The factors affecting neurogenesis after stroke and the role of acupuncture

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Stroke induces a state of neuroplasticity in the central nervous system, which can
lead to neurogenesis phenomena such as axonal growth and synapse formation, thus
affecting stroke outcomes. The brain has a limited ability to repair ischemic damage
and requires a favorable microenvironment. Acupuncture is considered a feasible
and effective neural regulation strategy to improve functional recovery following
stroke *via* the benign modulation of neuroplasticity. Therefore, we summarized
the current research progress on the key factors and signaling pathways affecting
neurogenesis, and we also briefly reviewed the research progress of acupuncture
to improve functional recovery after stroke by promoting neurogenesis. This study
aims to provide new therapeutic perspectives and strategies for the recovery of motor
function after stroke based on neurogenesis.

KEYWORDS

stroke, neurogenesis, axon growth, NGF, Rho A, slit, acupuncture

1. Introduction

Despite considerable efforts over the last decades, stroke remains the leading cause of death
and disability worldwide, placing a severe economic burden on countries (1, 2). Functional
recovery following a stroke is exceptionally limited, leaving the affected individual with life-long
neurological deficits (3). This lack of functional recovery can at least in part be attributed to the
restriction of neurogenesis (4, 5).

Neuroplasticity is a native ability of the brain to adapt to individual developmental growth.
The historical view of the central nervous system (CNS) as a static organ has shifted in recent
years (6). We now realized that CNS remains plastic and has some regeneration capacity to
rebuild neural circuits after acute injuries, such as stroke (6, 7). Stroke induces a state of
neuroplasticity. This period of enhanced plasticity provides an opportunity for neurogenesis,
such as the sprouting of new axons, the formation of new synapses, and the remapping of
sensorimotor functions, which is associated with motor recovery (8). The reconstruction of
neural circuits is generated in the sensorimotor cortex, thalamus, brain stem, and spinal cord
(9, 10).

The compensatory repair capacity of the brain for ischemic injury is limited and a favorable
microenvironment is needed (11). After a stroke, the reconstruction of neural circuits is
restricted by the presence of many inhibitory factors that inhibit neurogenesis in the local
microenvironment, the lack of growth factors, as well as the formation of glial scars in the injured
area (12). An enriched microenvironment exerts a significant influence on neurogenesis (13, 14).
As such, improving the microenvironment of the injured site, thus promoting neurogenesis and
the reconstruction of neural circuits, has been the focus of intense research.

Acupuncture is a form of physical stimulation therapy that originated in traditional Chinese medicine. Needles are inserted into the skin or deep tissues at specific locations (acupoints) on the body, and stimulation is enhanced by specific needling techniques or by electricity to restore body balance, prevent, and treat disease (15). Modern neuroanatomical evidence has demonstrated that there are abundant nerve endings in the acupuncture meridian and acupoint areas of the body, while the achievement of therapeutic effects of acupuncture mainly depends on the nervous system (16, 17). A growing number of clinical studies have shown that acupuncture can effectively improve recovery from stroke (18, 19). The mechanism of acupuncture effects may be related to neuroplasticity.

Here, we summarized our current understanding of the key factors and signaling pathways that affect CNS neurogenesis. Meanwhile, we briefly overviewed the research progress of acupuncture in improving motor function after stroke by accelerating neurogenesis. We hope to emphasize that neurogenesis could be modulated by potential strategies to improve functional outcomes after stroke and explore the feasibility of acupuncture in promoting motor function recovery after stroke. The aim is to provide new therapeutic opportunities for post-stroke motor rehabilitation based on neurogenesis.

2. Enhancing factors of neurogenesis

Neurogenesis is the process through which neural stem cells (NSCs), or more generally neural progenitor cells (NPCs), generate new neurons (20). The adult CNS contains NSCs that can continuously generate neurons, astrocytes, and oligodendrocytes (21, 22). Under normal conditions, the NPCs are in a quiescent state in the adult brain. After an ischemic injury, NPCs proliferate, differentiate, and migrate to the ischemic region to replenish neurons in the damaged area, release the anti-inflammatory cytokines to limit the deleterious inflammatory environment, and form the new

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Abbreviations: AAV, adeno-associated virus; ADSCs, adipose-derived stem cells; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BDNF, brain-derived neurotrophic factor; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CASP3, caspase-3; CNS, central nervous system; CSPGs, chondroitin sulfate proteoglycans; DCC, colorectal cancer; DAG, diacylglycerol; EA, electroacupuncture; Eph, erythropoietin-producing hepatocellular; ERK, extracellular signal-regulated kinase; Shroom3, F-actin-binding protein; GFRα, GDNF family receptor alpha; GDNF, glial cell lineage-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; GV, Governor Vessel meridian; GAP43, growth-associated protein-43; IP3, inositol triphosphate; IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; MA, manual acupuncture; miRs, MicroRNAs; MAPK, mitogen-activated protein kinase; MAG, myelin-associated glycoprotein; NGF, nerve growth factor; NPCs, neural progenitor stem cells; NSCs, neural stem cells; NeuN, neuronal nucleus; NT-3/4, neurotrophin-3/4; NMDAR1, N-methyl-D-aspartate receptor 1; NgR, Nogo-A receptor; OMgp, oligodendrocyte-myelin glycoprotein; p75NTR, p75 neurotrophin receptor; Pir B, paired immunoglobulin-like receptor B; PIP2, phosphatidylinositol 4,5-bisphosphate; PI3K, phosphoinositide-3 kinase; PLC-γ, phospholipase C-γ; POSH, plenty of Src homology 3 domains; PSD-95, postsynaptic density protein 95; Akt, protein kinase B; Robo, Roundabout; Shc, Src homologous and collagen; SVZ, subventricular zone; Trk, tyrosine kinase; UNC5, uncoordinated-5.

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neuronal connections to promote the recovery of nerve function and resist ischemic injury (23–25). Neurogenesis in the brain of adult mammals has been clearly demonstrated that mainly occurs in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (21, 22, 26). After the cerebral ischemic injury, multiple growth factors and a variety of proteins are increasingly expressed to promote the proliferation of NSCs or NPCs, and thus facilitate neurogenesis, which includes the production of new neurons, glia, axons, myelin sheaths, or synapses (6, 22, 27).

2.1. Growth factors

Growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell lineage-derived neurotrophic factor (GDNF), neurotrophin-3/4 (NT-3/4), and insulin-like growth factor 1 (IGF-1) play an important role in neuronal survival, differentiation as well as axonal regeneration of damaged CNS neurons (28–30). Their downstream signaling cascades, mainly including phospholipase C-γ (PLC-γ), phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathways, have also been involved in promoting neurogenesis after stroke (31). The activation of MAPK/extracellular signal-regulated kinase (ERK) pathway starts from RAS. The activated RAS protein recruits downstream RAF proteins located in the cytoplasm and further activates downstream MEK through its CR3 structural domain at the C-terminus, which in turn activates MAPK and ERK. ERK phosphorylates and activates the downstream transcription factor CREB to regulate the expression of target genes that contribute to neuronal differentiation and survival (32). PI3K plays an important role in regulating cell survival, axon growth, and cytoskeleton remodeling by recruiting protein kinase B (Akt) to activate mammalian targets of rapamycin (mTOR), and phosphorylation and inhibiting apoptosis-promoting proteins such as Bad and GSK3β (33, 34). PLC-γ is activated to convert extracellular stimuli into intracellular signals by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PIP2) to generate second messengers inositol triphosphate (IP3) and diacylglycerol (DAG). DAG activates PKC, while IP3 induces calcium release from intracellular calcium depots (35, 36) (Figure 1).

