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Advanced rehabilitation in ischaemic stroke research

Jixian Wang ,[1] Yongfang Li,[1] Lin Qi,[2] Muyassar Mamtilahun,[2] Chang Liu,[2] Ze Liu,[2] Rubing Shi,[2] Shengju Wu,[2] Guo-Yuan Yang [2]

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[1]Department of Rehabilitation Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medical, Shanghai, China
[2]Med-X Research Institute and School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China

Correspondence to
Dr Guo-Yuan Yang;
gyyang@sjtu.edu.cn

ABSTRACT

At present, due to the rapid progress of treatment technology in the acute phase of ischaemic stroke, the mortality of patients has been greatly reduced but the number of disabled survivors is increasing, and most of them are elderly patients. Physicians and rehabilitation therapists pay attention to develop all kinds of therapist techniques including physical therapy techniques, robot-assisted technology and artificial intelligence technology, and study the molecular, cellular or synergistic mechanisms of rehabilitation therapies to promote the effect of rehabilitation therapy. Here, we discussed different animal and in vitro models of ischaemic stroke for rehabilitation studies; the compound concept and technology of neurological rehabilitation; all kinds of biological mechanisms of physical therapy; the significance, assessment and efficacy of neurological rehabilitation; the application of brain–computer interface, rehabilitation robotic and non-invasive brain stimulation technology in stroke rehabilitation.

INTRODUCTION

For most of the last century, doctors advised less activities and movements after stroke attack. In the 1950s, Twitchell began studying rehabilitation patterns for patients who had a stroke, and he found if hand function had recovered by 4 weeks after stroke, there was a 70-percentile chance that all or most functions would be recovered. He also noted that most recovery occurred in the first 3 months, and there would be only a slight recovery after 6 months.[1] Although clinical rehabilitation therapies achieved some good results, the lack of an ideal animal model of ischaemic stroke and residual neurological dysfunction made it difficult to study the mechanism of rehabilitation therapy. These included increased plastic changes leading to neuronal rewiring, neurogenesis and new forms of synapse formation, accompanied by transcriptional and translational changes in the affected cells. The second issue was the lack of quantitative or at least semiquantitative means of assessing the effectiveness of rehabilitation. Fortunately, in recent years, great progresses were made in the establishment of rehabilitation model and treatment mechanism after stroke injury.

Development of an animal model of rehabilitative ischaemic stroke

Animal models of ischaemic stroke are essential tools for studying the pathological mechanisms of cerebral ischaemia and developing new therapeutic options.[2–5] Before preparing for modelling, we need to make everything clear, such as should the whole brain or focal ischaemia model be used. Should the left or right hemisphere be used for ischaemia modelling? Should permanent cerebral ischaemia model or cerebral ischaemia-reperfusion model be used? Should male or female animals be used? Should old rats or hypertensive rats or hyperglycaemic rats or hyperlipaemia rats be used? The most widely used models of the middle cerebral artery occlusion (MCAO) in rodents could be divided into two main categories and described as below: thromboembolic models and non-thromboembolic models.

The physiopathological changes in the acute phase (24 hours) of ischaemic stroke were mainly acid-base balance and electrolyte disorder, neurotoxicity, calcium overload and oxidative stress. The subacute phase of ischaemic stroke (72 hours) was characterised by cerebral oedema, blood–brain barrier (BBB) disruption and inflammation. In the recovery period of ischaemic stroke (after 7 days), the main manifestations were angiogenesis, nerve regeneration and glial scar formation.

Model of MCAO by suture

The model of MCAO is considered to be the closest model to ischaemic stroke in humans and is used in more than 40% of study of neuroprotective experiments.[6] There is no obvious causal relationship between MCAO model and brain injury; however, brain ischaemia model prepared by craniotomy could also cause bacterial infection in the brain. Thus, rodent MCAO

by the endovascular suture method in rodents is a widely acceptable and highly standardised small animal model due to its simpler procedure and stable infarct volume. In 1981, Tamura *et al* first established a rat model of permanent occlusion of MCA.[7] To simulate recanalisation after stroke in clinical patients, Koizumi and Koizumi J performed a rat ischaemia-reperfusion model in 1986 by occluding the MCA with a suture and then removing the suture.[8 9] This model is proper for the study of permanent or transient cerebral ischaemia mimicking human ischaemic brain injury. Subsequently, Yang *et al* established a mouse model of MCA ischaemia and ischaemia-reperfusion injury.[10] The establishment of this ischaemic mouse model has laid a worthy foundation for the application of rodent transgenic model of cerebral ischaemia, and for gene, molecular and stem cell therapy. At present, most laboratories around the world are using modified transient MCA thrombus models; however, this model cannot fully induce the pathogenesis of clinical patients who had an ischaemic stroke. A large proportion of patients with ischaemic stroke are often accompanied by hypertension, hyperglycaemia, hyperlipidaemia and metabolic diseases, and these risk factors are also important reasons for the high incidence of ischaemic stroke. Therefore, rodent model of ischaemic brain injury with above diseases has attracted more attention.

MCAO model by thrombus method

The thrombotic MCAO model is the most popular model for the study of thrombolytic drugs. However, it is difficult to control the site of embolised brain vessels. This model can be traced back to the rat model of thromboembolic cerebral infarction first described by Kudo *et al*.[11] Chopp's team established rat and mouse thromboembolic models and conducted in-depth studies on MRI changes in the thromboembolic models. It was found that perfusion-weighted imaging and diffusion-weighted imaging showed decreased cerebral blood flow and hyperintense areas after injection of thrombus into the innervated region of the MCA. At 2 hours after ischaemia, injection of recombinant tissue plasminogen activator (rt-PA) rapidly restored blood flow to preischaemic levels.[12 13] This type of model can also be used to study the dynamics of injury and repair in thrombotic stroke. The combination of rt-PA and the proteasome inhibitors PS-519 or bortezomib has been shown to reduce infarct volume and improve neurological function without increasing the risk of bleeding in a rat model of thromboembolism.[14] Another study demonstrated that the combination of glycoprotein IIb/IIIa receptor antagonists and full or half-dose rt-PA reduced infarct volume and improved neurological function.[15] In thromboembolic models, clots can be obtained from spontaneous, or thrombin induced thrombosis, either from autologous or from heterologous blood.[16] In 2016, Kamel *et al* further developed the thromboembolic stroke model by combining the atrial fibrillation model with the thrombus model.[17]

Proximal or distal MCAO

Distal MCAO (dMCAO) was extensively used in stroke research. The technique is somewhat difficult to learn but the lesion size is stable. Rodents were placed in the left lying position and an incision was made between the external canthus of the right eye and the external auditory canal. Blunt separation and distraction of the temporal muscle. The temporal nerve, artery and vein damage should be avoided, and the zygomatic arch was exposed and most of them should be removed. Drill a small hole on the stull, which is close to the arcuate margin. The MCA generally sends out several branches, hook the MCA with Dumont curved forceps then permanently ligate it with the forceps tip of the electrocoagulator. Damage is restricted to the cortex if blood flow is interrupted distal to the striatal branches of the MCA, whereas occlusion proximal to these small arteries result in both striatal and cortical injury.[18] The reperfusion could be induced in this model if using suture ligation. This model is simple, the infarct size is fairly constant and there is no reperfusion. However, the disadvantage of this model is the need for craniotomy, which may cause brain tissue damage and local inflammation.

