Current Neurology and Neuroscience Reports (2019) 19: 43
https://doi.org/10.1007/s11910-019-0959-2

NEUROREHABILITATION AND RECOVERY (J KRAKAUER AND T KITAGO, SECTION EDITORS)

Pharmacological Enhancement of Stroke Recovery

Amit Kumar [1,2] · Tomoko Kitago [1,2]

Published online: 30 May 2019

Abstract

Purpose of Review This review aims to discuss the recent literature relating to drugs for stroke recovery and to identify some of the challenges in conducting translational research for stroke recovery.

Recent Findings Advances in our understanding of neural repair mechanisms in pre-clinical stroke models have provided insights into potential targets for drugs that enhance the repair/recovery process. Few drugs that act on serotonergic and dopaminergic systems have been tested in humans with mixed results. The FOCUS trial, a phase III study of early administration of fluoxetine for stroke recovery, failed to replicate the promising results of the FLAME trial, but outcome measures differed between the two trials. Another drug that has recently been shown to have potential to promote motor recovery after stroke is maraviroc, an inhibitor of C-C chemokine receptor 5 that is involved in learning and memory.

Summary Various drugs, including modulators of neurotransmitters, axonal growth inhibitor blockers, and growth factors, have been examined in preclinical and clinical studies for their ability to promote neural repair, particularly in the motor system. Neuroplasticity, broadly defined as the capacity of the brain to undergo biochemical, structural, or functional changes, is heightened early after stroke when behavioral improvements are observed. Further studies are needed to determine which of these neuroplastic processes are causal to recovery and therefore appropriate targets for drugs to promote recovery. There has also been little focus on trying to distinguish processes that promote true behavioral recovery versus those that improve task success through use of compensatory strategies. Incorporation of sensitive and detailed outcome measures that assess movement quality as well as task success in both preclinical and clinical studies are needed to further elucidate appropriate drug targets and improve the translation of preclinical findings into successful clinical trials.

Keywords Stroke · Stroke recovery · Neuroplasticity · Neuroprotection · Pharmacotherapy

Introduction

Recent advances in our understanding of neural repair mechanisms (post-stroke changes mediating behavioral recovery) in animal models of stroke now offer potential therapeutic targets for drugs that enhance stroke recovery. Presently, the cornerstone of treatment for recovery after stroke is neurorehabilitation, in which behavioral training is provided through cognitive, speech, occupational, and physical therapy. Recovery is most often incomplete with neurorehabilitation alone, and there is evidence to suggest that current rehabilitation methods have little impact on motor recovery after stroke [1]. A variety of outcome measures have been used in both preclinical and clinical studies to assess recovery, which can lead to disparate results. Behavioral assessments may capture recovery at the level of impairment (e.g., strength and motor control) or activity (e.g., task-based measures such as pellet reaching in rodents, or activities of daily living in humans). While improvement in activity is often emphasized in clinical neurorehabilitation and remains an important goal, most task-based measures lack assessment of movement quality and are unable to distinguish between true recovery (impairment reduction) and behavioral substitution (use of alternate compensatory strategies) [2, 3].

This review focuses on several pharmacological approaches that are under investigation for promoting neural repair and neuroprotection after stroke. Finally, we discuss important considerations in the development of

This article is part of the Topical Collection on *Neurorehabilitation and Recovery*

✉ Tomoko Kitago
tok7002@med.cornell.edu

[1] Burke Neurological Institute, 785 Mamaroneck Ave., White Plains, NY 10605, USA

[2] Weill Cornell Medicine, New York, NY, USA

pharmacological strategies for stroke recovery, including the specific timing of these strategies and the combination of pharmacological agents with rehabilitative training.

Spontaneous Biological Recovery After Stroke: Lessons to Learn

The brain's response to stroke involves a series of spatial and temporal dynamic changes at the molecular, cellular, structural, and functional levels. The acute phase involves neuronal excitotoxicity and cell death within the infarct core and peri-infarct region. This is followed by a sub-acute phase (days to weeks) of heightened neuroplasticity, defined as the capacity of one or more units of the brain to undergo biochemical, structural, or functional changes in response to intrinsic or extrinsic signals. During this subacute period, a number of endogenous processes are highly active, including increases in levels of growth factors, axonal sprouting, dendritic remodeling, and changes in cortical excitability and synaptic plasticity. Some of these processes have been shown to mediate improvements in behavior, while others have been shown to correlate with improved behavior but are yet to be proven as processes causal to recovery. In pre-clinical models, many of the assessments used are measuring activity (task-based measures) and are unable to distinguish between impairment reduction and compensation. Therefore, improvements in behavior seen in these models may or may not represent true recovery [4–10]. The use of measures that quantify impairment and movement quality, rather than solely focusing on task success, is needed in future studies to examine the effect on true recovery [3].

The period of heightened neuroplasticity also gives rise to a "sensitive period" early after stroke [11, 12], during which there is increased responsiveness to rehabilitative training. For example, in rodents with experimentally induced stroke, exposure to an enriched environment with skilled reach practice led to significantly greater behavioral improvements when started at 5 or 14 days, but not 30 days [13]. In the chronic phase, spontaneous biological recovery ends with stabilization of post-stroke behavioral deficits.

The brain has a remarkable capacity for neuroplasticity after stroke [14, 15], with different cellular and molecular mechanisms engaged at different phases of stroke recovery [16]. Drugs that enhance recovery in one phase may be either ineffective or even harmful at another phase of recovery [17, 18]. For example, the acute phase of injury involves cell death and excitotoxicity, which requires a therapeutic strategy focused on neuroprotection (defined as the ability to protect neurons from injury and cell death) and countering excitotoxicity, but once neurons survive this phase and the excitotoxicity has subsided and the environment becomes predominantly inhibitory, the therapeutic priority should shift from neuroprotection to neural repair and enhancing excitability in the peri-infarct region. Surprisingly, most of the therapeutic efforts have been monotherapies targeting neuroprotection or neural repair/recovery in an isolated fashion. Furthermore, given the many repair-related processes that are active during spontaneous biological recovery, combinatorial repair-based strategies targeting multiple pathways may be needed to achieve clinically significant functional recovery.