2.1.1. NGF

NGF is present in tissues mainly in the form of precursors and promotes the survival and differentiation of neurons in the nervous system (37). TrkA is a specific receptor for NGF (38). Activation of TrkA leads to the phosphorylation of tyrosine residues in its structural domain, which recruits signaling molecules and activates multiple signaling pathways including PLC-γ, MAPK, and PI3K (38, 39). Among them, phosphorylation of Y490 and Y785 is the most common (40). Phosphorylated Y490 recruits Src homologous and collagen (Shc) to activate MAPK and PI3K pathways, while phosphorylated Y785 recruits the PLC-γ1 pathway (41, 42).

It has been demonstrated that NGF can protect sensory neurons and promote neurogenesis in the damaged area after stroke (43). On the one hand, NGF could increase the survival of NPCs through the activation of TrkA, in turn, induces axonal outgrowth (the elaboration of axonal filopodia and branches) and myelination (38,
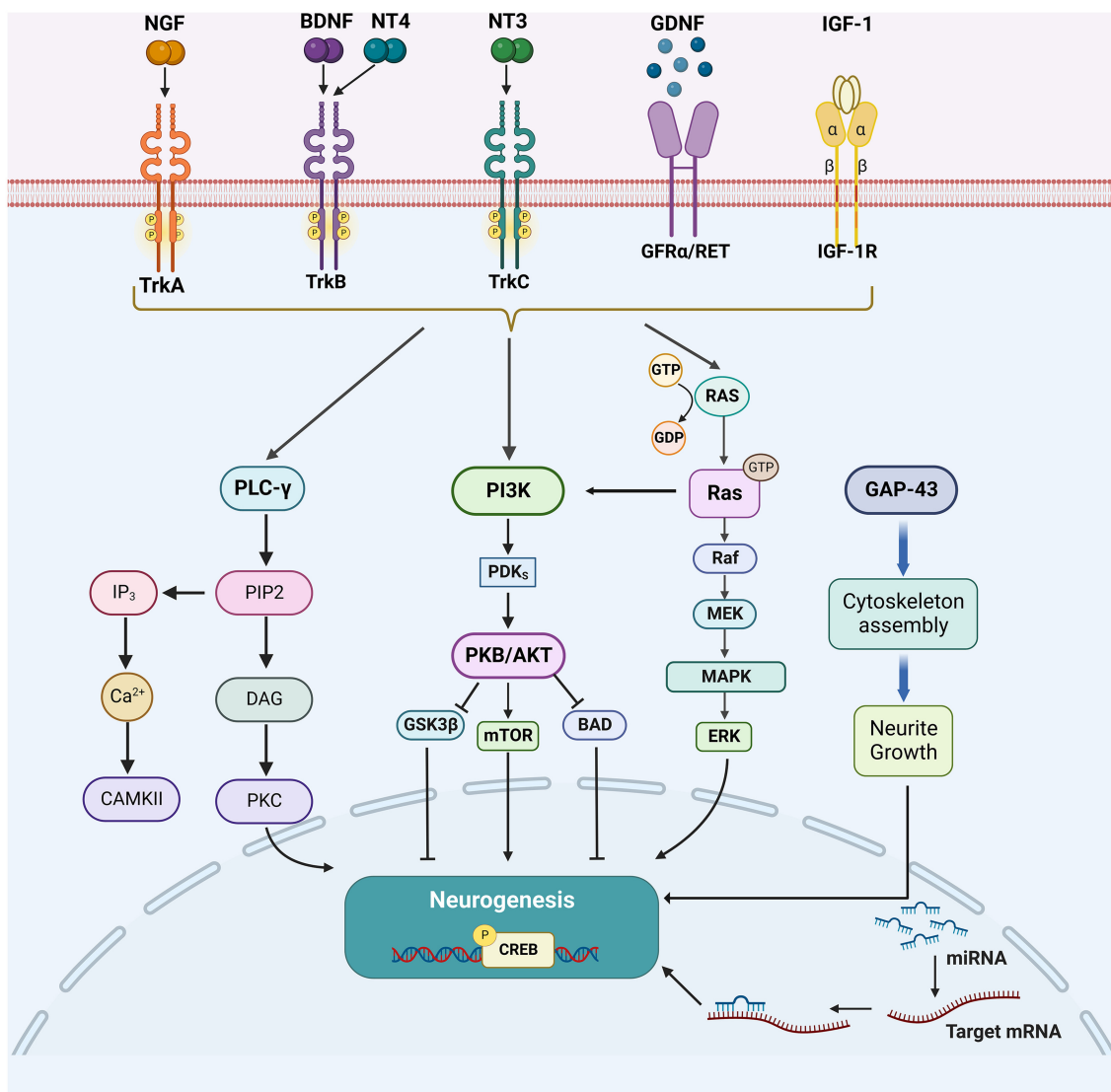
FIGURE 1
The enhancing factors of neurogenesis and their major downstream signaling pathways. After the cerebral ischemic injury, multiple growth factors (NGF, BDNF, NT3/4, and GDNF), other growth proteins (GAP-43 and IGF-1), and miRNAs could promote neurogenesis. Their downstream signaling cascades, mainly include PLC-γ/PKC, PI3K/Akt, MAPK/ERK, and Wnt/β-catenin pathways.

39). On the other hand, for the survival of newly generated neurons after stroke, the NGF is also involved in angiogenesis after acute stroke in rats (44). It demonstrates that NGF is involved in multiple processes in neurogenesis after stroke.

2.1.2. BDNF

BDNF is the most abundant and most studied neurotrophic factor (NTF) in the brain (29). BDNF can activate three signaling proteins, MAPKs, PI3Ks, and PLC-γ, by interacting with tropomyosin receptor TrkB (45). It has also been found that BDNF promotes neurogenesis by activating the JAK/STAT pathway in Schwann cells (46).

A study has shown that BDNF has a neuroprotective effect on hippocampal neuronal damage caused by hypoxic and glucose-deficient conditions (47). BDNF not only has an important role in promoting neuronal survival but also promotes neurogenesis after

ischemic stroke (48). Therefore, modulation of BDNF expression is promising for neurogenesis and protrusion generation in damaged areas after stroke.

2.1.3. Neurotrophic factors

Multiple NTFs act together to promote axonal growth during neurological maturation (49). Among them, NT3 and NT4 have the most potential for neurogenesis after stroke (50). NT3 binds mainly to TrkC receptors specifically and NT4 binds mainly to TrkB specifically (51). NT3 is a neurotrophic factor that plays an important role in preventing the death of damaged neurons, enhancing neuronal survival and axonal regeneration, and inducing the differentiation of endogenous oligodendrocyte precursor cells into mature oligodendrocytes to restore myelin (52). Studies have shown that NT3 can be transported from muscle to sensory ganglia and spinal motor neurons in nerve, as well as to the CNS through

the bloodstream (30, 49, 53, 54). NT3 can promote axonal growth and synaptic plasticity in various locomotor pathways including the corticospinal tract and proprioceptive pathways and can induce axonal growth from the intact corticospinal tract across the midline to the innervated side (30, 49). In addition, Ann et al. have demonstrated that NTF synergistic neuronal responses after a combination of basic fibroblast growth factor, NT3 and BDNF delivered to the somata of retinal ganglion cells promoted greater survival and axon growth than did the sum of the effects of each NTF alone (55).

Regarding NT4, it has been shown that stroke mice knocking out the NT4 gene exhibit larger infarct foci, suggesting that NT4 can counteract ischemic brain injury (56). Another study has also shown that post-stroke neurological recovery from exercise is closely related to NT4 (57).