Endothelin 1 vasoconstriction

Intracerebral injection of the vasoconstrictor peptide, endothelin-1 (ET-1), has been used as a method to induce focal ischaemia in rodents.[19 20] ET-1 produced ischaemia by constricting blood vessels. ET-1 could be stereotaxically injected into parenchymal regions of the interest, to constrict local arterioles, or near the MCA. Restoration could be reached but at a much slower rate than with the intraluminal suture MCAO model. Lesion size could be adjusted by varying the concentration or volume of ET-1 to achieve reproducible injury.

Photothrombosis

This approach was originally proposed by Rosenblum and El-Sabban in 1977 and modified in rat brain by Watson in 1985 and laid the foundation for this model. Increased availability of transgenic mouse lines has further fueled interest in photothrombosis models.[21] A photosensitive Rose Bengal dye (0.15%; Sigma-Aldrich, USA) is injected systemically into animals, in which a section of skull has been removed or thinned. The underlying cortical blood vessels are exposed to a green laser (λ=532 nm) for 5 min or epifluorescent light source, generating singlet oxygen species that lead to platelet activation and microvascular occlusion. This model could be used to produce small infarcts in any cortical region without invasive surgery.[22] Ischaemic penumbra areas in the photothrombosis model could be identified by combining perfusion-weighted imaging, diffusion-weighted imaging and other MRI techniques.[23]

Table 1 Advantages and disadvantages of rodent ischaemic stroke models

Stroke models	Advantages	Disadvantages	Refs
Permanent/transient MCAO	Closest to human permanent or transient ischaemic brain injury, widely accepted and used, stable and repeatable	Fail to simulate ischaemic pathogenesis in patients with comorbidities	7–10
Thrombotic MCAO	Suitable for studies in thrombotic stroke, for example, thrombolytic drugs	Difficult to control the site of embolism	11–17
Proximal/distal MCAO	Stabled lesion size	Difficult to operate, craniotomy needed	18
ET-1 vasoconstriction	Relatively controllable lesion size, easy to operate	Slower restoration rate compared with MCAO by intraluminal suture	19 20
Photothrombosis	Induce infarcts in any cortical region, non-invasive	Small infarct volume	21–23
Bilateral common carotid artery ligation or coils	Suitable for studies of white matter injury induced by chronic ischaemia	/	24

ET-1, Endothelin-1; MCAO, middle cerebral artery occlusion.

Other cerebral ischaemia models

In addition to MCAO model, other relatively less used cerebral ischaemia models such as the chronic ischaemia model were also developed to some extent in recent years. Most models of chronic ischaemia use bilateral common carotid artery ligation or coils to reduce cerebral blood flow to study white matter injury caused by chronic ischaemia.[24]

The advantages and disadvantages of the above stroke animal models are compared in table 1.

Animal model suitable for poststroke rehabilitation

Rodent cerebral ischaemia models have been widely used in experimental research due to the abundant animal sources, simple operation and stable postoperative detection methods. Unfortunately, all neuroprotective agents that have been applied in the treatment of rodent cerebral ischaemia and were successful in the evaluation have failed in subsequent clinical trials. It is suggested that rodents have certain species limitations as stroke models. Larger animal models have more important scientific research value because of their relatively large brains and more complexity like humans. At present, animal models such as sheep, geriatric dogs and monkeys have been used in stroke research. These animal models can control the size of ischaemic lesions and subsequent neurological effects, and make it easier for animals to carry out rehabilitation training and show typical ischaemic lesion patterns by behavioural phenotyping, neuropathology, immunohistochemistry and MRI/SPECT(single photon emission computed tomography) imaging, which can range from simple scoring systems, to highly complex assessments of cognitive function, to fine motor tests.[23 24] Although large animal models more closely reflect the situation of ischaemic stroke in humans, the essential difference between large animal models and humans is that animals are on all fours while humans are on both feet, so the blood supply of the circulatory system to the brain is different. For large animal models, we need to pay more attention to the large individual differences in large animals, and the model has relatively high variability. In addition, the application of large animals for neurorehabilitation requires highly specialised animal-specific rehabilitation equipment and professional rehabilitation experimental researchers.[25] Because large animal models have larger brain tissue structures, it is advantageous to demonstrate the effects of neurorehabilitation indirectly by applying alterations in structural, functional, metabolic or diffusion tensor imaging. MRI/SPECT can noninvasively assess brain metabolism, so it has more application value.[26] Enriched habitation of animals is a novel therapeutic approach, similar to giving patients a more diverse living environment during rehabilitation, can be used for a variety of sensory, motor, social and visual stimulation, and can be used to evaluate the efficacy of exogenous cell transplants.[27] Mandatory or active physical training, specific training, combined central and peripheral, combined upper and lower limbs or left and right rehabilitation training equipment, microrehabilitation therapy robots and various intelligent electronic devices enable intensive, controllable and repeatable training methods become possible.[26 27] Considering the diversity of experimental models and results, it is very important and challenging to select simple, stable and effective experimental methods and evaluation methods. It is currently believed that forced exercise training (eg, treadmill) and skilled forelimb training may be more effective in stroke animals. Constraint-induced movement therapy is ineffective in animal models.[28]

The advantages and disadvantages of different species of animal stroke models are compared as follows (table 2).

