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REVIEW

Factors influencing brain recovery from stroke via possible epigenetic changes

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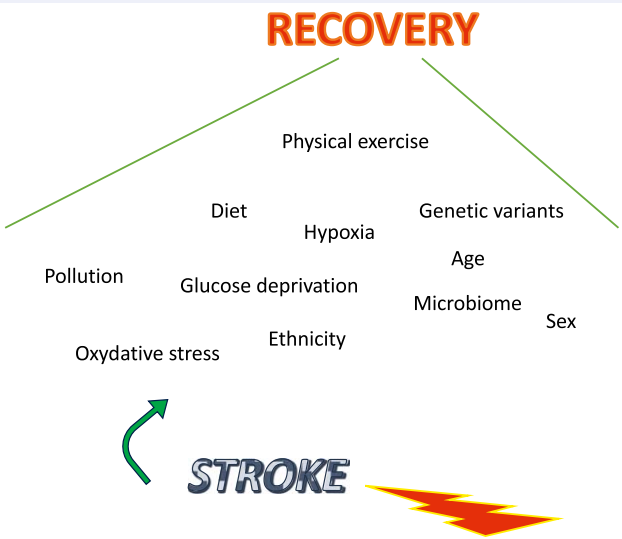
ABSTRACT

Aim: To examine epigenetic changes leading to functional repair after damage to the central motor system.
Data sources: A literature search was conducted using medical and health science electronic databases (PubMed, MEDLINE, Scopus) up to July 2023.
Study selection: Data were summarized for type of intervention, study design, findings including human and animal studies.
Data extraction: Data were extracted and double-checked independently for methodological quality. By means of the influence of environmental (calorie restriction or physical exercise) and other factors, epigenetic instructions were found to increase levels of *BDNF* and enhance synaptic neurotransmission, possibly leading to larger scale changes in structural and functional assets, which may end up to cognitive and motor repair after stroke.

PLAIN LANGUAGE SUMMARY

Recovery from stroke has a very high social cost. We explored in the literature what factors may influence stroke and its recovery. We found that several factors affect mechanisms of gene regulation. Some of them regards environmental interventions, like modifications in diet or programs of physical exercise. These may alter the production or degradation of proteins, modifying the cell function, in particular in neurons, leading to changes in cognition and behavior and modifying the path to recovery.

GRAPHICAL ABSTRACT



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BDNF; DNA methylation; environmental factors; epigenetics; gene expression; ischemic stroke; physical exercise and rehabilitation; recovery

1. Introduction

According to National Institute of Health, stroke is a condition that happens when blood flow is poor, blocking the circulation and leading to cell death. Stroke is a leading death and disability cause in many countries of the world, yet even though daily research is going on, there are a few proven treatments to assist patients

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during recovery. Thus, is necessary designing and finding new drugs and new therapeutic approaches in order to help stroke patients and also to reduce the daily costs of this long-term disability worldwide, which costs millions of dollars every year [1–3].

2. Experimental studies on stroke

Nevertheless, a lot of work/research has been done until now to understand the pathophysiology of stroke, yet a unique protective strategy for preventing this disease still does not exist. Stroke can result from many factors, some known such as atherosclerosis diseases, cardioembolic and others not fully elucidated [4–6]. Apparently, there are several molecular and genetic underlying mechanisms causing stroke, but also some environmental causes can lead to stroke disease [2,7,8]. Epigenetic alterations have been supported by several studies, in humans and animals, to be involved in stroke disease [6]. While studies on humans must rely on patients, whose conditions may be intrinsically different, animal studies may be controlled more strictly and several variables can be manipulated to dissect their contribution. Moreover, in addition to spontaneous stroke models, several animal models, usually in rodents, were developed to control issues like aging, timing (e.g., transient or permanent ischemia) and mode of delivery of the damaging stimulus (physical or pharmacological), as summarized in [9].

2.1. Role of epigenetic changes

Epigenetic changes and modifications are sensitive to environmental stimuli and these mechanisms might help the physiological process happening in living organisms [10]. DNA methylation, DNA editing RNA, non-coding RNAs, chromatin remodeling, histone modifications are all included in epigenetics mechanisms [11–13] and in changes occurring in the epigenome during the life [13,14]. DNA methylation has been linked to several disorders, among them also stroke [15] and is involved in gene regulation and cell differentiation [16]. DNA methylation is a reversible modification that inhibits the process of transcription promoting the binding of methyl-CpG-binding domain proteins. DNMTs are linked with the methyl group of cytosine in various genomic regions [11,14]. Increased DNA methylation at six CpG loci were distinguished in 80 Chinese adult patients with ischemic stroke and decreased DNA methylation at one additional locus. These data suggested an altered DNA methylation which might play a functional role in ischemic stroke [13]. Endres et al. [17] showed DNA methylation increases *in vivo* after middle cerebral artery occlusion in a rat model, suggesting cell death promotion. These data highlight that DNA methylation plays an important role in mechanisms of injury and repair in post-ischemic brain. Histones are composed of five major families, such as H1, H2A, H2B, H3 and H4 and are the basic units of chromatin. Histone H1 is known as the linker histone, the other four are the core histones. All histone code refers to posttranslational modifications for acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, deamination, adenosine diphosphate ribosylation, catalyzed by histone acetyltransferases and histone deacetylases [14,18]. Histone acetyltransferases and histone methyl transferases make changes in histones, including acetylation and methylation of lysine residues in the tails of H3 and H4 histones [10,19]. Previous studies done in animal models of stroke have shown a decrease of H3 and H4 acetylated histone connected to brain injuries [20,21]. A study done in an animal model of stroke involving middle cerebral artery occlusion demonstrated a decrease in the ischemic brain tissue induced by histone H3 acetylation. Interestingly, these data showed that the pharmacological inhibition of histone deacetylases promotes expression of neuroprotective proteins [22]. Also, the manipulation of histone acetylation prevents oxidative stress thanks to neuroprotective antioxidant enzymes, such as peroxiredoxins [23].