2.1.4. GDNF

Among the many endogenous regulatory molecules, GDNF is particularly notable as it is produced by glial cells and neurons and is a member of the transforming growth factor β superfamily, which plays an important role in neuronal differentiation during normal development (58). GDNF family ligands bind to specific GDNF family receptor alpha (GFRα), all these form receptor complexes and signal through the RET receptor tyrosine kinase and activate downstream PLC-γ, MAPK and PI3K/Akt signaling pathways (59, 60).

GDNF can promote the survival and recovery of several types of mature neurons after CNS injury. The RET receptor induces calcium (Ca^{2+}) signaling and regulates neocortical NPCs migration through the PLC-γ binding domain Tyr1015 (60). In one study, a fusion protein, PEP-1-GDNF, was injected intravenously into rats with stroke, and GDNF was found to significantly reduce infarct size, promote proliferation and differentiation of hippocampal dentate gyrus cells, and improve behavioral function (61). Beker et al. (62) demonstrated that GDNF is effective in inducing long-term neural recovery, peri-infarct brain remodeling, and contralateral neuroplasticity. In addition, activation of GDNF pathways may enhance hippocampal neurogenesis and thus promote neuronal survival (63). Therefore, GDNF is an important target for regulating neurogenesis after cerebral ischemia.

2.1.5. Other growth factors

Growth-associated protein-43 (GAP-43) is the main protein of the axonal growth cone that promotes axonal sprouting during the development and regeneration of the nervous system (64, 65). It promotes the accumulation of F-actin in neural protrusions and contributes to the formation of the cytoskeleton (66). It also promotes the release of presynaptic membrane neurotransmitters, cytokinesis, and circulation of synaptic vesicles, promotes synapse formation, stimulates axon outgrowth and extension, inhibits axon necrosis and growth cone retraction, promotes oligodendrocyte and astrocyte differentiation, and thus promotes neurogenesis (64). The axonal sprouting process is accompanied by high expression of GAP-43 after ischemic stroke (67). GAP-43 in neurons is a substrate for caspase-3 (CASP3) (68), a protease involved not only in apoptosis but also in fine-tuning the formation of new synaptic contacts (69). It was found that GAP-43 and CASP3 are involved in the neurogenesis of lesions after ischemic stroke (70).

Insulin-like growth factor-1 (IGF-1) is a growth factor primarily produced by the liver in adults and plays a crucial role in cell proliferation, maturation, and survival (71). In addition, it has important effects on early CNS development and neuronal plasticity (72). In the CNS, IGF-1 exerts its action by binding to its receptor (IGF1R), a membrane-bound glycoprotein composed of two alpha and two β subunits (73, 74). Once IGF-1 binds to IGF1R, the tyrosine kinase structural domain on the β subunits activates the PI3K/Akt1/mTOR and MAPK/ERK pathways to induce their downstream effects (75, 76). Adeno-associated virus (AAV)-mediated IGF-1 overexpression was found to promote long-term functional recovery in mice with focal ischemia by promoting neovascularization and neurogenesis (77). IGF-1 was also found to regulate the survival and migration of bone marrow mesenchymal stem cells in an ischemic environment and improve neurological recovery after ischemic stroke (28). It suggests that IGF-1 may be a safe and potentially effective treatment for a variety of CNS disorders including ischemic stroke.

2.2. MicroRNAs

MicroRNAs (miRs) are a family of 20–25 non-coding RNAs that play an important role in the pathogenesis of ischemic stroke and are important factors in the regulation of axonal growth in neurons (78). The main miRs that promote neurogenesis are miR-133b/30b/132/124/146 (79–83), etc. These molecules can affect a variety of biological processes related to post-stroke axonal growth and synaptic function regulation by targeting hundreds of proteins in a range of cellular and molecular targets and multiple regulatory networks.

Different miRNAs regulate neurogenesis differently. For example, miR-133b promotes neurogenesis by activating MAPK/ERK1/2 and PI3K/Akt signaling pathways (79). The miR-146 family (miR-146a and miR-146b) could promote the differentiation of NSCs into neurons by regulating the Notch1 signaling pathway (83). MiR-30b promotes axon outgrowth of retinal ganglion cells by inhibiting Sema3A-mediated caspase-3 and p38MAPK signaling pathways (80). In addition, miR-124 can activate the Wnt/ß-catenin pathway by targeting DACT1 to promote NSCs proliferation and differentiation to neurons (82). It has also been shown that miR-124 can reduce glial scar formation in M2 microglia and promote neurogenesis in mice after stroke through STAT3 signaling (84). Therefore, miRs may serve as innovative gene therapy candidates for neurogenesis (85).

3. Inhibiting factors of neurogenesis

The limited ability to neurogenesis after cerebral ischemia is mainly due to ischemic injury-producing factors that inhibit neurogenesis. The microenvironment within the nervous system is critical for the survival and regeneration of damaged nerves. To some extent, inhibitory factors are thought to possibly play a more important role.

Locally to the injury, the formation of hard glial scarring by glial cells prevents nascent axons from crossing (86). Most of these inhibitory molecules induce the activation of RhoA/ROCK pathway in neurons (87). RhoA/ROCK is one of the most widely studied signaling pathways and is primarily responsible for regulating

cytoskeleton organization, cell growth, cell migration, proliferation, and development (87). Inhibition of this pathway can promote axon growth and behavioral recovery in rats after stroke (87, 88).

3.1. Myelin proteins

There is a consensus that myelin is a major barrier to inhibiting neurogenesis (87). The myelin sheath of PNS appears to be removed faster and more effectively than that of CNS, mainly due to differences in the surrounding microenvironment (89, 90). The myelin sheath of PNS is Schwann cell, which can produce more neurotrophic factors and promote the growth of PNS axons (91). However, the myelin sheath of CNS is oligodendrocyte, which produces more neural growth inhibitory factors and is not conducive to the regeneration of CNS axons (92) (Figure 2). Some factors that inhibit axonal growth such as Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp) are mainly expressed in CNS myelin (93).

3.1.1. Nogo-A

NOGO-protein family Nogo, especially Nogo-A plays a key role in CNS neurogenesis. Nogo-A and its receptors are widely present in the CNS of mammalian, and they are strongly associated with axonal growth inhibition and neuronal damage caused by ischemic brain injury (94, 95). It was found that spontaneous axonal plasticity and functional recovery after stroke may be limited by Nogo-A (96). Studies have shown that Nogo-A is expressed in several regions of the nervous system to varying degrees after cerebral ischemic injury, and it can inhibit the structural and functional recovery of the corticospinal tract to a certain extent, while the recovery of neurological function can be effectively promoted by antagonizing Nogo-A (95, 97–99).

One of the mechanisms by which Nogo-A exerts these effects is that Nogo-A binds to its receptor (NgR) complex. Nogo-66 stimulates the receptor complex composed of NgR1 and its related proteins LINGO and p75 neurotrophin receptor (p75^{NTR}) or TROY to activate the downstream Rho A/ROCK actin disruptor cofilin (a major effector of growth cone cytoskeleton disassembly) signaling pathway, preventing actin cytoskeleton aggregation in the growth cone, eventually leading to collapse of growth cones and inhibition of axonal growth (100, 101). The study further confirmed that inhibition of Nogo-A/NgR1 expression at the gene level or antagonism of its function at the protein level could reduce Rho A/ROCK signaling pathway activation and promote neurological recovery in post-stroke animals (97, 102). The same receptor complex and downstream mechanisms appear to be involved in the growth-inhibitory effects of other myelin-associated proteins, such as MAG and OMgp (103). NgR is mainly expressed in neuronal cytostomes and axons in the cerebral cortex, hippocampus, and dorsal root ganglia, as well as in activated microglia/macrophages in the CNS (101). There are three isoforms of NgR, namely NgR1, NgR2, and NgR3, among which NgR1 is the first receptor with high affinity to Nogo-66 in the extracellular segment of Nogo-A (104). The results of existing studies demonstrate that this receptor and its complex have a more direct effect on axon growth (105, 106). Neurogenesis was effectively promoted by inhibiting the expression of NgR1.TAT-NEP1–40, an

antagonist of NgR1, can protect neurons and promote the recovery of neurological functions after stroke (107, 108).