New focus on animal models of cerebral ischaemia

Time course of cerebral ischaemia

MCAO models are widely used in the study of ischaemic stroke, including permanent MCAO model and transient

Table 2 Advantages and disadvantages of large animal and rodent models for poststroke rehabilitation

Animals	Advantages	Disadvantages	Refs
Large animals	Closer to human ischaemic stroke, easier to perform rehabilitation training and evaluating, suitable to test different rehabilitation methods	Large individual differences and model variability, high requirements in rehabilitation equipment and researchers	25–28
Rodents	Abundant animal sources, stable postoperative detection methods	Results are difficult to translate into clinics due to species limitations	

MCAO model, which have distinctly different pathological mechanisms. Questions remain about how to choose a permanent ischaemia model or an ischaemia-reperfusion model, and studies focusing on ischaemia-reperfusion are currently much more common than permanent ischaemia studies. Prompt revascularisation after cerebral ischaemia is the most direct and effective treatment; however, it can also cause secondary damage to the ischaemic brain. Therefore, many studies have used ischaemia-reperfusion models to develop neuroprotective agents. Researchers generally use the 90–120 min ischaemia-reperfusion model, mainly because the 30–60 min ischaemia-reperfusion rats only have mild embolism and greater variability with no cognitive impairment.[29] In contrast, reperfusion of cerebral vascular occlusion for more than 3 hours can lead to increased infarct volume and mortality.[30] Reperfusion after 90–120 min of vascular occlusion can induce relatively stable neurological dysfunction and reperfusion injury, with high modelling success rate, low animal mortality and long survival period, which can meet the experimental requirements.[31] Notably, compared with rats, mice were more susceptible to occlusion time.[32] Reperfusion alters the physiological and pathological processes after ischaemia, resulting in significant differences of ischaemic changes in the acute (24 hours), subacute (3–7 days) and chronic recovery (14–28 days) phases of ischaemic stroke.

The importance of animal strains, sex and age

Animal strains,[33] sex and age[34] and the need to pay attention to ischaemic stroke risk factors in modelling have been published.[33–36] Epidemiological surveys have shown that the age of onset of ischaemic stroke plays an important role. Statistics from China show that the median age of most ischaemic strokes is 65 years old, and there is a trend of increasing age. It is generally believed that ageing-induced metabolic changes associated with cerebrovascular dysfunction increase the severity of cerebral ischaemia-hypoxic injury compared with younger animals. However, a study of ischaemic stroke in aged rats suggested that neural stem cell transplantation reduced ischaemic brain injury in aged rats while increasing angiogenesis and neurogenesis, indicating that the ageing-related microenvironment does not hinder the beneficial response to neural stem cells during cerebral ischaemia.[37] Therefore, it is recommended to use animals in the middle and late stages as research subjects.[38] Since there are no major differences in human ischaemic stroke by sex, experiments should be considered when using equal numbers of males and females.

Underlying diseases and risk factor-related animal models

The incidence of ischaemic stroke increases when patients have underlying conditions such as hypertension and hyperglycaemia; in addition, the incidence of hyperlipidaemia also increases with age. These underlying diseases often lead to higher mortality and morbidity risk of ischaemic stroke, among which hypertension is the leading risk factor. Clinical studies have shown that hypertension reduces the BBB integrity, aggravates white matter damage and oedema, and exacerbates ischaemic injury outcomes. Therefore, spontaneously hypertensive rats exhibit various vascular morphological changes and larger brain infarct volume.[39]

Epidemiological data showed more than 50% of patients who had ischaemic stroke have hyperglycaemia,[40] while diabetic stroke patients tend to be younger.[41] Hyperglycaemia induces ischaemic stroke by exacerbating endothelial dysfunction, promoting early arteriosclerosis, systemic inflammation, capillary basement membrane thickening or increasing lactate production.[39] Diabetes is closely related with the occurrence and development of brain microvascular diseases. Studies show that hyperglycaemia induces more severe brain infarction and oedema, exacerbates sensorimotor and cognitive impairment and hinder neurological recovery.[42 43]

Pathological mechanism difference between permanent ischaemia and ischaemia-reperfusion

There are significant differences between the pathological mechanisms of permanent ischaemic stroke and ischaemia-reperfusion injury. Permanent ischaemic stroke mainly is a primary hypoxic-ischaemic injury, where small blood flow reduction does not result in significant functional or metabolic disturbances.[44] After the onset, the ischaemic core gradually expands towards the peri-infarct area overtime and the infarct volume reaches a maximum.[45] The impact of ischaemic-reperfusion model on brain tissue depends on the time course of ischaemia. Ischaemia less than 30 min causes brain damage; however, mostly recoverable, while ischaemia longer than 30 min to 2 hours would lead to irreversible brain tissue death. If the reperfusion process begins at 3 hours after stroke onset, it will lead to a more severe reperfusion injury and secondary neural cell death.[10 46] The reperfusion injury has close relation with processes including cell apoptosis

and inflammation, while permanent brain ischaemic injury is more associated with neurotransmitter receptor, ion channels, growth factors and other pathways.[47] The mechanism differences between two models still require further clarification.

The asymmetry of brain

Asymmetry exists in the adult human brain, often with one hemisphere taking the lead. For most right-handers, left hemisphere is the centre of language, known as the dominant hemisphere. The dominant hemisphere usually has more complex functions. Besides, the ischaemic pathological outcomes differ in dominant and non-dominant hemispheres in both human and other vertebrates.[48–50] Ischaemia in dominant hemisphere results in more severe neurological dysfunction, while animals with ischaemia in non-dominant hemisphere tend to have quicker neurological recovery.[48 50] Interestingly, the biochemical processes, behaviours and even the expression of growth factor encoding genes have differences when stroke occurs in different hemisphere. Therefore, brain asymmetry needs to be taken into consideration in ischaemic stroke studies.

Oxygen glucose deprivation model of brain cells

In vitro models are commonly used in mechanism studies of ischaemic stroke. Many models include coculture of different types of cells such as astrocytes with endothelial cells, neurons with endothelial cells and so on. There is even a triple-cell coculture system. By coculture, it is possible to mimic some properties of BBB and study how different types of cells interreact under ischaemic conditions.[51]

There are three representative in vitro ischaemic models: oxygen glucose deprivation model (OGD), excitotoxicity model and oxidative phosphorylation blocking model. Most in vitro ischaemic models are based on OGD model, using chemicals or enzymes to induce glucose deprivation and hypoxia.[52] OGD model has many advantages because it allows researchers to study animal and human cells directly.[53] Second, the studies using excitotoxicity models have used N-methyl-d-aspartate or glutamate, as part of ischaemic injury.[54 55] Third, oxidative phosphorylation blocking methods mainly use chemicals such as sodium azide, rotenone and antibiotics to inhibit the electron transport chain.[52] In vitro studies, though important, must combine with in vivo studies to mimic the real human ischaemia.

The advantages and disadvantages of the in vitro cerebral ischaemia model are shown in table 3.

New techniques for monitoring posterior neural network remodelling and circuit formation

In recent years, technologies for monitoring neuronal cell integration and remodelling, repairing neural circuits, and forming new neural circuits have been advancing rapidly. A classic method of studying the restructuring process of the whole brain is functional MRI (fMRI), which allows monitoring of the neural circuits restructuring process in the same animal anatomy of the macrolevel, but the spatial and temporal resolution level is still low.[56] These new technologies extend tremendous possibilities for analysing plasticity processes in experimental ischaemic animals and identifying and localising key factors for novel stroke therapies.