2.2. Stroke, a multi-factorial disease

A lot of research has been done to understand the mechanisms, which might contribute to stroke conditions. The reduction in blood supply decreases the oxygen levels and also decreases the glucose levels, creating stress due to nutrients deprivation, inducing hypoxic stress [24]. The imbalance between free radicals and antioxidants in the body creates oxidative stress, which is associated with some factors such as hyperlipidemia, obesity, cigarette smoking, hypertension [25,26], all risk factors which increase the probability of stroke. However, a healthy lifestyle is very important as daily exercise will help to maintain body–brain in a well-balanced function [27]. Several studies demonstrated a link among synaptic plasticity and the motor and cognitive functions [28]. Hence, internal and external conditions may impact on stroke appearance and recovery.

To understand the pathophysiology of stroke it is important to study and understand the epigenetic mechanisms that may act on this condition. How do these mechanisms happen, and which are the main factors playing a role? Do they interact with each other and how? Why it is important to study these mechanisms? In this short review we want to summarize the environmental factors, such as environmental pollutant and toxicants. We next focus on ischemia as a signal, oxidative stress, which can influence stroke, its effect and recovery. Also,

we briefly explain the therapeutic approaches such as physical exercise and rehabilitation, that recently are gaining more space in the daily rehabilitation of stroke patients (Table 1).

2.3. Factors influencing stroke & its outcomes

Several studies have been done in order to understand the epigenetic signals/factors, and even though it is established that they are quite stable, previous research has shown that these factors can be modified over time [36,87]. Even though research makes daily progress in this field, the underlying molecular mechanisms of the alteration of epigenetics mechanisms by environmental factors/signals, are still not fully understood.

2.4. Type of risk factors

The risk factors for stroke can be divided in two main groups: modifiable (e.g., diet, air pollution) and non-modifiable (e.g., race-ethnicity, age, sex).

2.5. Non-modifiable factors

Non-modifiable factors such as age play a role in stroke risk factors. Notably, aging appears to be one of the major risk factors for stroke, in addition to other diseases [29]. Women are more susceptible to stroke during young age due to hormonal factors, pregnancy and post-partum state, or the use of hormonal contraceptives, compared with young age men, while later the risk is reversed. However, due to longer lifespan of women, then stroke occurs more in women than in men in general [4]. Also, racial-ethnicity disparities among Afro-American and Hispanic/Latino Americans compared with Caucasian people play a role in stroke. The stroke risk incidence is double at Afro-American compared with white people and Hispanic/Latino – American also have an increased stroke risk [34]. These differences could be due to several reasons, such as economic and cultural issues. Furthermore, genetic variability of different gene disorders play an important role as well in stroke risks [35].

2.6. Modifiable factors

Modifiable factors, such as nutrition and diet, called behavioral factors, play a very important role in stroke risk factors. It is not easy to define which is the best diet/nutrition for the daily life, but previous research has shown that a diet rich in fruits and vegetables reduces the risk of stroke [37]. Furthermore, the stroke risks are somehow decreased with potassium intake and are increased with salt intake [38]. Besides diet, physical activity plays an important role, as it has been demonstrated that physical inactivity is linked to poor health and high risk of stroke [4]. Thanks to technology, nowadays is possible to remind and to encourage patients to keep doing physical activity for the post-stroke recovery. Keeping Active with Texting After Stroke app, a text message was sent to patients to remind them of their physical activity over a period of 12 weeks. The results were very promising as patients felt supported and connected [83]. Furthermore, the influence of environment plays a crucial role, as the presence of green parks incites/promotes an active lifestyle, helping in the reduction of stroke incidence.