Another mechanism by which Nogo-A exerts axonal growth inhibition is through binding to paired immunoglobulin-like receptor B (Pir B), which affects multiple protein functions involved in microfilament depolymerization and restriction of axon growth *via* downstream plenty of Src homology 3 domains (POSH) signaling molecules (109, 110). It has been shown that POSH formed an inhibitory complex by binding to F-actin-binding protein (Shroom3), which activated the POSH/Shroom3/ROCK signaling pathway, leading to a decrease in Myosin II expression and inhibition of axonal growth (111, 112). The study confirmed that the knockdown of Pir B caused more axon regeneration than the knockdown of NgR1, suggesting that Pir B plays a more important role in myelin inhibition (113). By antagonizing the action of Nogo-A and Pir B, it could inhibit POSH expression and suppress the activity of downstream molecules Shroom3/ROCK/Rho A, which effectively reverses the inhibition of its axonal growth (111, 114, 115). These results suggest that the Nogo-A/Pir B signaling pathway has an important role in axonal growth inhibition due to cerebral ischemia injury.
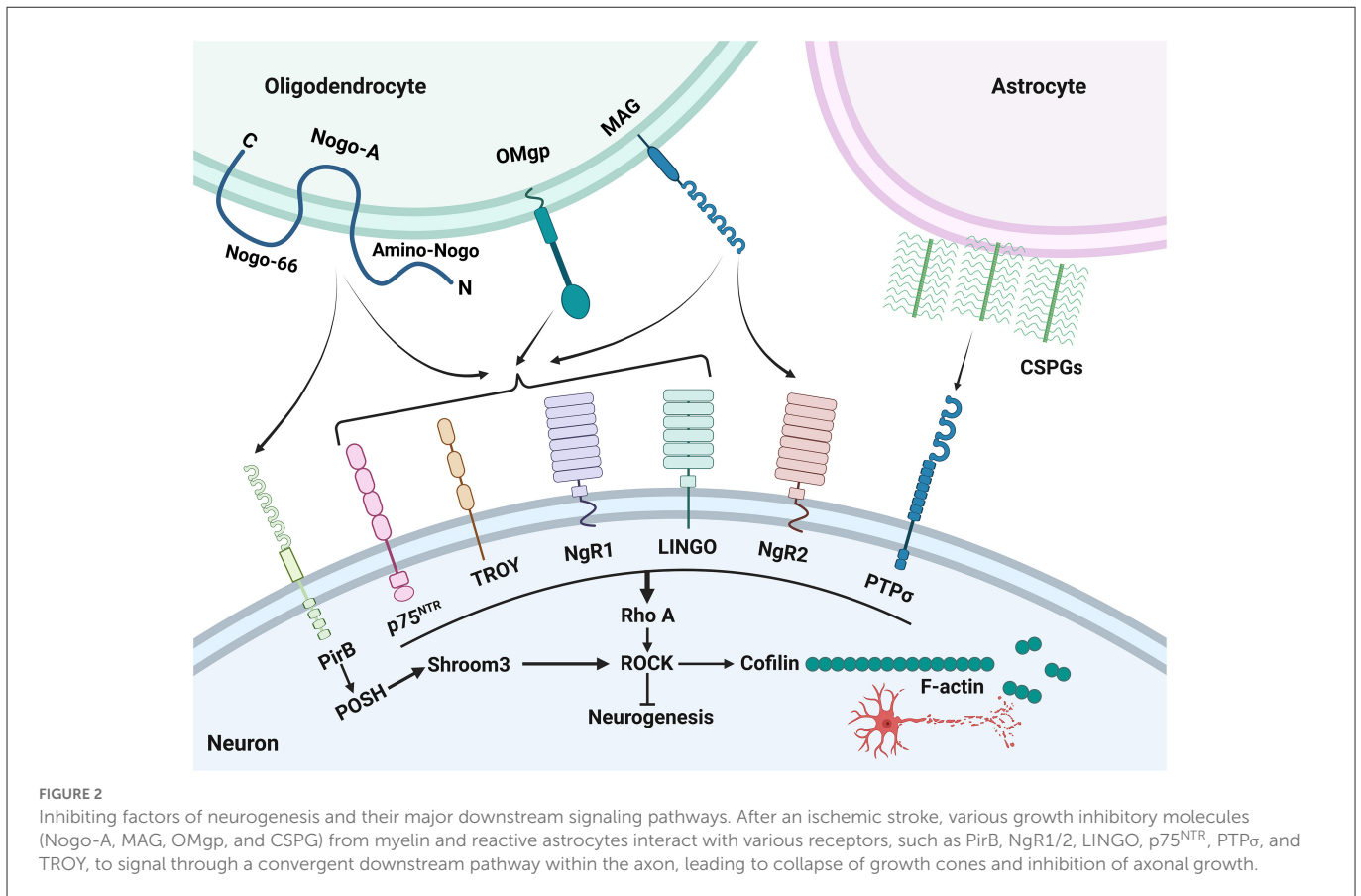
3.1.2. MAG and OMgp

MAG is a member of the immunoglobulin superfamily. MAG is present in the preaxial membrane and unmyelinated regions of the CNS and PNS and is therefore well suited to interact with axonal receptors. It is both a ligand for axonal receptors required for the maintenance of myelinated axons and a receptor for axonal signals that promote oligodendrocyte differentiation, maintenance, and survival (116). Peripheral injection of a mouse monoclonal antibody against MAG resulted in significant preferential motor reinnervation in mice after transection of the femoral nerve, suggesting that interference with the rejection function of MAG facilitates the reinnervation of motor neurons to the correct pathway (117). It was also found that MAG levels could be reduced after MCAO, therefore mitigating axonal injury and improving neurological function in adult mice after cerebral ischemia (118).

OMgp is expressed not only through oligodendrocytes but also at high levels in various neurons. OMgp is the protein responsible for myelin partial inhibition, inducing growth cone collapse and inhibiting neurogenesis (119). Both MAG and OMgp interact with NGR with approximately the same relatively high affinity (120). There are relatively few studies on the effect of OMgp on axonal growth after stroke compared to Nogo-A and MAG.

3.2. Glial scar

Glial scar formation and altered astrocyte function are important pathological features of ischemic stroke. After a stroke, astrocytes proliferate reactively and later form a physical barrier of glial scarring with microglia, macrophages, and extracellular matrix (86). Glial fibrillary acidic protein (GFAP) is a characteristic marker of astrocyte activation and glial proliferation and constitutes a major component of the glial scar (121). The study has found that GFAP-positive reactive astrocytes significantly increased in the cortical infarct zone after ischemic stroke, resulting in enhanced expression of chondroitin sulfate proteoglycans (CSPGs) and formation of glial scar (86).