Optical imaging equipment includes photoacoustic microscopy, photoacoustic tomography, confocal microscopy, two-photon microscopy, optical coherence tomography, scanning laser acoustic microscopy and so on. Their advantage is the outstanding high spatial resolution, which can visualise small areas in a specific brain region and show the spatial relationship between the functional organisation and these smaller areas. For example, two-photon calcium imaging could record the activity of individual neural cells in the neural cell network and allow the functional analysis of specific subtypes of brain tissue. However, neural cell activity at a level of cellular resolution is limited to a small field of view to examine. The collective dynamics of different brain regions is not available. Recent advances in two-photon microscopy allow simultaneous imaging of neural cell networks at cellular resolution level in the multiple areas of active animals that are not even directly connected.[57–59] Commonly used optical imaging uses the principle that active brain tissue reflects less light than inactive tissue. Thus, the most active regions appear as the darkest regions. Currently, researchers have applied this principle of optical imaging to show functional connectivity disruption in rodent ischaemic stroke models.[60 61] Another technique for studying sensory movement is millisecond-timescale voltage-sensitive dye (VSD), which measures electrical activity with relatively high spatial and temporal resolution.[62] The sensorimotor cortex of the forelimb of mice is a target of stroke, and VSD imaging can show the function of the sensorimotor cortex.[63] VSD imaging could even show new

Table 3 Advantages and disadvantages of in vitro ischaemic stroke models

Stroke models	Advantages	Disadvantages	Refs
Oxygen glucose deprivation	Directly study cells and intracellular interactions via coculture	Only mimic a part of ischaemic pathophysiology and injury	53 54
Excitotoxicity model Oxidative phosphorylation blocking model	Directly study cells and intracellular interactions via coculture	Only mimic a part of ischaemic pathophysiology and injury	55 56

sensory representations in the motor cortical regions of the forelimb before stroke, accompanied by high levels of dendritic spines as seen by two-photon microscopy. The recently developed exogenous, especially genetically encoded fluorescent indicators of neural cell activity such as GCaMP and YC-Nano, has revolutionised the targeted expression of fluorescence, with higher signal levels and even transgenic lines.[64] Dynamic flavoprotein fluorescence imaging targeting oxidative metabolism can be used for optical localisation in central nervous system tissues,[65 66] which may also be a useful tool for experimental stroke studies. Wide-area calcium imaging is also a powerful tool to study the remodelling of the whole cerebral cortex at a high-resolution level over time, monitor the animal's rehabilitation process, or record rehabilitation training after stroke.[67] A new non-invasive strategy to study motor cortical remodelling over time after stroke in the same animal is a technique called light-based motility mapping. This technique uses light to stimulate neural cells, either by blocking neurotransmitters or by directly activating light-sensitive channels.[68 69] The optogenetic method is also a good and effective method to observe the neuronal activity. Early methods of stimulating neuronal activity with light include stimulating Drosophila neurons with selective light through coexpressing Drosophila photoreceptor genes.[70] Subsequently, channelrhodopsin-2 (ChR2) was cloned, and light stimulation showed specific selectivity for ChR2-expressing neurons.[71] In recent years, neuroscientists have been quick to apply the possibilities of this new technique to live experiments. It has been widely promoted to study the activation and inhibition of specific neurons.[72–74] But optogenetics remains an invasive process for many in vivo experiments. Because light sources must be located close to neuronal tissue, targeting deep brain regions or diffuse neuronal populations remains a significant challenge.

So far, a few studies have applied optogenetics to the study of experimental stroke, mainly using optogenetics to dynamically observe neuronal activity[75] and as a therapeutic approach to promote neuronal activity aimed at promoting functional recovery.[76] Optogenetic stimulation of the ipsilateral primary motor cortex in ChR2 transgenic mice promoted functional recovery of the striatum and somatosensory cortex after stroke.[77] Selective stimulation of neurons in the lateral cerebellar nucleus resulted in a sustained recovery in stroke mice during the rotarod test.[78] Optogenetics has also been used to promote the excitatory output of transplanted neural stem cells and to increase the use and movement of the affected forelimb in a rat stroke model.[79] Although optogenetics has revolutionised the field of neuroscience, examination of the deeper subcortical regions of the brain remains a challenge because light must somehow be delivered to tissues that often require invasive implantation of optical fibres, resulting in collateral damage to surrounding brain tissue. An emerging approach to overcome spatial limitations is magnetogenetics. It relies on a principle known as thermal relaxation,[80] which means that alternating magnetic fields can heat small magnetic nanoparticles to activate cell-expressed heat-sensitive TRPV1 channels, raise plasma membrane temperature, and initiate calcium influx via heat-sensitive ion channels. This technology is currently being explored.

At present, a new technique for monitoring neural circuits has been developed by combining anatomy and molecular biology. Li *et al* have injected two different combinations of cholera toxin B (CTB) fluorescence tracer to the forelimb sensorimotor cortex at different time points.[81] Injection of a CTB tracer at stroke and other different tracers at 7 or 21 days after stroke can study molecular changes in newly emerging neural cells in the periinfarct cortex. Neurons expressing only the second tracer were those that missed axonal projections to the injection site when the first tracer was injected, thus representing neurons that had established a new projection pattern after stroke. The laser can capture two types of neural cells, single-labelled and double-labelled, to define the transcriptional characteristics of neurons budding in the cortex around the infarct area.

The characteristics of the above different new detection techniques are as follows (table 4).

Timing of neurological rehabilitation

Neurological rehabilitation training appears to have a window of time for treatment, and how to choose is crucial. Ultra-early training (24 hours) may aggravate brain injury after focal cerebral ischaemia in rats, and the possible molecular mechanism is that rehabilitation therapy may promote cytotoxicity.[82] The mechanism could also influence the effect of rehabilitation therapy on promoting the repair of neural cells in the acute phase (2–5 days). However, brain neural cells showed a higher sensitivity to rehabilitation therapy treatment in the subacute phase (5–14 days) after stroke decreased with time.[83 84] But some studies suggest that only early training can improve symptoms. At the beginning of training 1–5 days after ischaemic stroke, the volume of infarction decreased, and cognitive and motor function improved. Rehabilitation starting 1–7 days after haemorrhagic stroke enhances functional and plasticity.[85] There is no relationship between treatment frequency and treatment effect at present, but if training is suspended, the improvement of function will be lost.[86]

Individual neural rehabilitation paradigms can be combined to improve outcomes and tissue recovery. The rehabilitation training and environmental stimulation of injured forelimbs increased dentate neurogenesis in rats with cortical infarction, which is related to improving the performance of water maze.[87] Combining the rich environment and running wheel training can increase the survival rate of transplanted cells.[88] Forced induced exercise therapy facilitated function recovery, dendritic branch formation and neuroplasticity of haemorrhagic stroke rat model, while forced use of the damaged limbs at 1 day after haemorrhagic stroke led to better outcome recovery.