2.7. Environmental pollutant/toxicants

However, the environmental pollution, such as air pollution, which can be considered an important and modifiable risk factor, plays a considerable role in stroke risk factors [42]. The exposure to air pollution has been associated also with DNA methylation and vascular cell adhesion molecule-1 expression, suggesting a possible development of cardiovascular diseases [43]. Persistent differences in DNA methylation in the insulin-like growth factor-2 gene, can be seen in individuals exposed to famine, six decades later, compared with the same-sex siblings who did not, in the epigenetic example of Dutch Famine study [39]. Also, studies done in animals have demonstrated that the maternal diet is crucial to environment factors including DNA methylation. Lillycrop et al., [40] studied if feeding pregnant rats with a protein-restricted diet or leaving them under low nutrition caused changed to epigenetic factors in the livers of the offspring. The Dutch Famine natural experiment and the studies done in animals show that maternal diet, nutrition and environment affect gene expression and epigenetic mechanisms [36,41]. Previous experimental studies suggest environmental toxicants, such as maternal smoking or heavy metal exposure, are associated with epigenetic alterations [44]. *BDNF* is a gene which plays a key role in neurodevelopment and synaptic plasticity, in overall neuronal health [88], being also a gene of interest in the study of neurological effects of environmental toxicant [44]. Prenatal smoking is apparently involved in brain and behavior variation in adolescence. Toledo-Rodriguez et al., [45] demonstrated that prenatal smoking is linked with DNA hypermethylation in the *BDNF-6* exon, leading to a long-term effect modification in brain plasticity and development in adolescence. Furthermore, maternal exposure to heavy metals is associated with poor growth in childhood [32] and hematopoietic system toxicity [46]. The association between prenatal lead exposure and epigenome-wide DNA methylation in umbilical cord blood from 268 mother-infant pairs was investigated showing that prenatal red blood cells (RBC) lead levels were associated

Table 1. Summary of the key factors influencing stroke recovery, discussed herein.

Factor	Main effects	Ref.
Age	Higher risk in older persons	[31,79,82,29,79,82]
Sex	Variable, depends on age	[4,45,48]
Ethnicity	Lower risk in Caucasian	[32]
Genetic variants	Variable	[33]
Nutrition/diet	Higher risk if high sodium, lower if high potassium intake	[29,34,35,39–41]
Environmental pollution (including smoke)	Deleterious	[37,38,40,42,44–47]
Hypoxia/glucose deprivation	Worsen the effects of stroke	[24,51,52,60–62,67,68,71,73,74]
Oxidative stress	Worsens the effects of stroke	[25,26,77,78,80,81,84,86–92,95,97,99]
Microbiome	Changes related to stroke severity	[109,117–119,121–126]
Physical exercise	Beneficial for recovery	[4,27,36,99,138–140]

with different DNA methylation in umbilical cord blood. Interestingly there was a sex difference, such as RBC lead levels were associated with DNA methylation more in female compare to male infants [47]. Noteworthy, these data are in line with the previous studies regarding the sex differences in lead exposure [32]. Heavy metal exposure have been studied in animals, showing that perinatal lead exposure on dose-and sex-specific responses in DNA methylation acts on the epigenome in a locus specific fashion, indicating that sex is a factor in epigenetic response [33].

2.8. Hypoxia & glucose deprivation

Hypoxia is a very important factor, as oxygen is essential for metabolism and its physiological functions. HIF is a heterodimeric transcription factor consisting of one oxygen-sensitive (either HIF-1α, HIF-2α or HIF-3α) subunit and one constitutively expressed HIF-1β subunit. It is a regulator of oxygen detection and adaptation at the cellular level (Figure 1) [89]. The cellular oxygen homeostasis including genes involved in oxygen consumption are controlled by genes activated by HIF [89,90]. Low oxygen tension activates the HIF transcription complex which controls a diverse range of cellular processes such as erythropoiesis, angiogenesis, cellular metabolism at increasing oxygen delivery to tissues [3]. Apparently, the release of a certain amount of adenosine occurs early following a stroke *in vivo* and during hypoxia and oxygen and glucose deprivation *in vitro* [48,49]. There are four different subtypes of adenosine receptors; A_1, A_{2A}, A_{2B} and A_3, thus, adenosine being an important neuromodulator in the CNS, it plays an important role in the neuroprotective response of cells to decreased oxygen [49,91]. Tonic activation of A_1 receptors has an important role in the recovery, after reoxygenation, of the metabolic alterations caused by transient hypoxia. In rat hippocampal slices, the ability of A_1 receptors to control neural metabolism could be the key mechanism for adenosine brain neuroprotection, indicating that the metabolism in hippocampal brain tissue can be controlled by A1 receptors [92]. Previous studies reviewed by Cunha, [2001] [93] have shown that A_{2A} have a role in facilitating neurotransmitter release. Apparently, A_{2A} receptors are less abundant than A_1 receptors in the hippocampus [94], however they role is very important in neuroprotection [93]. This could happen because of their ability in controlling glutamate release in hippocampus, which is under the control of inhibitory A_1 and facilitatory A_{2A} receptors [95–97]. A_1 and A_{2A} receptors are co-localized in a subset of glutamatergic nerve terminals of the rat hippocampus [97]. A selective dose of A_{2A} receptor antagonists administered before ischemia in gerbils protected hippocampal pyramidal cells by reducing the ischemia [50]. Furthermore, when adenosine release is suppressed, a significant reduction in hippocampal injury was shown during hyperglycemic ischemia, and improved Morris water maze performance was detected in rats with cerebral ischemia induced by four vessels occlusion [51]. Adenosine A3 receptors (A3R), which were also found in hippocampus neurons [95], were examined in ischemic brains with non-hypotensive conditions [52]. Selective A3R agonist 2-chloro-N^6-(3-iodo-benzyl)-adenosine-5′-N-methyluronamide (CI-IB-MECA) or vehicle was administered in control and A3R knockout animals that were subjected to focal ischemia by middle cerebral artery (MCA) ligation. The results suggest that the A3R activation has a protective effect against cerebral ischemia, and A3R agonist CI-IB-MECA given intracerebroventricularly reduced cerebral infarction improving locomotor activity in stroke animals [52]. However, further research is needed to prove the restorative effects of adenosine. Ginseng is known to be used as a traditional medicine to treat a large range of disorders, among them cardiovascular diseases. The cell-penetrating cysteine-dense microprotein TP1 is beneficial in the coordination of attempting anti-stress and cardiovascular advantages [98]. Ginsentide TP1 has been shown to diminish vascular dysfunction in cardiovascular disease patterns and to minimize the molecular pathogenesis of hypoxia-induced vascular endothelial dysfunction [98]. For daily life function, besides oxygen,