Drugs to Enhance Stroke Recovery

Many drugs capable of enhancing repair-related processes (post-stroke changes mediating true recovery) and neuroplasticity (all post-stroke changes) are currently under study for stroke recovery at various stages of the translational pipeline. Here, we focus on recent pharmacological approaches that show promise for enhancing recovery from stroke, including drugs that act on neurotransmitters (serotonergic, dopaminergic, GABAergic, glutamatergic), drugs targeting axonal growth, and growth factors.

Selective Serotonin Reuptake Inhibitors One of the more promising recent advances in the pharmacological enhancement of stroke recovery has come with positive trials of SSRIs for stroke recovery. SSRIs, which are widely used in the treatment of depression, influence numerous biological processes involved in learning, memory, and neuroplasticity including hippocampal neurogenesis [19], secretion of neurotrophic factors such as BDNF [20], modulating excitatory/inhibitory balance in the brain [21, 22]. Fluoxetine has been demonstrated to reopen the critical period of ocular dominance plasticity in the visual system of adult rodents [22]. Preclinical studies of motor recovery after stroke with SSRIs have yielded mixed results, with some showing no effect [23, 24] and others showing benefits of SSRIs [25, 26] on training and recovery. Evidence from clinical studies has generally been more positive, with initial, small-scale studies showing benefits for stroke recovery [27, 28] and enhancement of motor performance in chronic stroke patients [29]. In the recent FLAME trial (Fluoxetine for Motor Recovery After Acute Ischemic Stroke), the SSRI fluoxetine was found to significantly enhance motor recovery after stroke in humans [30]. This study was a double-blinded, placebo-controlled phase II trial involving 118 patients with recent ischemic stroke causing moderate to severe hemiparesis. Patients were excluded if they met criteria for depression based on a standard depression assessment. Patients were randomly assigned to either fluoxetine (20 mg once per day, orally) or placebo for 90 days starting 5–10 days after stroke. All patients received rehabilitation as part of their clinical care, though this was not standardized for the study. Importantly, the fluoxetine group had a significant, 10-point increase in their Fugl-Meyer motor score compared to the placebo group, as well as an increase in the proportion

of patients with modified Rankin scores 0–2. Additionally, the fluoxetine-treated group showed lower rates of depression during the course of the study, which raises the question of whether the antidepressant effects of the medication could have led to greater motivation during rehabilitation, leading to indirect effects on motor recovery. The results of the FLAME trial, while promising, have not been replicated in larger phase III studies to support the routine use of SSRIs for post-stroke recovery. FOCUS, a recently completed phase III randomized, placebo-controlled trial with 3127 patients with stroke, showed no benefit of fluoxetine over placebo at 6 months [31]. However, the main outcome measure in this trial was the modified Rankin scale measuring overall disability; thus, an effect of fluoxetine specifically on motor recovery was not examined. Two other trials of early fluoxetine administration for recovery—AFFINITY and EFFECTS—are currently ongoing [32]. Notably, other related SSRIs have also been found to improve neurological recovery in patients [33•].

Dopaminergic Drugs Dopamine is another monoaminergic neurotransmitter that is linked to many neurobiological functions including synaptic transmission, reward processing, and regulation of movement [34–36]. Dopaminergic drugs are already widely used for the treatment of Parkinson's disease, with established safety profiles. Dopaminergic input to the motor cortex contributes to neuroplasticity and motor learning in preclinical studies [37, 38] and in patients with stroke [39]. One of the earliest clinical trials of dopaminergic therapy for stroke recovery was a randomized, placebo-controlled trial of 53 patients with recent stroke, which found that patients given 100 mg of L-Dopa, a precursor of dopamine, prior to physiotherapy for 3 weeks had significantly greater motor improvements measured by the Rivermead Motor Assessment compared with the placebo group [40]. However, subsequent small trials of dopaminergic drugs for stroke recovery have been largely negative [41, 42]. The results of the Dopamine Augmented Rehabilitation in Stroke (DARS) trial, which randomized 593 patients with recent stroke to co-careldopa or placebo for 6 weeks in combination with routine occupational and physical therapy, are unpublished but reportedly showed no benefit of dopaminergic therapy in the proportion of patients walking independently (abstract presentation—ESOC 2015). The mixed results of these dopamine studies may be due to the small sample sizes, differences in timing of drug administration in relation to therapy, and the different outcome measures used. Genetic polymorphisms in dopamine-related genes (catechol-O-methyltransferase, and dopamine receptors (DR1, DR2, and DR3) have also been suggested to affect motor recovery after stroke [43•] and may contribute to variable effects of dopaminergic drugs on recovery.

Modulation of Excitation/Inhibition with GABAergic or Glutamatergic Drugs After stroke, changes in excitability have been demonstrated in the peri-infarct region and in remote regions with structural or functional connections to the infarcted area [44]. The peri-infarct region becomes hypoexcitable after stroke due to increases in extracellular γ-aminobutyric acid (GABA) caused by impairment in GABA transporter function leading to increased tonic GABA signaling mediated by extrasynaptic $GABA_A$ receptors [45]. Pharmacological strategies to block this pathologically upregulated GABA signaling, or to increase excitatory glutamatergic signaling have been explored as means to re-establish a balance between excitation and inhibition and promote functional recovery. Clarkson and colleagues demonstrated that delayed administration of an α5-$GABA_A$ receptor antagonist (or genetic knockdown of $GABA_A$ receptor subunits) led to improved functional motor recovery [46]. Interestingly, treatment with the α5-$GABA_A$ receptor antagonist starting on the day of stroke, but not day 3, led to increased stroke volume, suggesting a time-dependent effect of this strategy.

Enhancing glutamatergic signaling is another way to upregulate neuronal excitability. In a preclinical study, a positive modulator of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor signaling led to improved recovery of motor function [47] mediated by peri-infarct increases in BDNF. BDNF is one of the crucial factors involved in improving the plasticity mediating memory and cognition [48, 49]. Glutamatergic AMPA and N-methyl-D-aspartate (NMDA) receptors also play a critical role in long-term potentiation and learning and memory. Memantine, an NMDA receptor antagonist used in the treatment of Alzheimer's disease, was found to have benefits for motor recovery in a mouse model [50]. Mice that received memantine for 28 days beginning 2 h after experimental stroke showed greater improvements in forelimb control compared with the placebo group. Infarct size, motor maps, and behavior were not significantly different with memantine treatment, suggesting that the effect of the drug was not neuroprotective, but related to recovery. Increased BDNF expression was found in peri-infarct areas and has been reported by others [48].