Table 4 The new techniques for monitoring posterior neural network remodelling

New techniques	Characteristics	Refs
functional MRI	Monitor whole brain neural circuit restructuring, relatively low spatial and temporal resolution	57
Two-photon calcium imaging	Record individual neural cell activities, high spatial resolution, limited cellular resolution field (<1 mm^2)	57
Two-photon microscopy	Real-time imaging neural cell networks at cellular resolution level in live animals	58–60
Millisecond-timescale voltage-sensitive dye	Measure electrical activity of sensory movement, relatively high spatial and temporal resolution	64 65
Dynamic flavoprotein fluorescence imaging	Targeting oxidative metabolism	66 67
Wide-area calcium imaging	Monitor the whole cerebral cortex remodelling, animal's rehabilitation process and training at a high-resolution level over time	68
Light-based motility mapping	Monitor motor cortical remodelling over time	69 70
Optogenetics	Selectively stimulate or inhibit specific neurons in live experiments, invasive process with spatial limitations	71–80
Magnetogenetics	Initiate calcium influx via heat-sensitive ion channels	81
Cholera toxin B fluorescence tracer injection tracing	Monitoring neural circuit reconstruction after stroke	82

The impact of neuroplasticity on rehabilitation

There is a growing evidence to support various forms of plasticity triggered after stroke and their potential contribution to recovery. As mentioned above, these plasticity reaction include alive neural network and reconnection of axon branches,[89] recruit of synapse after injury,[90] extracellular matrix remodelling,[91] activation of endogenous neuron/glial cells and migration of neural cells/glial precursor cells to injury area.[92] These processes prompt the high degree of plasticity observed during development, which reiterated the pervasive view that regeneration of central nervous system may partly depend on high plasticity during development In regenerative neuroscience.[93 94]

Application of experimental neurological rehabilitation

Functional recovery is closely associated with the brain plasticity of patients. Degree of functional improvement depends on initial defect, size, quality and location of infarct lesion, as well as the sex and age of the patient, all of which would affect the outcome of reconstruction and repair of damaged area.

Experimental stroke model is well developed, its method is relatively simple, and the outcome is relatively stable. The neurological effects caused by stroke will appear after several minutes of decreased cerebral blood flow. This kind of model has obvious advantages over animal models of chronic neurodegenerative diseases. Therefore, we believe that stroke study has a bright prospect, especially to promote the plasticity of synapses and neural network, thus leading to the recovery of neural function. The latest progress in the field of stroke rehabilitation is to emphasise that the adult brain has significant plasticity, which promotes the rehabilitation of stroke. For example, the plasticity mechanism of the developing nervous system is similar to that of the adult brain after stroke.[63 83 95 96] Therefore, it is possible for us to enhance the brain's natural recovery ability by understanding the mechanism of functional recovery. Although the behaviour improvement after stroke is unlikely to be the same as that pattern before stroke due to the loss of neural cells with highly specific functions, clinical and biomedical scientists call the enhancement of sensory and motor ability after stroke as rehabilitation. As human and animal behaviour assessment programmes rarely determine the extent to which improvements reflect true rehabilitation, behavioural compensation or both,[97] so scientists in the stroke research field are interested in understanding how these compensatory processes lead to recovery.

The motor and sensory cortex can be loosely organised into somatic functional maps and has highly activity dependent plasticity. The motor map reflects the coupling of specific motor cortex neurons and muscles, while the sensory map reflects the pairing of body parts and sensory cortex neurons. The motor map can learn and express actions, representing a 'motor engram' or a memory trace.[98] The motor engram will be lost when the stroke damages the cerebral cortex. Therefore, the only way to restore motor function after the stroke may be to replace the damaged conduction pathway.[97]

Several factors cause plasticity changes in the human brain after stroke. Foremost, there is a surprising amount of synaptic diffusion and redundant neural connections in the central nervous system. Second, new structural and functional circuits can be formed through the reprojection between related cortical regions.

Previous studies have shown that neural cells involved in complex functions, such as memory conduction pathways or memory imprint, are not limited to a single brain

region but distributed in the entire cerebral cortex.[99] Although the structure of brain conduction pathway has been defined, its function is like a spatially distributed computing machine which can send signals along multiple paths. The extensive conductive pathways with the rich connections of neuron cells may contribute to recovery after ischaemic brain injury.

Although the classical view is that the sensory and motor function of the body part is controlled by neural cells in the contralateral hemisphere of the brain, there are also ipsilateral pathways, such as the right brain can partially control the right side of the body and the left brain can partially control the left side of the body.[100] One of the ways in which brain function recovers from ischaemic injury in humans is by exploiting the existing distribution of neural networks involving brain regions that are functionally upstream and downstream of the embolisation-affected region.[101]

Human imaging studies showed that if there is relatively normal sensory area activation on the ipsilateral hemisphere of the ischaemic brain, the rehabilitation of the patients is most likely to be successful, while if the ischaemic insulted area is large and manifests as bilateral cortical area activation, the motor functions of patients generally hardly can be restored.[101] Therefore, bilateral cortical activation may indicate that compensatory mechanisms cannot be restored.

Neurorehabilitation promotes adaptive brain plasticity after stroke, including circuit reorganisation[102] and activation of endogenous stem cells. Recent data suggest that forced limb use promotes the migration and survival of neonatal cells in the subventricular zone in elderly subjects.[103] Furthermore, neurogenesis around the cortex is involved in the reorganisation of motor maps and the improvement in behavioural performance that if resulted from skilled forelimb training indicated a causal relationship between neurogenesis and functional recovery. In turn, lack of physical activity limits endogenous cell-based repair mechanisms after stroke.

Although inappropriate task integration may cause maladaptation that suppresses or affects rehabilitation,[103] neurorehabilitation is a promising approach for motor function restoration after stroke. However, few rehabilitation studies have been conducted in experimental settings, possibly because of the complexity of study design and uncertainty in experimental methods, especially in studies that are difficult to quantify. Additionally, rehabilitation training is fundamentally different in rodents and patients who had a stroke. For example, therapists instruct and help patients with kindness while the rodents are trained on test apparatus based on rewarding/aversive stimuli, which at worst may lead to applying additional stress on experimental animals or disguising the effects of treatment. The speed and completeness of spontaneous recovery in stroke rodents differ compared with patients who had a stroke.[104] Therefore, it is crucial to choose an appropriate experimental rehabilitation therapy.