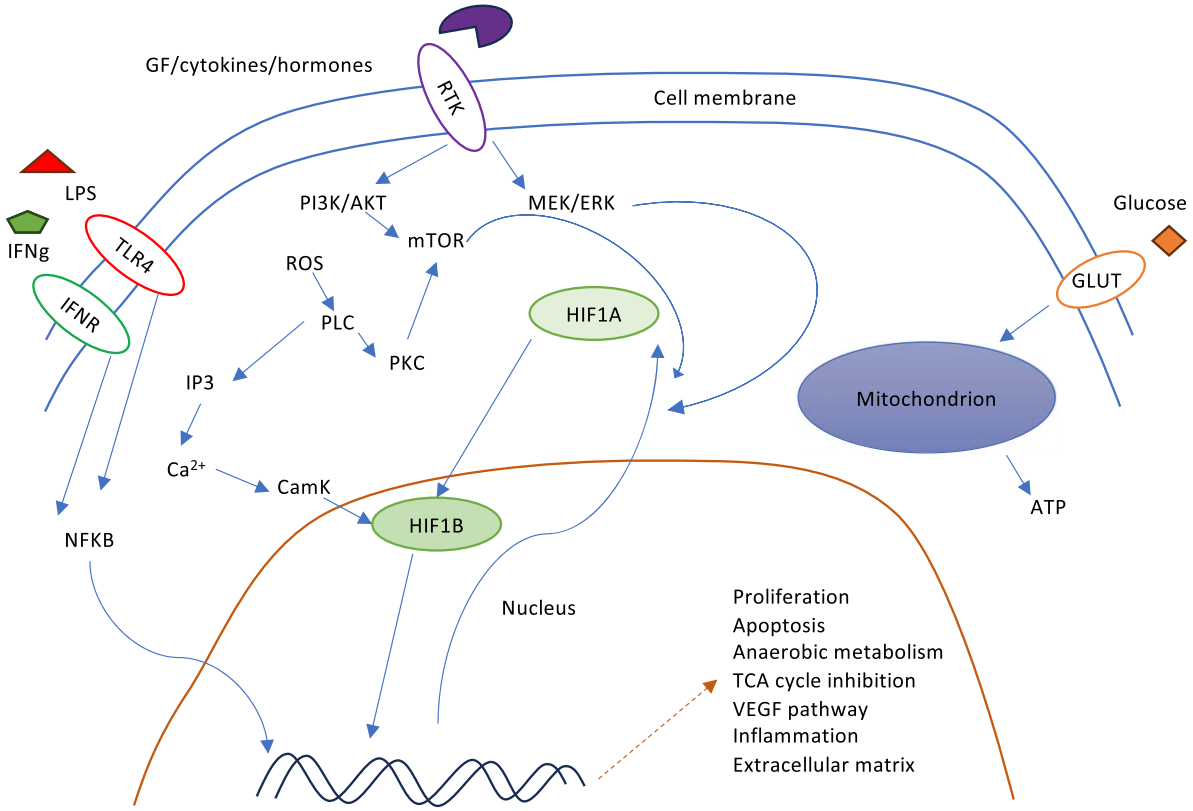

Figure 1. A simplified of the HIF pathway. HIF is activated by different extracellular stimuli, that act through several receptors. In turn, these modify the cell responses and metabolism, mainly acting through regulation of transcription of specific genes. Details are described in the articles cited in the text.
AKT: AKT serine/threonine protein kinase; ATP: Adenosine trisphosphate; CamK: Ca2+/calmodulin-dependent protein kinase; ERK: Extracellular signal-regulated kinase; GF: Growth factor; GLUT: Glucose transporter IFNg: interferon-gamma; HIF1A/B: Hypoxia inducible factor 1 alpha/beta; IP3: Inositol-triphosphate; LPS: Lipopolysaccharide; MEK: Mitogen-activated protein kinase kinase; mTOR: Mammalian target of Rapamycin; NFKB: Nuclear factor kappa-light-chain-enhancer of activated B cell; PLC: Phospholipase c; PI3K: Phosphatidyl-Inositol 3-Kinase; ROS: Reactive oxygen species; RTK: Receptor tyrosine kinase; TLR4: Toll-like receptor 4.