FIGURE 2
Inhibiting factors of neurogenesis and their major downstream signaling pathways. After an ischemic stroke, various growth inhibitory molecules (Nogo-A, MAG, OMgp, and CSPG) from myelin and reactive astrocytes interact with various receptors, such as PirB, NgR1/2, LINGO, p75^{NTR}, PTPσ, and TROY, to signal through a convergent downstream pathway within the axon, leading to collapse of growth cones and inhibition of axonal growth.
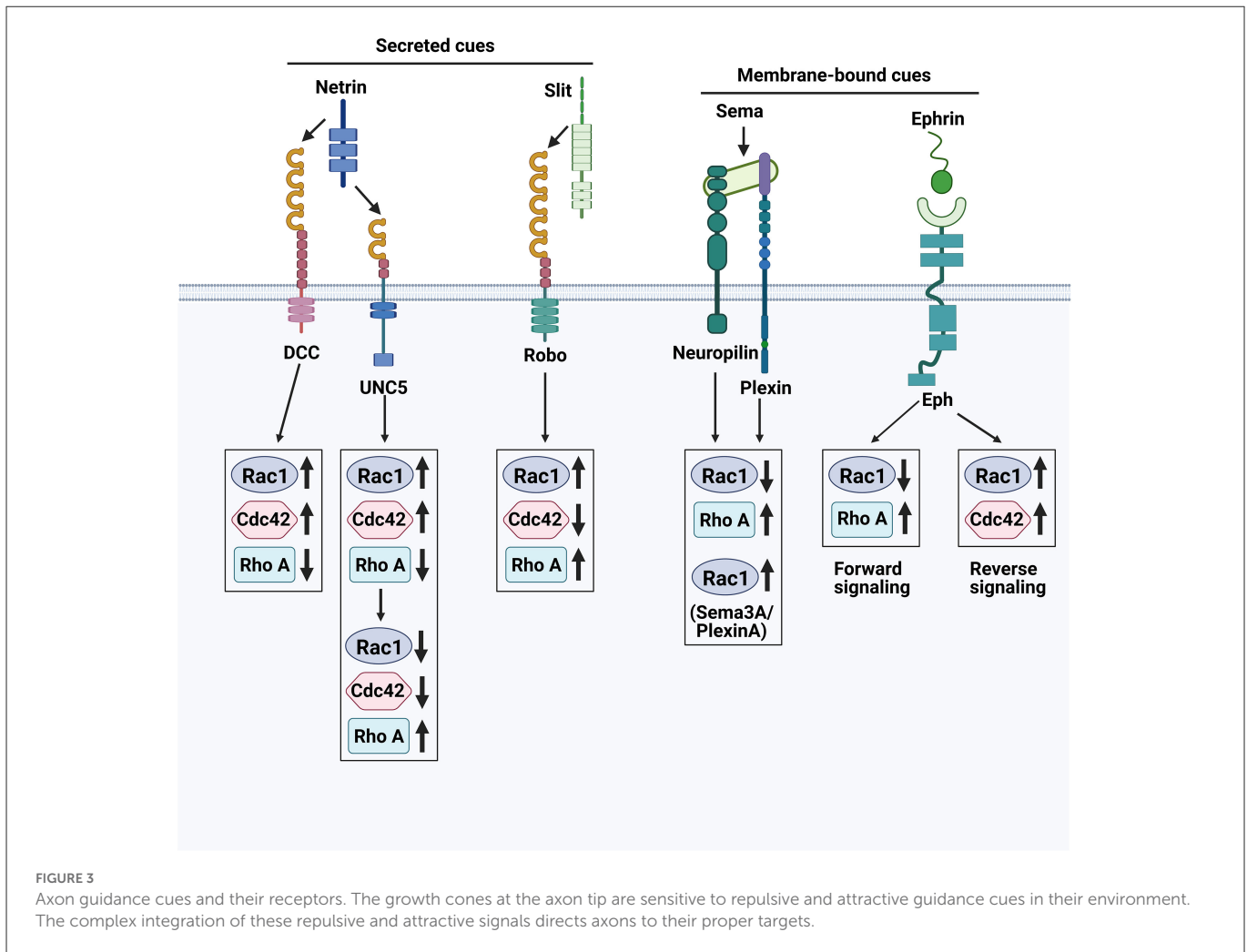
Glial scar is two-sided in nature. When an ischemic stroke occurs, the dense glial scar can isolate the area of injury from the surrounding tissues and impede the diffusion of large amounts of neurotoxic substances released from the infarcted area to the peripheral areas, thus effectively controlling further tissue infection, maintaining extracellular ion and fluid homeostasis, preventing overwhelming inflammatory and growth factor responses, and scavenging free radicals (122). However, in late cerebral ischemia, astrocytes increase in number, cytosolic hypertrophy, and protrusions increase and lengthen, creating a physical barrier in space that not only prevents reconnection between neurons but also works in concert with myelin-associated inhibitory factors to impede regeneration of injured axons, thus preventing recovery of CNS function in the chronic phase of ischemic stroke (86, 123). Astrocytes are able to upregulate several neuroinhibitory factors such as CSPGs, tyrosine-protein kinase-B2, and Slit protein C, impeding neuronal axon extension and synaptic regeneration, which is detrimental to the re-establishment of neural network structure and recovery of neurological function (123, 124).

It has been shown that CSPGs are the most important component of the glial scar that hinders regeneration after CNS injury, and their combination alone or with other extracellular matrix causes axonal extension toward the site of injury to stop at the glial scar, and that CSPGs reduce the plasticity of axonal growth (125). CSPG acts as an axon growth inhibitor by binding to PTPσ receptors to activate the downstream Rho A/ROCK pathway (12). Another study found that after nerve injury, the regeneration of injured axons and partial recovery of function was effectively promoted by eliminating

CSPGs in the brain and spinal cord (125, 126). The above information suggests that reducing CSPGs has an important role in the recovery of motor function after a stroke.

4. Axon guidance cues

Species with bilateral symmetry possess a midline axis, a feature that becomes very important in vertebrates, especially humans (127). Newborn neurons have to decide whether or not to cross the midline or toward which direction they should send their axons to Flanagan and Van Vactor (128). Ramon y Cajal, the "father of neuroscience," observed a very irregular structure at the distal end of the axon, which he called the "growth cone." The growth cone is a very active structure composed mainly of cytoskeletal elements with high dynamics (e.g., microtubules, actin, and microfilaments) and numerous other proteins (129, 130). The growth cone is often equipped with one or more receptors to enable an appropriate response to axon guidance molecules that give developing neurons navigation to connect with distant targets (131, 132). In recent years, scientists have progressively confirmed the above theories and finally identified the classical guiding cues that give directionality to navigating axons: Netrins, Slits, Semaphorins (Sema), and Ephrins as well as their cognate receptors: deleted in colorectal cancer (DCC) and uncoordinated-5 (UNC5), Roundabout (Robo), Plexin and Neuropilin, and erythropoietin-producing hepatocellular (Eph), respectively (133, 134). Axon guidance molecules play a key role

FIGURE 3
Axon guidance cues and their receptors. The growth cones at the axon tip are sensitive to repulsive and attractive guidance cues in their environment. The complex integration of these repulsive and attractive signals directs axons to their proper targets.

in the development of the nervous system and can regulate the regenerative capacity of neurons during neurological diseases.

The effects of Netrin on nerve axons are mainly manifested by inducing the migration of growth cones, orienting the axons, promoting the growth of growth cones, and prolonging the axons (135). Four types of Netrins were found in animal spinal cords: Netrin-1, Netrin-2, Netrin 3, and Netrin-4. Netrin-1 has both repulsive and attractive receptors. The UNC-5 homolog is a repulsive receptor that mediates the rejection of axons by Netrin-1 and inhibits the growth and extension of sensory nerve fibers; DCC is an attractive receptor that mediates the attraction of axons by Netrin-1 and promotes the growth and extension of sensory nerve fibers (135).