Application of trending technology in stroke rehabilitation

Application of brain–computer interface in rehabilitation

Brain–computer interface (BCI) is currently a hot spot in clinical neuroscience research, and its primary purpose is to help patients who have lost motor function regain motor control. The potential of this innovative research area to apply in poststroke rehabilitation training and help patients regain some fundamental life functions should be enormous. Cerebral ischaemic injury is usually an acute isolated event rather than a chronic neurodegenerative process. Since most neural networks not affected by cerebral infarction remain relatively intact, this provides a basis for the realisation of brain–computer interfaces for stroke rehabilitation. Current research has made it possible to control primate movements by deciphering cortical electroencephalogram (EEG) activity, or to enable paralysed patients to control robotic limbs and computer cursors through cortical signals recorded by high-density microelectrode arrays and electrocorticography grids,[105–109] the closed-loop system of movement has begun to explore the primate's ability to control limb function by using cortical signals to stimulate spinal circuits to induce upper limb movements.[110] Early arrays required direct implantation into the brain through a craniotomy, a procedure that can lead to tissue inflammation and neuronal damage. Therefore, it is essential to develop micro-invasive methods to avoid brain tissue damage. Studies indicated the feasibility of long-term recording of brain activity from a vein using a passive stent-electrode recording array. With cerebral angiography, superficial epidermal veins implanted in the motor cortex were achieved and neural recordings from freely moving sheep were demonstrated for up to 190 days. Vascular cortical EEG was comparable to recordings from epidural surface arrays. The lumen of the vein was kept open during implantation.[111] Non-invasive methods such as EEG-based signal acquisition are also increasingly used in neurorehabilitation research. With the rapid development of non-invasive technology, it is possible to replace implantable arrays with non-invasive methods in the future,[112] which makes it easier to popularise. However, these methods are still limited by the complexity of the interface between tissue and electronic devices and the ability to accurately decipher the cortical synthetic neural output. As the ability to interpret cortical signals becomes more precise and robotics becomes more sophisticated, brain–computer interfaces showed promising potential that brings revolutionary changes on the rehabilitation of function in stroke-induced hemiplegia or speech impairment patients. A study by the University of California, San Francisco (UCSF) regained the ability to speak to a severely paralysed man with aphasia by using brain–computer interface technology.[113] This study is part of the brain–computer interface restoration of arm and voice study to assess the potential of cerebral cortical EEG recordings and custom decoding techniques for communication and mobility.

Blackrock Neurotech company, a global leader in brain–computer interface technology, recently declared that its groundbreaking brain–computer interface device, Move Again, has been awarded a breakthrough device designation by FDA in America. This system consists of an array of chips implanted in the brain and can decode signals of expected movements from neuronal activities. Then, these signals were transmitted wirelessly to external devices, finally allowing immobile patients to control mouse pointers, keyboards, mobile devices, tablet computers, wheelchairs or prosthetic devices. Additionally, the implantable brain–computer interface product Stentrode of American Synchron Company has also been approved for clinical trials.[114] The Stentrode device enters the brain through the ends of cervical vessels. This implanting operation can be easily performed during routine cerebral angiography without robot assistance. The implant is fully internalised with no wires coming out from the head or body. Patients can quickly start using this device right after the implanting operation. Through encoding the brain thinking signals and controlling external devices wirelessly, this device can help patients to carry out daily life tasks, such as sending messages, emailing, online shopping and receiving telemedicine, which facilitates patients' communication and promotes patients' ability to live independently. As we all know, approximately 80% of patients who had an acute stroke have upper limb dysfunction, and approximately 60% of the patients still suffer from upper limb dysfunction 6 months after stroke. Currently, a multicentre randomised controlled phase III clinical trial of vagus nerve stimulation combined with rehabilitation training in the treatment of upper limb dysfunction after stroke is being carried out in 19 stroke rehabilitation institution in the UK and the USA. The primary aim of this clinical trial is to compare the efficacy between vagus nerve stimulation combined with rehabilitation training and rehabilitation training alone, and whether vagus nerve stimulation combined with rehabilitation training can be safer and more effective to promote upper limb function recovery in patients who had a stroke.[115] In a word, studies of brain–computer interface, deep brain stimulation, peripheral nerve stimulation or combined stimulation technologies are gradually being transformed into clinical practice. We believe that these innovative technologies will contribute substantially to neurorehabilitation in the near future.

Applications of rehabilitation robots in rehabilitation

The rehabilitation robot system was introduced in the field of stroke in the 1990s, using a combination of devices with actuation, perception, automation and artificial intelligence-based capabilities.[116] The rehabilitation robot technologies include various kinds of robotic devices used to improve sensorimotor functions of human bodies, such as hands, arms, legs, ankles and so on.[117–120] Currently, robotic equipments are under developed in ways of combining movements of different rehabilitation sites, such as the associated movement of hand and upper arm, upper and lower limbs,[121–123] or combining with electric stimulation.[124] Rehabilitation robots can also help to recommend adjunctive training therapies and assess patients' performance in sensorimotor functions.[125] One advantage of rehabilitation robot is to free rehabilitation therapists from heavy physical labour. As rehabilitation therapists are severely understaffed in developing countries, patients can also have good access to rehabilitation training with the assistance of rehabilitation robot. Studies showed that rehabilitation training through robots was an effective assistant therapy for patients who had a stroke with movement disabilities. Based on the patient-centred conception, the research and development of wearable rehabilitation robot is developing rapidly. The major advantages of rehabilitation robot include: (1) good matching and close fitting with human segments and joints; (2) easy accepted in patients as designed around human bodies and functions; (3) is a biomechanical electronic combined working system with various functions; (4) patients can have sensorimotor and cognitive interactions with robots anf (5) substantial better therapeutic effects can be produced with the combination of advanced computer technologies, such as virtual reality, artificial intelligence and metaverse.

