by altering the ENS, in a brain-to-gut axis direction. The communication would occur through molecules released by cells in the brain (neurons and/or glia) and targeting cells in the ENS. To date, only one group has conducted two separate studies to evaluate the changes in the ENS consequent to brain ischemia, in a model of MCAo in mice.[177,178] The intriguing evaluation has revealed that three days only after MCAo there was a loss of enteric neurons, both submucosal and myenteric, with a proximo-distal gradient (60% in the colon and 40% in the ileum, compared to controls). In the study, this phenomenon was correlated to galectin-3, released by microglia in the brain during stroke and involved in intestinal signaling. Indeed, galectin-3 knock-out mice did not show this feature. The data were confirmed in vitro by exposing primary cultures of myenteric neurons to exogenous galectin-3. In the second study, the same authors went further in their investigation by 1/evaluating the difference in enteric neurons loss in focal versus global cerebral ischemia and 2/deciphering the changes in the major subpopulation of enteric neurons after stroke. The different outcomes evidenced after focal or global ischemia point a valuable attention on the different signaling dependent on infarct volume in the brain. However, changes in neuron subpopulations need more careful examination. The method for counting enteric neurons, as performed by the authors, is not the current gold standard. Dissection of the plexus (submucosal and myenteric) would have allowed a better appreciation of abnormalities, from the number of neurons/ganglia to the number of neurons/surface.[179,180] Along the same observation, the appraisal of the changes in neuronal subpopulation needs a more stringent methodological approach. One additional doubt emerges from the fact that the neuronal marker HuCD, used to count neurons, appears homogeneously expressed in the cell body, while it is now known that when neurons are unhealthy the expression pattern does change in favor of a nuclear staining.[179–181] More accurate evaluation is necessary to unravel the previously unrecognized link between stroke and ENS abnormalities, which will help understand the intestinal outcomes of pathophysiological processes after stroke.

The brain-gut axis: A possible therapeutic target for brain remodeling in stroke?

Data from last decade clearly evidence the involvement of the brain-gut axis in neurological disorders, including IS.[160–163] Commensal bacteria seem to play a role in the pathogenesis, the course and the outcome of stroke by modulating immune responses, as evidenced in preclinical studies.[169,173] In humans, the beneficial effects of probiotics on the immune system have been convincingly demonstrated.[182] Although the actual link between microbiota, brain remodeling and the underlying mechanisms are yet to be characterized, the manipulation of the gut microbiota appears as a promising future (co)-therapy in IS.

Conclusion

Through this review, it has been realized that understanding the mechanisms of neurological recovery from stroke will permit to define therapeutic targets to assign the right treatments to the right patients. Since cellular and molecular mechanisms are often inaccessible in humans, animal models and imaging methods will help to understand, predict, and guide therapy.

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