Slit directs the targeted growth of the growth cone through a concentration gradient (136). It is more sensitive to the action of peripheral protrusions of sensory neurons than to the action of central protrusions (137). Robo is the primary receptor for Slits and the Slit/Robo signaling pathway is one of the most important regulatory pathways for axon guidance, which is formed by the binding of Slit and Robo receptors (138). It mediates axonal rejection, neurogenesis, and migration during the development of the CNS and peripheral nervous system (138, 139).

Sema mediates axon guidance through chemical repulsion, and its receptors are mainly Neuropilins and plexins (140). Sema3A binds to its receptor Neuropilins-1, which not only reverses the direction of

axonal growth, but also prevents the formation of axonal terminals and inhibits axonal extension (141).

The Eph receptor ligand is Ephrin. The Eph receptor and its ligand Ephrin are collectively known as the Eph family proteins (142). Ephrin- Eph signaling can regulate neuronal plasticity (143). Ephrin-A5 and EphA5 regulate the projection and location of nerve fibers (144). Ephrin-A5 was found to be induced in reactive astrocytes in the peri-infarct cortex and is an inhibitor of axonal sprouting and motor recovery in stroke patients (143).

Rho GTPases are known for their role in the regulation of cell motility and cytoskeletal structure, with the most frequently studied members being mainly RhoA, Rac1 and Cdc42 (145). Studies have shown that activation of RhoA, Rac1, and Cdc42 can lead to the formation of different actin-based structures—respectively stress fibers, lamellipodia and filopodia (146). Different axon guidance cues can regulate axon regeneration by modulating these three molecules in response to changes in the microenvironment, as shown in Figure 3.

5. Acupuncture for post-stroke neurogenesis

Above summary of the factors affecting neurogenesis after stroke clearly demonstrates the complex response of the organism in the

process of neurogenesis after injury. Targeting only one single factor for activation or inhibition thereby often results in an overkill situation and causes side effects (147, 148). From the perspective of treatment, there is an urgent need for stroke neuromodulation therapy without side effects, especially ones that can improve functional recovery by regulating the own regulatory mechanisms and facilitating brain repair of the body. Although as a traditional physical stimulation therapy, acupuncture is integral to modern medical practice and is considered a feasible and effective neural regulation strategy to improve functional recovery following stroke *via* the benign modulation of neuroplasticity (18, 19, 149). The research regarding the mechanism of effects of acupuncture on neurogenesis or axonal growth is accumulating (Table 1).

5.1. Acupuncture for enhancing factors

Electroacupuncture (EA) can improve the symptoms of neurological deficits and promote the recovery of motor function in post-stroke rats. The mechanism may be related to the upregulation of BDNF by EA at *Neiguan* (PC6) and *Zusanli* (ST36) to promote neuronal growth, and the downregulation of Sema3A and NRP-1 to reduce the inhibitory effect on axonal regeneration (150) (Figures 4A, B). Studies have shown that EA at *Baihui* (GV20) and *Dazhui* (GV14) significantly improved functional recovery by enhancement of proliferation and differentiation of NSCs *via* upregulating the BDNF and TrkB expression (151, 152). In addition, EA may promote synaptic plasticity after stroke by protecting and improving synaptic ultrastructure in the rat ischemic cerebral cortex and increasing the expression of synaptophysin P38, GAP-43, NGF and BDNF (153). Manual acupuncture (MA) at *Taixi* (KI3) and *Taichong* (LR3) can promote functional recovery as well as learning and memory abilities after ischemic stroke by enhancing BDNF and SYN expression and synaptic structural reconstruction in the ipsilateral hippocampus after I/R (154). EA on trigeminal innervation points [GV20 and *Yintang* (GV24$^+$)] is an effective therapy for poststroke cognitive impairment and is associated with neuroprotection and synaptic plasticity-mediated in relevant brain regions in the MCAO rat model (155). EA reversed I/R injury-induced BDNF, TrkB, N-methyl-D-aspartate receptor 1 (NMDAR1), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR), γ-aminobutyric acid type A receptor, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), neuronal nucleus (NeuN) and postsynaptic density protein 95 (PSD-95) expressed in the prefrontal cortex and hippocampus.

It was also found that EA at GV20 and *Shuigou* (GV26) enhanced the permeability of the blood-brain barrier in the prefrontal cortex and induced the uptake of NGF by prefrontal neurons (156). Nape cluster acupuncture has neuroprotective and restorative effects in rats with post-ischemic stroke sequelae, and its mechanism may involve effective upregulation of BDNF and NGF protein expression (157). EA treatment at *Quchi* (LI11) and ST36 acupoints exerted neuroprotective effects on I/R-injured rats through the proliferation of GFAP/vimentin/nestin-positive reactive astrocytes and secretion of potentially reactive astrocyte-derived BDNF and may also be related to the activation of cortical PGC-1α/Irisin (FNDC5)/BDNF pathway by electroacupuncture (158, 159). In addition, EA ST36 and LI11 could also increase the expression of PI3K, p-Akt, p-Bad, Bcl-2, BDNF, and GDNF, which exerted neuroprotective effects by activating the PI3K/Akt pathway (160, 161) (Figure 4A).

EA at *Jinsuo* (GV8) and *Fengfu* (GV16) caused proliferating endogenous NSCs to migrate from the SVZ region to the damaged area of the striatum in rats' post-stroke, thereby promoting neurogenesis in the brain striatum (162). EA promotes the proliferation and differentiation of endogenous NSCs by upregulating the Wnt/β-linked protein signaling pathway and the secretion of neurotrophic factors, thereby ameliorating neurological defects and producing a therapeutic effect on cerebral ischemia (163, 164) (Figure 4A).

EA at LI11 and ST36 can promote endogenous NSCs differentiation *via* exosome-mediated miR-146b, thereby improving neurological dysfunction after ischemic stroke (165). EA at GV20 enhances neurobehavioral functional recovery against ischemic stroke *via* targeting of SOX2-mediated axonal regeneration by miR-132 (166). EA at GV26 and GV20 could activate the HIF-1α/VEGF/Notch 1 signal pathway to facilitate angiogenesis after ischemic stroke *via* exosomal miR-210 (167).

5.2. Acupuncture for inhibiting factors

The protective effect of EA at GV14 and GV20 on hypertensive I/R cerebral injury rats might be closely related to down-regulating central nervous system myelin growth inhibit ion mediated factors Nogo-A receptor NgR protein expression (168). EA stimulation of acupoints of pericardium meridian can down-regulate the expressions of cerebral Nogo-A and NgR1 mRNA in cerebral ischemia rats, which is conducive to nerve repair after cerebral ischemia (169). EA can downregulate the RhoA/ROCK pathway to promote axonal regeneration (170). EA at LI11 and ST36 could significantly improve neurological deficit scores following stroke *via* inhibited Nogo-A/NgR/RhoA/ROCK signaling (171) (Figure 4C).

Taken together, acupuncture can promote neurogenesis after stroke by enhancing axonal growth factors as well as decreasing axonal growth inhibitory factors, thus promoting neurological recovery. More and more in-depth studies are worthwhile to enrich the multi-target mechanism of clinical acupuncture for post-stroke motor dysfunction.