glucose as well is fundamental as the main source of energy for sustaining homeostasis in the brain. Neurons mainly utilize glucose and its transportation is done through different glucose transporters, across the blood-brain-barrier (BBB) into the brain for keeping normal functions [99–101]. However, when stroke occurs these functions fade, since oxygen and glucose are interrupted followed by a decrease of glucose in the brain [53]. Thus, an enhancement of glucose uptake is beneficial for the cells as a neuroprotective mechanism during brain ischemia. Apparently, nicotine has a negative role by decreasing glucose transport across BBB in ischemic brain [54], thus worsening the reduced glucose level in an ischemic brain [53]. Global glucose metabolism was reduced by nicotine in healthy male volunteers [102], and in freely-moving rats [103]. Short and long-term exposure of nicotine on ischemic brain glucose were tested in an *in vitro* ischemic stroke model of oxygen-glucose deprivation (OGD) of primary mouse cortical neurons, as neurons are mostly involved in glucose utilization. The exposure of neurons to nicotine/cotinine significantly decreased glucose uptake after OGD [53]. However, astrocytes play an important role in brain metabolism, as well, increasing their glucose uptake during ischemia [55,104]. Chen et al., [2014] [56] optimized a method to measure glucose uptake by astrocytes during oxygen-glucose deprivation. They used the fluorescent glucose analog, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG). The primary astrocytes incubation was done at 37°C in buffer containing 25–200 μM 2-NBDG. The effects of OGD on the uptake of 2-NBDG in astrocytes were described for the first time, showing that the concentration of 2-NBDG in reactive astrocytes was higher compared with the astrocytes not exposed to OGD, also suggesting that 2-NBDG can be used to detect glucose transport in astrocytes exposed to OGD [56]. After thrombolysis the ischemic stroke-reperfusion (S/R) injury is decisive in the protection of brain function. A murine S/R model was established to test the vasodilation induced by

ultrasound (US)-stimulation microbubble cavitation to reduce S/R injury through sonoperfusion. Oxygen-loaded microbubbles (OMB) in combination with US stimulation was used to ensure sonoperfusion and local oxygen therapy to reduce the brain infarct size. The data showed that OMB cure helps in the reduction of brain infarction and in the activation of neuroprotection to inhibit S/R injury [57].