Drugs Targeting Other Neurotransmitter Systems Noradrenergic drugs such as atipamezole and reboxetine, although tested only in small studies in the sub-acute and chronic stroke populations, have also shown promising results [51, 52]. In addition, cholinergic drugs such as donepezil, which enhances not only the level of neurotransmitters but also the duration of their activity, have shown some positive results in patient trials, but more studies are required to assess their efficacy [53, 54].

Targeting Axonal Growth Axonal sprouting and the formation of new connections have been linked to behavioral recovery after experimental stroke [8–10]. For instance, GDF10 (growth and differentiation factor 10) delivery starting 1 week after stroke has been shown to improve forelimb motor tasks such as grid walking, cylinder, and pasta handing tasks in mice through a unique transcriptome-mediated enhancement of axonal sprouting. SiRNA-mediated knockdown of GDF10 was shown to block axonal sprouting and reduce behavioral recovery [8]. Similarly, another study from Carmichael and colleagues also implicated axonal sprouting as one of the key mediators of post-stroke behavioral recovery by knocking down Ephrin A5, which is expressed in reactive astrocytes in peri-infarct cortex and inhibits axonal sprouting. This blockade of ephrin A5 was shown to induce new projections in premotor, motor and prefrontal circuits and to mediate recovery in forelimb motor tasks such as grid walking, cylinder task through these connections [10]. Together, these studies have shown axonal sprouting as an event causal to behavioral recovery, thereby providing evidence in favor of axonal sprouting and new projections as part of neural repair mediating the recovery. Interestingly, a number of other structural and biochemical changes such as changes in cortical maps, formation of new circuits, angiogenesis, and neuronal excitability occur after stroke, but it is still not clear if these processes truly mediate behavioral recovery. Thus, another potential therapeutic strategy to promote recovery is to block the activity of endogenous axonal growth inhibitors. These inhibitory proteins include Nogo-A, myelin-associated glycoprotein (MAG), oligo-myelin glycoprotein (OMgp), and chrondroitin sulfate proteoglycans (CSPGs), which block the formation of new connections after stroke. Administration of monoclonal antibodies to these inhibitory proteins can promote axonal growth and behavioral recovery [55]. In a rodent model, the anti-MAG antibody GSK249320 administered intravenously starting within 24 h post-stroke was associated with improved composite neurological function and forelimb reaching, though this benefit was not seen when the therapy was initiated at 7 days post-stroke [56]. Furthermore, using immunohistochemistry, the drug was found to reach the ipsilesional hemisphere. These results and a primate study, suggesting efficacy of this approach [57], led to its testing into human patients in clinical trials. Despite these promising preclinical results, a recent phase IIb trial of 134 patients randomized to 2 IV infusions of GSK249320 or placebo demonstrated no benefit of the antibody in improving gait velocity [58••]. The authors discuss the choice of outcome measure, patient selection criteria, too low dose of the antibody, interspecies differences in stroke recovery, and lack of CNS target engagement as possible reasons for the negative results of the clinical trial. This highlights the difficulty of translating promising preclinical results into successful therapeutic strategies in humans.

Growth Factors Administration of neurotrophic factors (such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and basic fibroblast growth factor (bFGF)) or growth-promoting cytokines can stimulate multiple growth programs and activate a multitude of repair processes including neurogenesis, angiogenesis, axonal sprouting, and myelination. Additionally, these neurotrophins have been shown to play important roles in protecting neurons and improving behavioral outcomes, including restoration of coordinated hindlimb movement after spinal cord injury in rats and improved cognitive function in the Morris water maze test in mice after traumatic brain injury [59–61]. Indeed, treatment with neurotrophins such as stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF) has been shown to lead to significant improvements in animal models of stroke in the sub-acute [62, 63] and later phases (3–6 months) of recovery [64–67]. However, systemic administration of growth factors will also have unwanted side effects that are related to their specific actions, such as hematopoiesis or cell proliferation. Focused delivery of growth factors to targeted peri-infarct brain regions is desirable but has not yet been achieved.

C-C Chemokine Receptor 5 Signaling Inhibiting C-C Chemokine Receptor 5 (CCR5) has been demonstrated to enhance synaptic plasticity, learning, and memory in the hippocampus [68]. Though CCR5 is undetectable in neurons in normal cortex, its expression is highly upregulated after stroke [69]. In this recent study in mice, neuron-specific CCR5 knockdown in premotor cortex led to improved post-stroke motor functions, measured using the grid-walk and cylinder tests, as well as preservation of dendritic spines in the peri-infarct area and axonal projections to contralateral sensorimotor cortices. The investigators also showed that administration of maraviroc, an FDA-approved oral CCR5 antagonist currently used for treatment of human immunodeficiency virus (HIV), beginning 24 h post-stroke led to improved recovery of motor function compared with vehicle [69]. When maraviroc was administered 3 weeks after stroke, greater motor improvements were seen on the grid-walk test but not on the cylinder test, suggesting that this drug could be beneficial in subacute and chronic stages of stroke. A phase II clinical trial testing the efficacy of maraviroc in patients with subacute stroke is now underway (Clinicaltrials.gov NCT03172026).

Neuroprotective Strategies: Is There Potential for Clinical Utility?

Neuronal cell death is one of the immediate events following stroke. Stroke injury leads to massive cell death in the infarct core and significant damage to neurons in the peri-infarct area. In fact, there has been a good effort, at least in pre-clinical

studies, to protect damaged neurons mainly in the peri-infarct area, which if not protected would eventually die [70–72]. These neuroprotective strategies target acute processes such as excitotoxicity, glutamate release, NMDA receptor activation, Ca^{2+} influx, mitochondrial dysfunction, free radical production, nitric oxide production, inflammation, and apoptosis leading to early cell death in the infarct core and peri-infarct region. Few of these pre-clinical studies have shown significant neuroprotection in different animal models of stroke [73]. Some of these agents include antioxidants such as ebselen, edaravone, tirilazad mesylate, citicoline, and NXY-059; calcium channel blockers such as nimodipine and flunarizine; glutamate antagonists such as MK-801/dizocilpine, aptiganel, CGS-19755, dextromethorphan, ZK200755 AMPA receptor antagonist, YM872, and magnesium sulfate; GABA agonists such as clomethiazole and diazepam; and nitric oxide inhibitor lubeluzole and multi-target agents such as statins, minocycline, and albumin. Notably, all of these drugs have failed to augment recovery in stroke patients in randomized clinical trials [74].