At present, much research of stroke rehabilitation robot is still focused on the motor function recovery of the limbs. Different types of robotic training models including active training, passive training and assistant training are used in clinical trials.[126] Rehabilitation physicians and therapists select the appropriate training pattern according to patient's condition and limb impairment assessment. For example, passive mode should be selected in patients with complete limb paralysis, where the patient's movements are completely controlled by the robot. Similarly, assist mode should be selected in patients with incomplete limb paralysis, where the robot helps the patient to perform the desired movement of the affected limb.[127 128] Therefore, the primary principle of stroke rehabilitation robot is providing sensorimotor feedback to promote the movement of impaired limbs.[129]

A systematic review assessing the effects of robot-assisted therapy on improving activities of daily living, arm function and arm muscle strength involved 1619 patients in 45 trial groups. Results found that using robot-assisted devices in rehabilitation settings slightly improved patients' activities of daily living, arm function and arm muscle strength. Adverse effects and treatment withdrawal were uncommon in robot-assisted arm training, suggesting that the use of robot-assisted arm training devices was safe and acceptable for most patients. Compared with traditional rehabilitation therapy, the intervention of rehabilitation robots could enhance the motor function of upper limb.[130 131]

In recent years, several robot-assisted neurorehabilitation systems have been developed to improve poststroke rehabilitation of hand movements, arm functions and gait. Such robot systems include moving image T-MANUS system, a robot platform with 2 df, which provides

horizontal movement of the elbow and shoulder joints. In a study comparing the therapeutic effects in patients who had a subacute stroke between moving image T-MANUS system and traditional rehabilitation methods, results showed that patients receiving robot-assisted treatment exhibited higher movement power and Fugl-Meyer Assessment (FMA) scores at the elbow and shoulder joints.[132] Moving imaging ME system contains a wrist-forearm orthosis and a robot attached to the affected arm. Usually, movements of the healthy forearm attached to the six-axis digitiser direct the robot to perform mirrored movements of the affected arm in a positive/passive mode, allowing subjects to perform shoulder and elbow movements in the horizontal plane. This system was tested on 21 hemiplegic patients and showed improvements in the FMA score of shoulder and elbow motor function,[127 133] Assistant rehabilitation and measurement guide system is known as another great robot system designed by rehabilitation institution of Chicago. The system allows patients to perform hand stretching task in both vertical and horizontal motions, where patients' hands or arms are attached to the splint and robot accordingly resists or assists the movement of impaired arms.[134 135] Results of preliminary clinical applications showed that robot-assisted rehabilitation could produce positive effects on the recovery of arm motor function in chronic hemiplegic patients.[136] Whole arm manipulator operating system is an adaptive manipulator with standard 4 df and torque-controlled actuators. This system allows active and passive control of robot arms simultaneously and recording assessing data of patients' motor function.[137] However, the robot-assisted rehabilitation system failed to figure out the patients' attention state and motor initiative. Combination of robot-assisted rehabilitation system and brain–computer interface system provided an opportunity to overcome these limitations, where the robots could perform movement control according to the EEG signals from the brain–computer interface system.[138 139]

Enough documented evidence has proved that robot-assisted rehabilitation can promote functional recovery of movement disorders in the past two decades since the pioneering study by Aisen *et al*.[140] Although types of assessing system varies, such as assessing functions of distal and proximal limbs, and clinical research patterns also show significant heterogeneity, it was generally believed that robot-assisted upper limb rehabilitation was safe and could greatly reduce the movement disorders of shoulder and elbow, where the motor function improvement showed statistical significance.[141 142] Noteworthily, some studies revealed that additional robot-assisted treatment to traditional therapies showed great therapeutic effects, although the effects also largely depended on the treatment stage.[143 144]

Applications of non-invasive brain stimulation in rehabilitation

Non-invasive brain stimulation (NIBS) techniques include transcranial magnetic, transcranial electric, transcranial ultrasonic stimulation, which are techniques that use physical factors such as magnetic, electric and acoustic factors to cross the skull and act on the brain, thereby regulating brain function and plasticity, and then affecting neurobehavior. Transcranial magnetic and electric stimulation have been widely used in clinical rehabilitation and have shown good results. However, due to the limited stimulation depth and spatial resolution of these two techniques, it is difficult to achieve accurate regulation, which limits their clinical application to a certain extent. In contrast, transcranial ultrasound has the advantages of stimulation depth and high spatial resolution and could become a promising brain regulation tool.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is known as one of the four '21st century brain science and technology' and is the most mature and commonly used tool in NIBS techniques. It is based on the principle of electromagnetic induction and electromagnetic conversion, penetrating the skull without attenuation of the magnetic field generated by the transient current of the stimulation coil. Induced currents generated by magnetic fields stimulate neurons to trigger a series of physiological and biochemical responses. By regulating neuronal activity, which in turn causes changes in brain function and behaviour. It ultimately improves the clinical manifestations of various neurological and psychiatric disorders. The Food and Drug Administration (FDA) of US approved reparative TMS (rTMS) for the treatment of depression in 2008, migraine in 2013, and obsessive–compulsive disorder in 2018. rTMS has also been recommended by the International Federation of Clinical Neurophysiology for the treatment of neuropathic pain, Parkinson's disease, poststroke dyskinesia and epilepsy, etc.[145] There is also increasing evidence that TMS improved motor dysfunction, aphasia, dysphagia, cognitive impairment and insomnia after stroke and becomes an important tool of stroke rehabilitation.

Clinically, there are two main stimulation modes of TMS used for treatment: rTMS and theta-burst stimulation (TBS), in which low-frequency rTMS and continuous TBS play an inhibitory role, while high-frequency rTMS and intermittent TBS play an excitatory role. According to the interhemispheric inhibition theory, the excitability of the affected brain is relatively reduced, while the excitability of the unaffected brain is relatively increased after stroke. Therefore, excitatory stimulation mode is generally used to stimulate the affected brain, while inhibitory stimulation mode is used to stimulate the unaffected brain. Stimulation sites of TMS are various and resulting in different effects: the most common stimulation site of TMS is the primary motor cortex (M1), which is used to treat motor dysfunction, dysphagia, spasm and complex regional pain syndrome after ischaemic stroke.[146 147] TMS acts on the dorsolateral prefrontal cortex (DLPFC) to improve cognitive impairment and depression after ischaemic stroke,[148] TMS stimulation of Broca's area is used to improve motor aphasia, while stimulation of Wernicke's

area is used to improve sensory aphasia.[149] Stimulation of the parietal lobe is usually used to improve hemineglect. Although TMS was widely used in the rehabilitation of patients who had ischaemic stroke, it is inconclusive which stimulation parameters such as frequency, number of stimuli, stimulation time and treatment course are the most effective. Large-sample clinical trials are needed for validation in the future.

Transcranial electric stimulation

Transcranial electric stimulation (tES) is to apply specific low-intensity currents to specific brain regions through electrodes, which can regulate synaptic plasticity, change cortical excitability and achieve the regulation of neural activity in the brain. It includes transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS). tRNS is the use of a pair of electrodes to deliver weak alternating current oscillating at random frequencies through the scalp.[150] It differs from tACS in that its frequency is a randomly acquired frequency range rather than a stimulus at a specific frequency.