2.9. Oxidative stress

The disturbance in the balance between the production of reactive oxygen species (ROS), which are the free radicals, and the antioxidant defenses that could lead to tissue damage, is known as oxidative stress [105,106]. Oxidative stress compromises the integrity of genome in the ischemic tissue, leading to the cell death in neurons, DNA lesions, impairment in neurological recovery after stroke [58]. Cerebral ischemia contributes to an increase in oxidative stress followed by an ischemic injury in white and gray matter and also by DNA damage [59]. Oxidative DNA damage can be caused by glial cells as well, such as microglia [58]. Regulation of microglial ROS production by voltage gated proton channels (VSOP/Hv1) is age dependent. The oxidative levels were decreased in young animals, while the level of oxidative stress was substantially increased in older animals, aged more than 6 months. Also, the VSOP/Hv1 neuroprotective effects are age dependent, as at the older stage it was possible to notice an infarct reduction [30]. Besides microglia, astrocytes are also susceptible to oxidative DNA damage caused by focal cerebral ischemia in the ipsilateral striatum. The investigation of immunohistochemical changes of single-strand DNA immunoreactivity, in the striatum from one up to 15 days after 90 min of focal cerebral ischemia, showed a relevant increase of single-strand DNA immunoreactive apoptotic neurons in the ipsilateral striatum 1–3 days after the lesion [60]. After the ischemic stroke occurs, within hours up to days, the blood–brain barrier (BBB) function is compromised in peri-infarct region and this damage is connected with astrocytes swelling, and impairment in normal brain function [61]. The peri-infarct region was the focus in the examination of the ultrastructure of early and delayed changes, 3 versus 72 h, to the BBB in young and aged photothrombotic animal model of stroke. Age has an effect of ischemia on pericytes, the astrocyte end-feet and the mitochondria were severely swollen at age and at time points [31]. These results are in line with a human study where the biopsy from patients showed gray matter capillaries had thinner walls. This was due to the loss of pericytes and the endothelial cytoplasm [107]. Transient Focal Cerebral Ischemia model study was used to assess the effect of endogenous enzyme apurinic endonuclease-1 (APE1) in the long-term recovery of gray and white matter. An APE1 conditional knockout mouse (cKO) line was generated under the control of tamoxifen-inducible Cre recombinase to assess the role of APE1. Oligodendrocyte degeneration and post-ischemic neuronal increased due to APE1 in cKO, demonstrating that APE1 protects against the ischemic lesion preserving gray and white matter [62]. Even though oligodendrocytes are less understood than other neuronal or glial cells, they are important for myelination, and axonal injury in multiple sclerosis [108]. They are object of study of DNA damage and repair in stroke disease [58,62]. *BDNF* due to its role in synaptic plasticity, is a promising neurotrophin for stroke therapeutic purposes. Neuroprotective role of *BDNF* in the oxygen and glucose deprivation-dependent oxidative stress response in the cerebral cortex and hippocampus of animal model was tested, showing that oxidative stress plays a role among hippocampus and the cerebral cortex ischemic lesion. Furthermore, *BDNF* has a neuroprotective effect in hippocampus and cerebral cortex [63]. The effect of *BDNF* and oxidative stress markers were shown in the subacute and chronic stroke patients [64]. As stroke patients often complain of neuropathic pain (NP), then the aim of the study was to characterize NP and to investigate whether there is a correlation between NP, oxidative stress and *BDNF*. Oxidative stress is linked to neural death and *BDNF* is believed to play an important role in the peripheral and central sensitization [65]. An alteration in markers of oxidative stress and in *BDNF* values was noticed in subacute stroke patients with NP. *BDNF* values were higher in subacute patients compared with chronic ones, and *BDNF* was higher in subacute patients with NP than in those without NP [64]. As the brain endothelial cells release *BDNF*, then it is plausible that oxidative stress contributes to *BDNF* release at higher exercise intensities [66,67]. In fact, testing *BDNF* and oxidative stress level at different exercise intensities, increased oxidative stress levels due to high intensity exercise explains *BDNF* release, which improves cognitive performance [68]. The biomarker of oxidative stress has been studied in human acute ischemic stroke patients. Specifically, the hyperacute plasma levels of oxidative stress, inflammation and tissue damage predict infarct growth. F2-isoprostanes (F2-isoP) are more stable and specific markers of oxidative stress compared with other biomarkers. The results showed that elevated hyperacute plasma F2-isoP are independent predictor of the occurrence of infarct growth, suggesting that oxidative stress has an important role in brain tissue injury and cell death [69]. The imbalance represented between the antioxidants and the production of reactive nitrogen species and reactive oxygen species, leads to oxidative

modifications of lipids, proteins and DNA [109]. Oxidative DNA damage is reversible and it usually occurs soon after ischemic stroke [58,110]. As this damage is reversible, it has become an important source of interest in research. Free radicals' generation and consequent oxidative stress have a role in the pathogenesis of acute ischemic stroke, in determination of the protein oxidative profile using protein carbonyl and in evaluation of the activity and expression of AChE mRNA in ischemic patients. Free radicals and ROS apparently are directly involved in biological cell damage and gene expression [70]. Polyphenols are a class of natural and synthetic antioxidant, mainly derived from food and beverages, and from medicinal herbs and are possible substances against oxidative-related diseases [25]. As oxidative stress is related to stroke damage, polyphenol enriched diets help as a therapeutic treatment [25]. Besides having a protective role against oxidative stress, polyphenols may play a role in regulating neurotrophins levels, such as *BDNF* and NGF. Several studies, in humans and animals, demonstrated the beneficial effects of polyphenols in regulation of neurotrophins, such as *BDNF* and NGF, also on their receptors TrkA, TrkB, reviewed in Carito et al., [111]. As we previously mentioned, polyphenols can be found also in the medicinal herbs, and one of them is *Salvia miltiorrhiza (SM)*, which in several countries has been used to treat cardiovascular diseases and stroke. SM has anti-inflammatory effects, antioxidant, antiapoptotic, reducing the impact of injury in stroke [71] and cardiovascular disease, reviewed in Chang et al., [112]. Oxidative stress levels have been tested also using the therapeutic method of magnetotherapy, which uses extremely low frequency (ELF) and an electromagnetic field (EMF). The ELF-EMF treatment effects were tested on the reduction of the oxidative damage of biomolecules after a stroke. Ischemic stroke patients had to perform a rehabilitation program consisting of aerobic exercise for 30 min, neurophysiological methods for 60 min and psychological therapy for 15 min, divided into control group and the aerobic exercise group. The combination of ELF-EMF with the aerobic exercise group showed significantly reduced oxidative stress parameters compared with the control group, improving the effectiveness of stroke rehabilitation [72].