The question then arises, why should we still search for neuroprotective drugs? Although repair-enhancing drugs have shown promising results in both animal models as well as human patients, their targets of actions are limited only to spared neurons or circuits. By limiting cell death and sparing brain regions and connections that are important for functional activity and recovery [75–77], successful neuroprotective strategies even when incomplete will provide more neural substrate that can be acted upon by rehabilitative training or by other drugs. A recent multi-center phase II clinical trial of a neuroprotective agent, 3K3A-APC (RHAPSODY trial), which affects multiple signaling targets, has shown some promise with respect to safety, dose tolerability, and neuroprotection [78••, 79]. 3K3A-APC is a protease-activated receptor 1 (PAR1) agonist, a modified version of native activated protein C (APC), which has protective effects on neurons and endothelial cells [80] to promote vascular integrity. In this study, patients undergoing thrombolysis with tissue plasminogen activator (tPA) or mechanical thrombectomy were randomized to receive drug or placebo. This dose determination and safety trial in 110 patients found that total intracranial hemorrhage rates and hemorrhage volume were reduced in 3K3A-APC-treated patients. However, the proportion of patients with favorable 90-day outcomes on assessments of disability and activity (modified Rankin scale score of 0 or 1 and Barthel Index ≥ 90) were not significantly different between groups. Further large-scale clinical trials are required in order to examine efficacy of the drug with respect to not only hemorrhage but also impairment reduction in patients.

As mentioned above, NMDA antagonists can inhibit post-stroke excitotoxicity in the acute phase by inhibiting pathological activity of NMDA receptors (NMDARs), but they also inhibit physiological synaptic activity which limits their therapeutic use. NMDARs are composed of NR1, NR2A-2D, and NR3A-B sub-units that form various receptor complexes in different combinations and have different roles. Importantly, the NMDAR sub-unit NR2B which is located extrasynaptically has been shown to be involved in pathological excitotoxicity, while synaptic NMDAR subunits such as NR1/NR2A and NR1/NR2C have been shown to play a significant role in physiological synaptic activity. Most of the NMDA antagonists tried before the last decade actually targeted synaptic NMDARs either in a competitive manner [such as D-2-amino-7-phosphovalerate (APV) and 3-(2-carboxypiperzin-4-yl) propyl-1-phosphonate (CPP)] or in a non-competitive manner [such as dizocilpine (MK-801), phencyclidine (PCP)] [81]. In the last decade, a huge interest in blocking the extra-synaptic NMDAR has led to the development of a number of effective and selective NR2B antagonists, which showed significant neuroprotective effects with minimal effects on physiological synaptic activity [82].

Besides NR2B antagonists, scientists have also shown enormous interest in screening/developing open channel non-competitive inhibitors with faster on/off NMDAR blocking kinetics [83, 84]. This property of blockers allows them to effectively interact with NMDARs in a rapid voltage-dependent manner and block the pathological synaptic activity while sparing the normal physiological synaptic activity by their fast dissociation [81]. Memantine has been shown to be one of the most thoroughly investigated drug in this class. Interestingly, memantine has been shown to enhance the level of brain-derived neurotrophic factor (BDNF) and improve motor recovery assessed through grid-walking performance and cylinder task in mice when given chronically in drinking water at 30 mg/kg beginning more than 2 h after photothrombotic stroke [50]. Trotman et al. [85] recently found that memantine provided neuroprotection (assessed using 28-point neurological score) only at lower concentrations (0.2 mg/kg) but failed to do so at higher concentrations (2–10 mg/kg) which raises the possibility of its off-target actions. Moreover, clinical studies are lacking.

One of the key problems associated with NMDAR targeting is the possible disruption of physiological synaptic plasticity. To alleviate this issue, a number of groups have recently focused on targeting players downstream of NMDAR synaptic activity [86–88]. One of the downstream events of excitotoxic signaling cascade is the formation of a signaling complex of NR2B with PSD-95 (post-synaptic density protein 95) and nNOS (neuronal nitric oxide synthase). This signaling complex leads to NO (nitric oxide) production and thereby causes excitotoxicity. Tymianski and colleagues used a synthetic peptide, called Tat-NR2B9c (also known as NA-1) which was able to specifically inhibit protein-protein interaction and disrupt NR2B-PSD-95-nNOS signaling complex and provided significant neuroprotection in rat models of ischemia [86]. In this study, treatment with Tat-

NR2B9c was found to show significant improvement in the 24 h composite neurological score. In clinical settings, NA-1 has already been successfully tested for its safety and efficacy in reducing the number of iatrogenic strokes in patients undergoing endovascular repair of brain aneurysms in phase 2 clinical trial (ENACT Trial) [89]. However, National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale scores at 30 days showed no differences between NA-1 and control groups; detailed assessments of particular impairments were not included in this study.

Another major focus of the therapeutic efforts is directed towards expanding the time window of treatment for stroke. With the success of late mechanical thrombectomy in appropriately selected patients in the DEFUSE 3 and DAWN trials [90••, 91••], one can envision potential benefits of using neuroprotectants that can prolong neuronal survival until reperfusion can be achieved or limit reperfusion injury. Of note, NA-1 has been shown to provide neuroprotection in middle cerebral artery occlusion (MCAO) model of ischemia in rats even with delayed treatments such as 3 h or even day 4 after MCAO [92, 93], which again supports the idea of using neuroprotectants to prolong the neuronal survival and expand the therapeutic window. With a similar goal, NA-1 is currently being tested in phase 3 clinical trial (ESCAPE-NA-1) as a neuroprotectant in patients with acute ischemic stroke undergoing endovascular revascularization.