6. Summary and prospect

The difficulty of central neurogenesis after cerebral ischemia in adults is the main cause of neurological dysfunction after stroke and promoting neurogenesis after stroke has become a hot button in cerebrovascular disease research in recent years. Promoting axonal sprouting, synaptic remodeling, and suppressing the central damage microenvironment will be an important pathway to improve neurological impairment after ischemia, which is also a key issue in stroke treatment.

Acupuncture can regulate post-ischemic neurogenesis at multiple levels and targets and can promote the expression of post-ischemic NSCs and other factors that are beneficial to neurogenesis. On the other hand, acupuncture can reduce the local inhibitory microenvironment in the injured center, providing a favorable microenvironment for neurogenesis and repair. However, the specific mechanisms of these factors are not yet fully understood, because the mechanism of neurogenesis after cerebral ischemia is complex and influenced by various factors, and most of the studies on the

TABLE 1 Summary of the studies investigating acupuncture's effects on functional recovery improvement after stroke *via* regulating neurogenesis factors.

Research group	Model	Acupoint	Acupuncture method	Stimulation parameter	Treatment course	Molecular and cellular results	Behavioral test
Zhou (150)	MCAO	PC6, ST36	EA	2 Hz/30 Hz, 5 mA	30 min/day, 3, 7, 14, 28 days	BDNF↑, Sema3A↓, NRP-1↓	Bederson score
Kim et al. (151)	MCAO	GV20, GV14	EA	2 Hz, 2 V	20 min/day, 10 days from 5 days after MCAO	NSCs↑, BDNF↑, VEGF↑, p-PI3K↑, p-ERK↑	Morris Water Maze (MWM)
Kim et al. (152)	MCAO	GV20, GB7	EA	3 Hz for 5 s, with 2 s intervals, at an intensity of the muscle twitch threshold	5 min/2 days, 2 w from 3 days after MCAO	BDNF↑, TrkB↑	Garcia scale assessments
Yi et al. (153)	MCAO	GV20, GV14	EA	Disperse-dense wave, 5–10 times/s, intensity in the rat quiet tolerance degree, about 3–5 V	30 min/day, 2 or 5 weeks	P38↑, GAP-43↑, NGF↑, BDNF↑	/
Xia et al. (154)	I/R	KI3, LR3	MA	/	30 min/day, 14 days	BDNF↑, SYN↑, PSD↑	NSS, MWM
Zheng et al. (155)	I/R	GV20, GV24^{+}	EA	2 Hz, 1 mA, 100 μs	10 min/day, 14 days	BDNF↑, TrkB↑, NMDAR1↑, AMPAR↑, GABA$_A$R↑, CaMKII↑, NeuN↑, PSD-95↑	MWM, Novel Object Recognition Test, Open Field Test
Zhao et al. (156)	I/R	GV20, GV26	EA	2/100 Hz, 2 mA	40 min/day, 6 days	NGF↑	Zea Longa neurological score, MWM
Zhang et al. (157)	MCAO	nape cluster acupoints	MA	Twisting Angle is 60°	15 min/day, 15 days	BDNF↑, NGF↑	Zea Longa neurological score
Tao et al. (158)	I/R	LI11, ST36	EA	Dense disperse wave of 1 or 20-Hz	30 min/day, 3 days	BDNF↑, GFAP↑, vimentin↑, nestin↑, Cyclin D1↑, CDK4↑, phpspho-Rb↑	Zea Longa neurological score, Catwalk gait, Rotarod test
Gu et al. (159)	I/R	LI11, ST36	EA	2 Hz/15 Hz, 2–4 mA	20 min/day, 7 days	BDNF↑, PGC-1α↑, FNDC5↑	Zea Longa neurological score, Balance Beam score
Chen et al. (160)	I/R	LI11, ST36	EA	Disperse wave of 1 and 20 Hz	30 min, once	BDNF↑, GDNF↑, PI3K↑, p-Akt↑, t-Akt↑, Bcl-2/Bax ratio↑	Zea Longa neurological score
Xue et al. (161)	I/R	LI11, ST36	EA	Disperse wave of 4 and 20 Hz	30 min/day, 3 days	PI3K↑, p-Akt↑, p-Bad↑, Bcl-2↑, Bax↓, cleaved caspase−3↓	Zea Longa neurological score
Yang et al. (162)	MCAO	GV16, GV8	EA	60 Hz 1 s and 2 Hz 3 s alternately at an intensity of 10 mA	20 min, once	BrdU+ cells↑, BrdU+/CRMP-4(+)↑, BrdU+/MAP-2(+)↑	/
Tao et al. (163)	MCAO	LI11, ST36	EA	1 Hz/20 Hz, at an intensity of the muscle twitch threshold (the muscle twitch threshold was about 0.01 mA)	30 min/day, 7 days	BDNF↑, Nestin↑, Notch1↑, NICD↑, Hes1↑, GDNF↑, D1↑, Cdk4↑, p-Rb↑, p21↓, p27↓	Zea Longa neurological score
Chen et al. (164)	MCAO	LI11, ST36	EA	disperse-dense waves of 1 or 20 Hz frequencies	30 min/day, 3 days	GFAP↑, Nestin↑, Wnt1↑, β-catenin↑, GSK3↓	Zea Longa neurological score
Zhang et al. (165)	MCAO	LI11, ST36	EA	1/20 Hz, 1 mA	30 min/day, 21 days	miR-146b↑, NeuroD1↑	mNSS
Zhao et al. (166)	MCAO	GV20	EA	1–2 mA, dense-disperse frequency of 2/10 Hz	30 min/day, 5 days	miR-132↑, SOX2↓	The rotarod test, limb placement test, body swing test, measurement of forelimb placing

(Continued)

TABLE 1 (Continued)

Research group	Model	Acupoint	Acupuncture method	Stimulation parameter	Treatment course	Molecular and cellular results	Behavioral test
Xu et al. (167)	MCAO	GV26, GV20	EA	3.85 Hz/6.25 Hz, 0.8–1.3 mA	30 min/day, 3 days	miR-210↑, CD34↑, HIF-1α↑, VEGF↑	Zea Longa neurological score
Tan et al. (168)	I/R	GV20, GV14	EA	3 V, 3 Hz	15 min/day, 28 days	NgR↓	Zea Longa neurological score
Xiao et al. (169)	MCAO	PC2, PC3, PC6, PC7	EA	2–4 V	30 min/day, 21 days	Nogo-A↓, NgR↓	Zea Longa neurological score
Chen et al. (170)	I/R	GV20, GV14	EA	Continuous-wave stimulation at 2 Hz (intensity, 1 mA)	30 min/day, 7 days	GAP43↑, BDNF↑, RhoA↓, ROCK↓	/
Huang et al. (171)	MCAO	LI11, ST36	EA	A dense disperse wave of 1 and 20 Hz (adjusted to the muscle twitch threshold)	30 min/day, 3, 7, 14 days	GAP43↑, RhoA↓, ROCK↓, Nogo-A↓, NgR↓	Zea Longa neurological score

Neiguan (PC6), Zusanli (ST36), Baihui (GV20), Dazhui (GV14), Taixi (KI3), Taichong (LR3), Yintang (GV24⁺), Shuigou (GV26), Quchi (LI11), Jinsuo (GV8), Fengfu (GV16), Tianquan (PC2), Quze (PC3), Daling (PC7).