The most used tES technique is tDCS, which can regulate neuronal activity in the cerebral cortex by acting on brain targets with constant, low-intensity direct current (1–2 mA). In general, the anode tDCS depolarises the resting potential of the cell membrane and increases cortical excitability, whereas the cathode tDCS hyperpolarises the resting potential and inhibits cortical excitability. In addition, it produces effects that are not limited to the stimulated area but also involve functional connectivity with nearby brain areas.[151] The application of anodal tDCS to the ischaemic stroke affected side of the brain, or cathodal tDCS to the stroke unaffected side of the brain, could promote motor function recovery after stroke.[152] Placing the anode in the DLPFC on the affected side and the cathode in the supraorbital margin on the contralateral side can effectively reduce poststroke fatigue.[153] It has also been investigated that tDCS placed in Broca's area and Wernicke's area attempted to improve aphasia after stroke, but the effect was uncertain.[154] In recent years, there is also some evidence confirming the effective role of tACS and tNS in stroke rehabilitation. Applying different frequencies of tACS to patients who had a chronic stroke, it was found that 20 Hz tACS can produce more heterogeneous modulation effects in brain regions, especially in movement-related regions, which may contribute to motor function recovery after stroke.[155] The application of alpha frequency tACS to the contralesional parietal posterior cortex reduced hemineglect symptoms in patients who had a stroke.[156] Application of tRNS stimulation improved FMA-upper extremit scores in patients who had a subacute ischaemic stroke.[157] So far, tES has not been clearly recommended for poststroke rehabilitation due to insufficient evidence-based evidence.

Transcranial focused ultrasound stimulation

Transcranial focused ultrasound stimulation (tFUS) is a disruptive non-invasive neuromodulation technique. It generally uses low-frequency low-intensity ultrasound parameters, which produce mechanical biological effects to regulate brain function without thermal effects and tissue damage. Compared with other non-invasive neuromodulation methods, tFUS has higher spatial resolution and precision control, and can target deeper brain regions to achieve painless non-invasive deep brain stimulation.

Transcranial ultrasound can regulate brain function through multiple pathways, such as promoting neural regeneration and angiogenesis, affecting astrocyte and microglial function,[158] and directly inducing neuronal activity thus modulating the excitability of neural tissues.[159] However, some studies have also confirmed that ultrasound can cause neuronal activation in the brain through the cochlear pathway using different parameters.[160] Human studies have demonstrated that ultrasound stimulation modulates neural excitability. Low-frequency tFUS applied to the human primary sensory cortex and found that it could improve sensory discrimination[161 162]; low-frequency ultrasound stimulation applied to the human visual cortex could produce visual evoked potentials similar to light stimulation.[163] Ultrasound can also act

Table 5 The newly tested methods/technologies for stroke treatment and rehabilitation

Newly tested methods/ technologies	Characteristics and applications	Refs
Brain–computer interface	Control movements and external devices via deciphering cortical EEG activity to help regain motor control and fundamental life functions	108–114
Rehabilitation robots	A combination of devices with actuation, perception, automation and artificial intelligence-based capabilities aimed to assess or improve sensorimotor functions	119–128
TMS	Non-invasively regulate neuronal activity via magnetic stimulation to promote motor dysfunction, dysphagia, spasm, sensory or motor aphasia, hemineglect, cognitive impairment and depression	145–149
tES	Promote motor function, poststroke fatigue, aphasia, hemineglect recovery via stimulating specific brain regions through electrodes using specific low-intensity currents.	150–157
tFUS	Regulate brain function via mechanical biological effects without thermal effects, can act on deep brain tissue	158–170

EEG, electroencephalogram; tES, Transcranial electric stimulation; tFUS, transcranial focused ultrasound stimulation; TMS, transcranial magnetic stimulation.

on deep brain tissue, and studies have found that it can regulate the thalamus causing changes in somatosensory evoked potentials (MEP).[164] Currently, clinical studies of ultrasound brain stimulation have involved patients with neurological and psychiatric disorders including depression, epilepsy, dementia, chronic pain and traumatic brain injury.[165]

We previously applied low-frequency low-intensity ultrasound to the ischaemic mouse brain. We found that tFUS could cause haemodynamic changes, improving cerebral blood supply. tFUS therapy also reduced BBB damage thereby achieving neuroprotection and improving motor function in the early stage of ischaemic stroke[166]; while it could affect microglial polarisation in the late stage of cerebral ischaemia and promote neurorepair by reducing inflammatory response, thereby promoting motor function recovery after stroke.[167] It has also been found that tFUS can improve motor function in stroke mice by regulating cerebellar function. Researchers applied low-intensity ultrasound to stimulate the lateral cerebellar nucleus of cerebellum in mice with MCAO and found that it can improve sensorimotor and balance function in stroke mice.[168 169] In clinical study, tFUS has been found to improve cognitive impairment after stroke. This study included 60 patients with cognitive impairment after stroke and found that the ultrasound treatment group scored significantly higher than the control group in terms of execution, naming, attention, language and delayed recall. They also demonstrated that the auditory event-related potential P300, known as the 'cognitive potential', and brain-derived growth factor were also changed.[170]

At present, there is a lack of clinical trials of tFUS in the treatment of stroke. Due to the large differences between animal models and clinical patients, there are still many problems to be solved in the clinical application of tFUS. Ultrasound, however, has demonstrated its powerful role in regulating brain plasticity, altering short-term excitability and connectivity of the brain, inducing long-term plasticity and regulating behaviour. Given its beneficial effects in other diseases and its unique advantages, it may have some potential over other brain stimulation techniques in improving stroke dysfunction.

The characteristics and applications of the above new technologies for rehabilitation therapy are shown in table 5.

CONCLUSIONS

As the ageing of population and the progress of stroke treatment, the number of patients who had a stroke with dysfunction is increasing and the rehabilitation of stroke is increasingly important. However, lacking ideal animal models, quantitative or semiquantitative methods in assessing rehabilitation effectiveness is still a major obstacle to the development of stroke rehabilitation. We comprehensively discussed rodent and large animal models as well as in vitro models for ischaemic stroke rehabilitation. Researchers should choose suitable models according to the study purpose, and take the animal strains, sex, age and ischaemic underlying diseases into account. Advanced stroke rehabilitation emphasises to promote neural network reconstruction and compensation based on the theory of brain plasticity via different and joint rehabilitation therapist technologies. Accordingly, techniques for monitoring neural network remodelling and circuit formation are increasingly developed and used in stroke rehabilitation, such as fMRI, two-photon microscopy, wide-area calcium imaging, optogenetics and so on. BCI, rehabilitation robot and NIBS, such as TMS, tES and tFUS, are now hot and promising technologies in this field, which exhibit great therapeutic effects with or without the combination of traditional therapies. We believe the development and application of these technologies will surely boost the progress of rehabilitation in stroke.

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ORCID iDs

Jixian Wang http://orcid.org/0000-0002-3660-3175
Guo-Yuan Yang http://orcid.org/0000-0003-3105-9307

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