Timing of Drug Therapies for Stroke Recovery

One must give drug therapies at the appropriate stages of recovery in order to maximize benefits and minimize harmful side effects. Neuroprotective agents that target acute excitotoxicity by either controlling glutamate release or glutamate receptors (NMDAR or AMPR) or Ca^{2+} influx not only decrease pathological plasticity (i.e., excitotoxicity) but could also exacerbate the pathological inhibitory peri-infarct environment in the sub-acute and chronic phases. Similarly, GABA-A antagonism can exacerbate excitotoxic cell death and increase stroke volumes when administered immediately after stroke, but delayed administration will lead to improved outcomes [46]. Another combinatorial approach is to give neuroprotective drugs (if and when there are neuroprotective drugs that are found to have clinical benefit) early and repair-enhancing drugs later. Indeed, simvastatin, a neuroprotective drug, when combined with the neurotrophin G-CSF was found to decrease the recovery time and reduce severity of neurological deficits and sensorimotor asymmetry in a rat model of intracerebral hemorrhage [94].

The majority of the studies mentioned above have been conducted in the acute and subacute phases of recovery. The question still remains open if these viable strategies are appropriate for promoting further recovery for patients with chronic stroke. Chronic impairments have been challenging to improve with training alone. In the VA ROBOTICS study, patients with chronic stroke who received 36 sessions of upper limb robotic-assisted therapy with > 1000 movements per session did not significantly reduce their motor impairment as measured with the Fugl-Meyer scale after 12 weeks of therapy compared to patients who received usual care or matched occupational therapy [95]. Small improvements on activity measures have been reported in the chronic stroke population with other types of therapy including constraint-induced movement therapy (CIMT) and mirror therapy (MT) [96, 97], though these improvements may reflect learning of compensatory strategies rather than impairment reduction. With very high-intensity therapy, it is still possible to achieve significant impairment reductions in chronic stroke patients, as seen in the studies by McCabe and colleagues (300 h of therapy over 12 weeks; 5 h per day, 5 days per week) [98], and the Queen Square Neurorehabilitation program (90 h of therapy over 3 weeks; 6 h per day, 5 days per week) [99]. However, this intensity of treatment would be difficult to implement in a general stroke population with currently available resources in rehabilitation centers. By combining behavioral training with pharmacological approaches to enhance plasticity, we can hope to elicit large reductions in impairment in the chronic phase of recovery even with lesser amounts of training.

There is a great interest in "reopening the sensitive period" in patients with chronic stroke. Investigations into events involved in opening and closing of the critical period during normal development, or the sensitive period during spontaneous biological recovery, have yielded insights into how to reinitiate these periods once that window has closed. Fluoxetine can reopen a critical period of visual cortex plasticity in adult animals [22], though the generalizability of this finding is called into question by the results of a study on motor recovery, where it was required to be given early after stroke to have beneficial effects on recovery [26]. Other pharmacological strategies for reopening the sensitive period that are under investigation include digestion of CSPGs and perineuronal nets involved in closure of critical periods during development [100, 101] and blocking Nogo, an inhibitor of axonal growth [102, 103].

Combining Drugs with Rehabilitative Training

Giving repair-enhancing drugs in isolation is unlikely to optimally enhance functional recovery; pairing these drugs with training may be needed to establish functionally relevant connections. Indeed, in an early study of amphetamines, Feeney et al. found that amphetamine improved behavioral outcomes only when paired with training [104]. Drugs that enhance plasticity need behavioral training to strengthen functionally relevant connections. This principle has been demonstrated

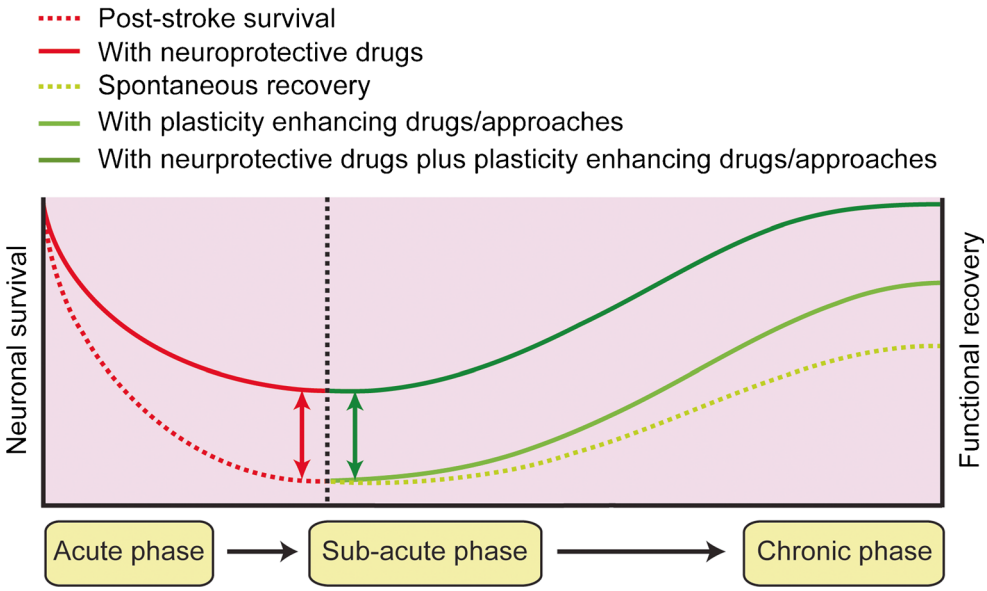

Fig. 1 The model illustrating a proposed treatment regimen where subjects should be treated first with a neuroprotective drug in the acute phase followed by plasticity-enhancing pharmacological/non-pharmacological approaches in sub-acute and chronic phases of recovery. Neuroprotective drugs would enhance the neuronal survival and thereby provide more neural substrates to be acted upon by plasticity enhancing pharmacological drugs in combination with neurorehabilitation to maximize functional recovery

with various drugs and training regimens [40, 42, 53, 105, 106]. While there remains much work to be done to optimize rehabilitative training regimens to maximize impairment reduction, some general principles are acknowledged: Training should be high-dose, emphasize movement quality, and be engaging [98, 99]. Future studies should also explore relative timing of drug and training interventions for short-acting drugs such as L-dopa, or for drugs such as SSRIs that can take time to achieve therapeutic effects.