2.10. Microbiome

It is well known now that there is a bidirectional communication between the gut microbiome and the brain, which might affect each-other function [113–115]. Thus, a diet rich in different type of microbial community is very crucial for our health and also, for disease prevention as shown in animal studies [116,117]. Previous epidemiological studies have shown that the exposure to microflora is linked to changes in life style bringing to the risk of development of diseases, such as autoimmune diseases [118]. Genetic factors, but also life style changes such as stress, exposure to drugs, bad nutrition and the cesarean delivery which prevent the child to obtain the natural vaginal and gut microflora during birth, could be the reason of the allergies, which might influence the epigenetics [119,120]. Behavioral and functional aspects of brain, including the emotional behavior, are affected by gut microbiota [121]. The release of several metabolites such as short-chain fatty acids (SCFAs) is generated by microbiota. The SCFA have vasorelaxant properties, helping the decrease in models of hypertension, where gut microbiota can contribute to vascular dysfunction, that increase the risk for stroke [73]. A rich and healthy diet is vital as it impacts gut-brain axis pathways related to stress responses. The SCFA is well studied in humans and animals and research has shown that they improve gut-brain axis and stress related cortisol level [122,123]. The microbiome can also be manipulated in order to enhance the brain function, since better cognitive control was noted at children on high fiber diet compared with children who had a diet low on fiber [124]. However, the microbiota might change gradually with age, possibly influencing the cognitive function [125]. Obesity, gastrointestinal and metabolic disorders can be caused due to the low or missing dietary fibers [126,127] but exercise helps to enhance SCFA already available [128].

During the acute phase of stroke, the microbial composition is apparently changed in the gut and is correlated with the severity of the lesions in the brain suggesting that restoring the health and balance of the intestinal microbiome could help to the treatment of stroke patients [74]. Any form of dysbiosis can create negative impacts on brain function, which is dependent on gut microbes for the necessary metabolic products [75,76]. Thus, gut-brain-microbiome axis is a bidirectional communication system, hence the dysbiosis might impact on brain function [75,129]. Previously, 22 bacterial groups/genera/species were examined, in 41 acute ischemic patients, showing that the dietary changes might affect gut microbial population in ischemic stroke patients. In addition, in their study, the prevalence of Type 2 diabetes was significantly higher in patients with stroke compared with control group, suggesting that the dysbiosis seems to be partially associated also with Type 2 diabetes in patients with stroke [77]. A group of 30 cerebral ischemic stroke patients and 30 healthy controls were involved to determine the characteristics of gut microbiota of stroke patients, evaluating the correlation between gut microbiota and clinical indexes. Their

results showed that the gut of cerebral ischemic stroke patients had more SCFA producer including *Odoribacter*, *Akkermansia*, *Ruminococcaceae*_UCG_005 and *Victivalis*, compared with healthy control group. These data suggest that the cerebral ischemic patients showed significant dysbiosis of the gut microbiota with enriched SCFA producer [78]. Similar evidence of stroke-induced gut dysbiosis can be found also in the studies done in animal model of stroke, where the gut microbiota and SCFAs mechanisms were tested. Middle cerebral artery occlusion ischemic animals were divided into different groups to investigate the effects of regulating the gut microbiota. Non absorbable antibiotics were tested, and supplementing the butyric acid, which was shown to have beneficial effects in neurodegenerative disorders, enhancing memory and restoring cognitive impairment [79,80]. The results confirmed the effect of butyric acid, which significantly reduced neurological impairment in cerebral ischemic rats, and enhanced gut microbiota diversity, reducing pathogenic bacteria. In conclusion, transplanting fecal bacteria rich in SCFAs and supplementing with butyric acid were found to be effective treatments for cerebral ischemic stroke [81]. Intestinal mucosal microbial communities were the subject of the study, analyzing and comparing across five gastrointestinal tract (GIT) sections: duodenum, jejunum, ileum, cecum and colon, between sham-operated and 24 h post-stroke mice. A significant difference of the microbial communities within the mucosa of GIT between sham-operated and post-stroke mice 24 h following surgery revealed that the mucosa of GIT induces robust changes to the intestinal mucosal microbiota [82].

2.11. Physical exercise

Exercise is indispensable for our daily life in order to maintain body–brain in a well-balanced function [130]. The plasticity of brain cognitive abilities is increased through daily exercise [131]. Also, the lack of exercise might be involved in several neurological diseases [132,133]. Exercise helps reducing depression [134], improves Alzheimer's disease patients decline [135], helps in epilepsy [136], anxiety [137] and other neurological diseases. Physical activity improved memory accompanied by neurogenesis and dendritic complexity in rodents, suggesting that these effects are related to synaptic plasticity [133].