mechanism of neurogenesis after stroke are focused on the enhancing factors of neurogenesis (such as growth factors), fewer studies on the effects of acupuncture on neurotransmitters, related inhibitory proteins and axon guidance cues. In the future, studies investigating the role of acupuncture on neurogenesis after stroke can particularly focus on axonal inhibitory factors and the complete pathway, which can better illustrate the mechanism of acupuncture initiation.
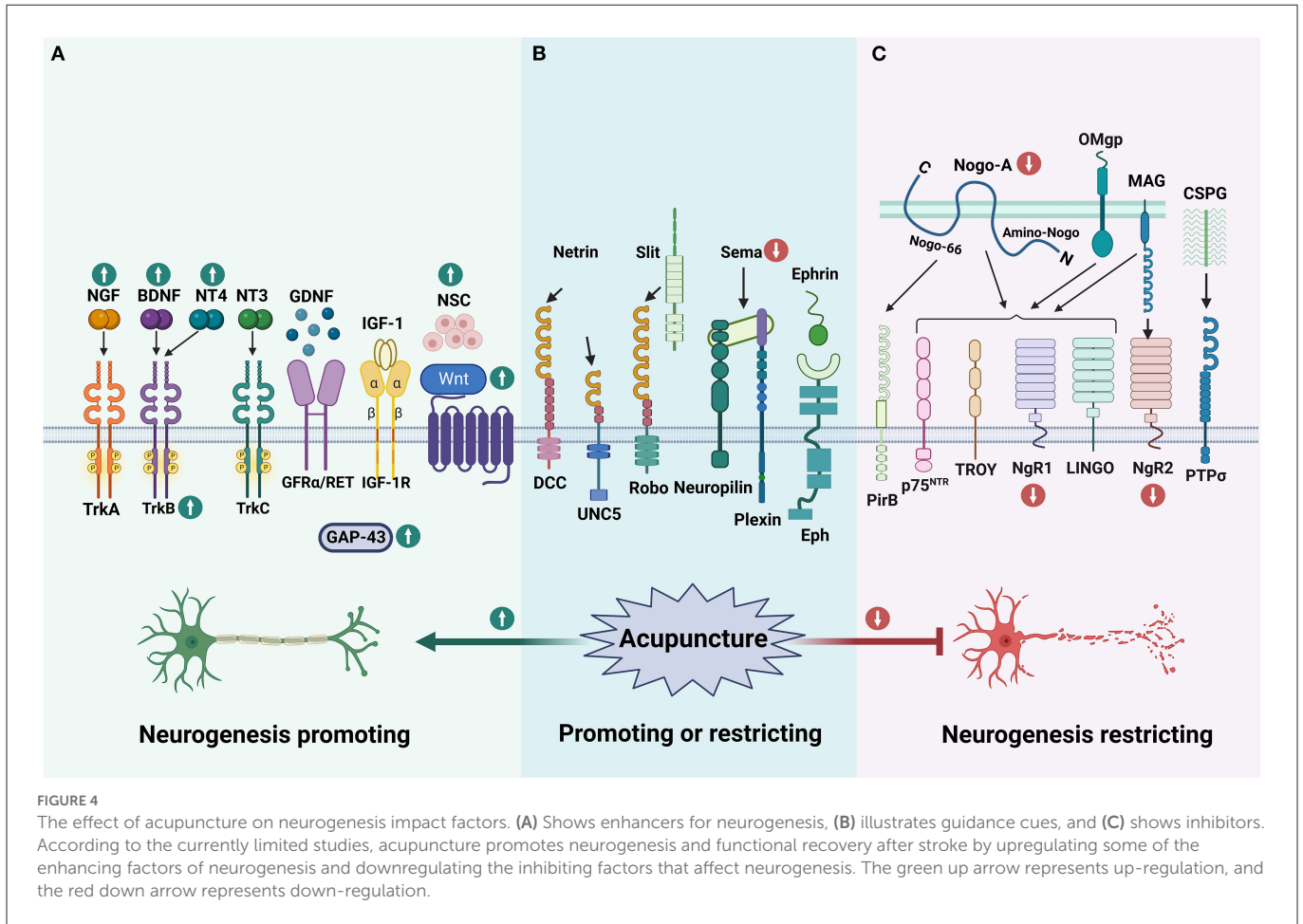
It appears that more researches have been done on EA than on MA, probably related to the stronger stimulation of EA and the easier control and fixation of stimulation parameters (172). Generally, an important factor of the effectiveness of acupuncture in clinical practice is the needling technique and MA is more commonly used in the clinic. However, there is a lack of research on the effects of MA on neuroplasticity. Future studies could attempt to compare the effects of MA and EA on neurogenesis to provide greater clinical guidance for acupuncture.

The acupoints used in each study varied. The most frequently applied acupoints are GV20, GV14, and ST36 (Table 1), which are located on Governor Vessel (GV) and stomach meridian. The GV runs along the middle of the back and connects to the brain, so points on GV are commonly used for brain disorders. The stomach meridian is connected to the stomach organ and is often considered to be the origin of energy, so points on this meridian are often used for conditions in which the body is deprived of energy. Although these three points are frequently used, few studies have compared them with each other or with other acupoints to determine which acupoint or combination of points produces the best effect on neuroplasticity after cerebral ischemia. In addition, differences in the frequency, intensity, and treatment course of EA might produce a variety of effects on neuroplasticity. Although many studies have used dense/sparse wave stimulation (Table 1), the number of studies comparing different stimulation parameters is relatively small. Therefore, both optimal stimulation conditions and therapeutic time windows for acupuncture need to be based on additional and more solid mechanistic studies in order to be supported by reliable data from preclinical studies.

Finally, most of the current acupuncture interventions have been studied through animal experiments, and there is a lack of large sample clinical studies, and further research is needed on how to effectively apply them to clinical practice. Therefore, in the future, we should carry out research on the interaction between various factors by acupuncture treatment. Using neuroimaging and other technical means, combine animal experiments with clinical practice to provide a theoretical basis for clinical acupuncture treatment of stroke.

7. Conclusion

The global burden of stroke is increasing every year due to population growth and aging trends. Stroke has become the most significant risk factor for human health worldwide. We reviewed studies on the mechanisms of neurogenesis after stroke, analyzed the role of various common factors on neurogenesis, and discussed the effects of acupuncture on neurogenesis and functional recovery after stroke. Stroke-induced neuroplasticity is a promising therapeutic target because it allows the brain in injured areas to re-establish neural connections and heal the damage caused by ischemia. Due to the complex interactions between various factors affecting neurogenesis, interfering with one factor alone

FIGURE 4
The effect of acupuncture on neurogenesis impact factors. **(A)** Shows enhancers for neurogenesis, **(B)** illustrates guidance cues, and **(C)** shows inhibitors. According to the currently limited studies, acupuncture promotes neurogenesis and functional recovery after stroke by upregulating some of the enhancing factors of neurogenesis and downregulating the inhibiting factors that affect neurogenesis. The green up arrow represents up-regulation, and the red down arrow represents down-regulation.

often leads to an overkill situation. Therefore, there is still a global need to develop better treatment options without side effects. Acupuncture is an ancient physical stimulation therapy that has been practiced in China for thousands of years and provides a benign regulation of the body through the stimulation of body acupoints. Acupuncture can promote functional recovery after stroke, and its mechanism of action is based on the modulation of neuroplasticity. However, there is still a lack of more comprehensive mechanistic evidence to fully demonstrate the role of acupuncture in the neurogenesis microenvironment.

In the future, based on neurogenesis mechanisms, the experimental design can focus on screening optimal factors of acupuncture treatment, particularly appropriate intervention time, needling techniques, acupoints, and acupuncture sessions, so as to provide more reliable mechanistic evidence for acupuncture strategy in functional rehabilitation after stroke.

Author contributions

Manuscript writing: J-DM and L-XM. Conception and design: J-DM, L-XM, ZZ, XQ, Q-YZ, L-HM, and T-YS. All authors conceived and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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