Conclusions

In conclusion, stroke-induced functional and structural remodeling in the central nervous system leads to a variety of pathophysiological changes but simultaneously opens a window of pro-adaptive therapeutic opportunity. Stroke leads to dynamic temporal and spatial changes at different phases of recovery. Therefore, it is important not just to focus on appropriate drugs but also to synergize appropriate drugs with appropriate phases of the post-stroke recovery. Moreover, instead of using either neuroprotective or repair-based drugs individually, it would be wise to combine both but only at their respective correct phases of the recovery. We propose a model of the treatment regimen (Fig. 1) where patients are first treated with a neuroprotective agent acutely and then repair-based pharmacological/non-pharmacological approaches at sub-acute and chronic phases of recovery. Further understanding from preclinical models of timing and regulation of various repair mechanisms will inform the search for appropriate therapeutic strategies at different time points after stroke.

Acknowledgements This work was made possible by grants from Goldsmith Fellowship to Amit Kumar for transition to independence.

Compliance with Ethical Standards

Conflict of Interest Amit Kumar and Tomoko Kitago each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Stinear CM, Byblow WD, Ackerley SJ, Smith MC, Borges VM, Barber PA. Proportional motor recovery after stroke: implications for trial design. Stroke. 2017;48(3):795–8. https://doi.org/10.1161/STROKEAHA.116.016020.
2. Kitago T, Liang J, Huang VS, Hayes S, Simon P, Tenteromano L, et al. Improvement after constraint-induced movement therapy: recovery of normal motor control or task-specific compensation? Neurorehabil Neural Repair. 2013;27(2):99–109. https://doi.org/10.1177/1545968312452631.
3. Corbett D, Carmichael ST, Murphy TH, Jones TA, Schwab ME, Jolkkonen J, et al. Enhancing the alignment of the preclinical and clinical stroke recovery research pipeline: consensus-based Core recommendations from the stroke recovery and rehabilitation roundtable translational working group. Neurorehabil Neural Repair. 2017;31(8):699–707. https://doi.org/10.1177/1545968317724285.
4. Alia C, Spalletti C, Lai S, Panarese A, Lamola G, Bertolucci F, et al. Neuroplastic changes following brain ischemia and their contribution to stroke recovery: novel approaches in neurorehabilitation. Front Cell Neurosci. 2017;11:76. https://doi.org/10.3389/fncel.2017.00076.