Apparently, exercise induces changes in acetylation and methylation which are connected to the regulation of synaptic plasticity, learning and memory [138]. Regarding learning and memory, several studies have been conducted to understand the role of DNA methylation on neuronal function and long-term memory, reviewed in Fernandes et al., [130]. An increase in hippocampal levels of DNA methyltransferases was shown, as well as DNA methylation was regulated in the adult nervous system, playing an essential role in memory formation [139]. Physical exercise improves cognitive performance and the *BDNF* plays a main role by promoting neuroplasticity and synaptic transmission [68,140]. The increase of *BDNF* production induced by exercise, was investigated in two groups of mice, training/exercising and control/no-exercising group, showing that *BDNF* as well plays a role in engaging changes in DNA methylation and histone acetylation [84]. In line with the previous findings is DNA methylation from the acute to the chronic phase after stroke in the peri-infarct motor cortex, and the same region of the contralateral hemisphere, in an animal model of stroke. Animals had to perform task-specific training (TST) and TST combined with DNA methylation in chronic stroke recovery. An infusion, 5-Aza-2′-deoxycytidine (5-Aza-dC), was done on the contralateral cortex, with and without TST. Functional recovery, axonal plasticity and the expression of *BDNF* were determined. The reaching skill ability improved due to the TST and TST combined with 5-Aza-dC, suggesting a promising approach for the recovery. Furthermore, the modulation of DNA methylation in the contralesionally cortex might increase the *BDNF* and involve axonal plasticity [85]. Paretic versus non-paretic skeletal muscle *BDNF* in stroke patients was tested, hypothesizing that *BDNF* is involved in the skeletal muscle changes post-stroke. Epigenetic regulation of *BDNF* would bring beneficial effects through exercise, resistive training, or aerobic exercise training. Comparing DNA methylation profile for *BDNF* and *BDNF* antisense RNA between paretic vs non-paretic skeletal muscle, stroke hemiparesis reduces *BDNF* skeletal muscle expression. Aerobic exercise training modulates methylation for *BDNF* in paretic skeletal muscle [86]. Furthermore, three aerobic exercise sessions at three (low, moderate and high) different intensities showed that the greatest release of *BDNF* following the best improvement in cognition performance after exercise happened with the high intensity exercise suggesting that *BDNF* release, which improves cognitive performance, is due to exercise-induced increases in oxidative stress and cerebral blood flow [68]. In humans, in sedentary men and women, where they had to exercise on an electromagnetically braked cycle ergometer, lead to important changes in DNA methylation in adult skeletal muscle. The biopsies showed a decrease of the genome methylation in skeletal muscle [141].

A bout of physical exercise increases the GLUT4 protein in skeletal muscle in animals. GLUT4 transcription mediated by the MEF2 binding, after the exercise, showed hyperacetylation of histone H3 at the MEF2 site. After

physical exercise the increase of GLUT4 mRNA was around twofold [142]. Gene expression in skeletal muscle is altered by acute exercise, and how insulin sensitivity influenced gene expression induced by exercise was tested. Micro RNA isolated from muscle biopsy taken at rest and 30 min after the completion of exercise were performed in healthy subjects. Exercise significantly altered 215 miRNAs, suggesting that insulin sensitivity is involved and confirming that physical exercise alters skeletal muscle gene expression [143]. Also, microRNAs have an important role in brain plasticity and memory, and have effects on differentiation and degeneration, on neurogenesis and regulate long-term synaptic plasticity. MicroRNAs have a role in transcriptional regulation in the field of cancer [130,144]. Changes in the methylation status relate to various types of cancers, as cancer is somehow related to increased methylation. Notably, previous research has shown that long-term physical activity might reduce the risk of cancers [145]. CACNA2D3 methylation was higher/more frequent in patients with gastric carcinoma, who lack physical activity, compared with patients, who had done physical activity [146]. These statements are also valid for the healthy participants, so the global methylation is higher in participants who had less physical activity compared with participants with higher physical activity [147].

3. Conclusion

Different factors play a crucial role in brain recovery after stroke. Experimental data start now to elucidate some pathways involved in stroke damage and recovery. However, there are various links missing among studies *in vitro*, in animal models and human clinical findings. For example, many experimental studies do not explore the issues of timing and duration of stroke. Since the genesis and possible resolution of stroke is clearly multifactorial, we can only hypothesize some links, which however should be put in a complex framework of interrelationships among various actors. Nevertheless, some points are emerging. A healthy diet plays an important role in daily life activities, and in the prevention of ischemic stroke. Nowadays, it is not clear which is the best diet, in general a healthy diet composed of vegetables and fruits is advised. Furthermore, the combination of a healthy diet with physical activities has beneficial effects on stroke recovery. Participation in daily, even moderate, physical activity has been shown to be very helpful or rather better is indispensable for the maintenance of a body-brain in a well-balanced function. The brain's cognitive abilities increase through daily exercise, and memory improvement accompanied by neurogenesis has been related to synaptic plasticity, proving its importance.

4. Future Perspective

Stroke has a rising prevalence in aging population, with a significant burden in term of monetary and social costs. In the last two decades, several lines of evidence, from animal experiments to human studies, pointed to a role for epigenetic factors in the process of stroke recovery. Some factors acting on the risk of stroke and on the process of recovery may be targeted, at a general level (e.g. environmental pollution) or at an organism level (like hypoxia/glucose deprivation, oxidative stress and microbiome). Particularly relevant appears the role of physical exercise. Political decisions on healthcare system should focus on modifiable risk factors, like environmental pollution or diet, and improve communication about non-modifiable risk factors, like ethnicity, age and sex, in a combined effort to combat the appearance of stroke and improve its resolution.

Author contributions

O Rroji: conceptualization, writing-original draft, writing-review and editing. C Mucignat: conceptualization, writing-original draft, writing-review and editing. Authors have equally contributed to the manuscript.

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The authors have no other competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript apart from those disclosed.

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