5. Ploughman M, Windle V, MacLellan CL, White N, Dore JJ, Corbett D. Brain-derived neurotrophic factor contributes to recovery of skilled reaching after focal ischemia in rats. Stroke. 2009;40(4):1490–5. https://doi.org/10.1161/STROKEAHA.108.531806.
6. Sanchez-Mendoza EH, Hermann DM. Correlates of post-stroke brain plasticity, relationship to pathophysiological settings and implications for human proof-of-concept studies. Front Cell Neurosci. 2016;10:196. https://doi.org/10.3389/fncel.2016.00196.
7. Font MA, Arboix A, Krupinski J. Angiogenesis, neurogenesis and neuroplasticity in ischemic stroke. Curr Cardiol Rev. 2010;6(3):238–44. https://doi.org/10.2174/157340310791658802.
8. Li S, Nie EH, Yin Y, Benowitz LI, Tung S, Vinters HV, et al. GDF10 is a signal for axonal sprouting and functional recovery after stroke. Nat Neurosci. 2015;18(12):1737–45. https://doi.org/10.1038/nn.4146.
9. Brown CE, Aminoltejari K, Erb H, Winship IR, Murphy TH. In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J Neurosci. 2009;29(6):1719–34. https://doi.org/10.1523/JNEUROSCI.4249-08.2009.
10. Overman JJ, Clarkson AN, Wanner IB, Overman WT, Eckstein I, Maguire JL, et al. A role for ephrin-A5 in axonal sprouting, recovery, and activity-dependent plasticity after stroke. Proc Natl Acad Sci U S A. 2012;109(33):E2230–9. https://doi.org/10.1073/pnas.1204386109.
11. Zeiler SR, Krakauer JW. The interaction between training and plasticity in the poststroke brain. Curr Opin Neurol. 2013;26(6):609–16. https://doi.org/10.1097/WCO.0000000000000025.
12. Zeiler SR, Hubbard R, Gibson EM, Zheng T, Ng K, O'Brien R, et al. Paradoxical motor recovery from a first stroke after induction of a second stroke: reopening a Postischemic sensitive period. Neurorehabil Neural Repair. 2016;30(8):794–800. https://doi.org/10.1177/1545968315624783.
13. Biernaskie J, Chernenko G, Corbett D. Efficacy of rehabilitative experience declines with time after focal ischemic brain injury. J Neurosci. 2004;24(5):1245–54. https://doi.org/10.1523/JNEUROSCI.3834-03.2004.
14. Jones TA, Adkins DL. Motor system reorganization after stroke: stimulating and training toward perfection. Physiology. 2015;30(5):358–70. https://doi.org/10.1152/physiol.00014.2015.
15. Grefkes C, Fink GR. Reorganization of cerebral networks after stroke: new insights from neuroimaging with connectivity approaches. Brain. 2011;134(Pt 5):1264–76. https://doi.org/10.1093/brain/awr033.
16. Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci. 2009;10(12):861–72. https://doi.org/10.1038/nrn2735.
17. Green AR, Hainsworth AH, Jackson DM. GABA potentiation: a logical pharmacological approach for the treatment of acute ischaemic stroke. Neuropharmacology. 2000;39(9):1483–94.
18. Kozlowski DA, Jones TA, Schallert T. Pruning of dendrites and restoration of function after brain damage: role of the NMDA receptor. Restor Neurol Neurosci. 1994;7(2):119–26. https://doi.org/10.3233/RNN-1994-7207.
19. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003;301(5634):805–9. https://doi.org/10.1126/science.1083328.
20. Bjorkholm C, Monteggia LM. BDNF - a key transducer of antidepressant effects. Neuropharmacology. 2016;102:72–9. https://doi.org/10.1016/j.neuropharm.2015.10.034.
21. Gerdelat-Mas A, Loubinoux I, Tombari D, Rascol O, Chollet F, Simonetta-Moreau M. Chronic administration of selective serotonin reuptake inhibitor (SSRI) paroxetine modulates human motor cortex excitability in healthy subjects. Neuroimage. 2005;27(2):314–22. https://doi.org/10.1016/j.neuroimage.2005.05.009.
22. Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, et al. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science. 2008;320(5874):385–8. https://doi.org/10.1126/science.1150516.
23. Windle V, Corbett D. Fluoxetine and recovery of motor function after focal ischemia in rats. Brain Res. 2005;1044(1):25–32. https://doi.org/10.1016/j.brainres.2005.02.060.
24. Jolkkonen J, Puurunen K, Rantakomi S, Sirvio J, Haapalinna A, Sivenius J. Effects-of fluoxetine on sensorimotor and spatial learning deficits following focal cerebral ischemia in rats. Restor Neurol Neurosci. 2000;17(4):211–6.
25. Li WL, Cai HH, Wang B, Chen L, Zhou QG, Luo CX, et al. Chronic fluoxetine treatment improves ischemia-induced spatial cognitive deficits through increasing hippocampal neurogenesis after stroke. J Neurosci Res. 2009;87(1):112–22. https://doi.org/10.1002/jnr.21829.
26. Ng KL, Gibson EM, Hubbard R, Yang J, Caffo B, O'Brien RJ, et al. Fluoxetine maintains a state of heightened responsiveness to motor training early after stroke in a mouse model. Stroke. 2015;46(10):2951–60. https://doi.org/10.1161/STROKEAHA.115.010471.
27. Dam M, Tonin P, De Boni A, Pizzolato G, Casson S, Ermani M, et al. Effects of fluoxetine and maprotiline on functional recovery in poststroke hemiplegic patients undergoing rehabilitation therapy. Stroke. 1996;27(7):1211–4.
28. Pariente J, Loubinoux I, Carel C, Albucher JF, Leger A, Manelfe C, et al. Fluoxetine modulates motor performance and cerebral activation of patients recovering from stroke. Ann Neurol. 2001;50(6):718–29.
29. Zittel S, Weiller C, Liepert J. Citalopram improves dexterity in chronic stroke patients. Neurorehabil Neural Repair. 2008;22(3):311–4. https://doi.org/10.1177/1545968307312173.
30. Chollet F, Tardy J, Albucher JF, Thalamas C, Berard E, Lamy C, et al. Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. Lancet Neurol. 2011;10(2):123–30. https://doi.org/10.1016/S1474-4422(10)70314-8.
31. Collaboration FT. Effects of fluoxetine on functional outcomes after acute stroke (FOCUS): a pragmatic, double-blind, randomised, controlled trial. Lancet. 2019;393(10168):265–74. https://doi.org/10.1016/S0140-6736(18)32823-X.
32. Graham C, Lewis S, Forbes J, Mead G, Hackett ML, Hankey GJ, et al. The FOCUS, AFFINITY and EFFECTS trials studying the effect(s) of fluoxetine in patients with a recent stroke: statistical and health economic analysis plan for the trials and for the individual patient data meta-analysis. Trials. 2017;18(1):627. https://doi.org/10.1186/s13063-017-2385-6.
33.• Savadi Oskouie D, Sharifipour E, Sadeghi Bazargani H, Hashemilar M, Nikanfar M, Ghazanfari Amlashi S, et al. Efficacy of citalopram on acute ischemic stroke outcome: a randomized clinical trial. Neurorehabil Neural Repair. 2017;31(7):638–47. https://doi.org/10.1177/1545968317704902 **This was a randomized placebo-controlled trial investigating the efficacy of citalopram to reduce stroke severity at 3 months. Citalopram was safe and led to a greater proportion of patients having a 50% reduction in NIHSS compared with placebo**.
34. Calabresi P, Picconi B, Tozzi A, Di Filippo M. Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci. 2007;30(5):211–9. https://doi.org/10.1016/j.tins.2007.03.001.

35. Schultz W. Getting formal with dopamine and reward. Neuron. 2002;36(2):241–63.
36. Costa RM, Lin SC, Sotnikova TD, Cyr M, Gainetdinov RR, Caron MG, et al. Rapid alterations in corticostriatal ensemble coordination during acute dopamine-dependent motor dysfunction. Neuron. 2006;52(2):359–69. https://doi.org/10.1016/j.neuron.2006.07.030.
37. Molina-Luna K, Pekanovic A, Rohrich S, Hertler B, Schubring-Giese M, Rioult-Pedotti MS, et al. Dopamine in motor cortex is necessary for skill learning and synaptic plasticity. PLoS One. 2009;4(9):e7082. https://doi.org/10.1371/journal.pone.0007082.
38. Hosp JA, Pekanovic A, Rioult-Pedotti MS, Luft AR. Dopaminergic projections from midbrain to primary motor cortex mediate motor skill learning. J Neurosci. 2011;31(7):2481–7. https://doi.org/10.1523/JNEUROSCI.5411-10.2011.
39. Floel A, Hummel F, Breitenstein C, Knecht S, Cohen LG. Dopaminergic effects on encoding of a motor memory in chronic stroke. Neurology. 2005;65(3):472–4. https://doi.org/10.1212/01.wnl.0000172340.56307.5e.
40. Scheidtmann K, Fries W, Muller F, Koenig E. Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomised, double-blind study. Lancet. 2001;358(9284):787–90. https://doi.org/10.1016/S0140-6736(01)05966-9.
41. Cramer SC, Dobkin BH, Noser EA, Rodriguez RW, Enney LA. Randomized, placebo-controlled, double-blind study of ropinirole in chronic stroke. Stroke. 2009;40(9):3034–8. https://doi.org/10.1161/STROKEAHA.109.552075.
42. Lokk J, Salman Roghani R, Delbari A. Effect of methylphenidate and/or levodopa coupled with physiotherapy on functional and motor recovery after stroke–a randomized, double-blind, placebo-controlled trial. Acta Neurol Scand. 2011;123(4):266–73. https://doi.org/10.1111/j.1600-0404.2010.01395.x.
43.• Kim BR, Kim HY, Chun YI, Yun YM, Kim H, Choi DH, et al. Association between genetic variation in the dopamine system and motor recovery after stroke. Restor Neurol Neurosci. 2016;34(6):925–34. https://doi.org/10.3233/RNN-160667 **This study suggested that genetic variation of dopamine-related genes may affect motor recovery after stroke and that COMT polymorphism could be useful for predicting motor recovery.**
44. Carrera E, Tononi G. Diaschisis: past, present, future. Brain. 2014;137(Pt 9):2408–22. https://doi.org/10.1093/brain/awu101.
45. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron. 2012;73(1):23–34. https://doi.org/10.1016/j.neuron.2011.12.012.
46. Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature. 2010;468(7321):305–9. https://doi.org/10.1038/nature09511.
47. Clarkson AN, Overman JJ, Zhong S, Mueller R, Lynch G, Carmichael ST. AMPA receptor-induced local brain-derived neurotrophic factor signaling mediates motor recovery after stroke. J Neurosci. 2011;31(10):3766–75. https://doi.org/10.1523/JNEUROSCI.5780-10.2011.
48. Meisner F, Scheller C, Kneitz S, Sopper S, Neuen-Jacob E, Riederer P, et al. Memantine upregulates BDNF and prevents dopamine deficits in SIV-infected macaques: a novel pharmacological action of memantine. Neuropsychopharmacology. 2008;33(9):2228–36. https://doi.org/10.1038/sj.npp.1301615.
49. Ranju V, Sathiya S, Kalaivani P, Priya RJ, Saravana Babu C. Memantine exerts functional recovery by improving BDNF and GDNF expression in 3-nitropropionic acid intoxicated mice. Neurosci Lett. 2015;586:1–7. https://doi.org/10.1016/j.neulet.2014.11.036.
50. Lopez-Valdes HE, Clarkson AN, Ao Y, Charles AC, Carmichael ST, Sofroniew MV, et al. Memantine enhances recovery from stroke. Stroke. 2014;45(7):2093–100. https://doi.org/10.1161/STROKEAHA.113.004476.
51. Beltran EJ, Papadopoulos CM, Tsai SY, Kartje GL, Wolf WA. Long-term motor improvement after stroke is enhanced by short-term treatment with the alpha-2 antagonist, atipamezole. Brain Res. 2010;1346:174–82. https://doi.org/10.1016/j.brainres.2010.05.063.
52. Zittel S, Weiller C, Liepert J. Reboxetine improves motor function in chronic stroke. A pilot study. J Neurol. 2007;254(2):197–201. https://doi.org/10.1007/s00415-006-0326-5.
53. Nadeau SE, Behrman AL, Davis SE, Reid K, Wu SS, Stidham BS, et al. Donepezil as an adjuvant to constraint-induced therapy for upper-limb dysfunction after stroke: an exploratory randomized clinical trial. J Rehabil Res Dev. 2004;41(4):525–34.
54. Barrett KM, Brott TG, Brown RD Jr, Carter RE, Geske JR, Graff-Radford NR, et al. Enhancing recovery after acute ischemic stroke with donepezil as an adjuvant therapy to standard medical care: results of a phase IIA clinical trial. J Stroke Cerebrovasc Dis. 2011;20(3):177–82. https://doi.org/10.1016/j.jstrokecerebrovasdis.2010.12.009.
55. Buchli AD, Schwab ME. Inhibition of Nogo: a key strategy to increase regeneration, plasticity and functional recovery of the lesioned central nervous system. Ann Med. 2005;37(8):556–67. https://doi.org/10.1080/07853890500407520.
56. Cash D, Easton AC, Mesquita M, Beech J, Williams S, Lloyd A, et al. GSK249320, a monoclonal antibody against the axon outgrowth inhibition molecule myelin-associated glycoprotein, improves outcome of rodents with experimental stroke. J Neurol Exp Neurosci. 2016;2(2):28–33.
57. Barbay S, Plautz EJ, Zoubina E, Frost SB, Cramer SC, Nudo RJ. Effects of postinfarct myelin-associated glycoprotein antibody treatment on motor recovery and motor map plasticity in squirrel monkeys. Stroke. 2015;46(6):1620–5. https://doi.org/10.1161/STROKEAHA.114.008088.
58.•• Cramer SC, Enney LA, Russell CK, Simeoni M, Thompson TR. Proof-of-Concept Randomized Trial of the Monoclonal Antibody GSK249320 Versus Placebo in Stroke Patients. Stroke. 2017;48(3):692–8. https://doi.org/10.1161/STROKEAHA.116.014517 **This was a phase IIb randomized placebo-controlled clinical trial investigating the efficacy of administering GSK249320, a humanized monoclonal antibody, to block the inhibitory myelin-associated glycoprotein to promote stroke recovery. The discussion of this negative study outlines some of the challenges of translating therapies from preclinical studies to humans.**
59. Mattson MP, Scheff SW. Endogenous neuroprotection factors and traumatic brain injury: mechanisms of action and implications for therapy. J Neurotrauma. 1994;11(1):3–33. https://doi.org/10.1089/neu.1994.11.3.
60. Rabchevsky AG, Fugaccia I, Fletcher-Turner A, Blades DA, Mattson MP, Scheff SW. Basic fibroblast growth factor (bFGF) enhances tissue sparing and functional recovery following moderate spinal cord injury. J Neurotrauma. 1999;16(9):817–30. https://doi.org/10.1089/neu.1999.16.817.
61. Longhi L, Watson DJ, Saatman KE, Thompson HJ, Zhang C, Fujimoto S, et al. Ex vivo gene therapy using targeted engraftment of NGF-expressing human NT2N neurons attenuates cognitive deficits following traumatic brain injury in mice. J Neurotrauma. 2004;21(12):1723–36. https://doi.org/10.1089/neu.2004.21.1723.
62. Lee ST, Chu K, Jung KH, Ko SY, Kim EH, Sinn DI, et al. Granulocyte colony-stimulating factor enhances angiogenesis after focal cerebral ischemia. Brain Res. 2005;1058(1–2):120–8. https://doi.org/10.1016/j.brainres.2005.07.076.
63. Kawada H, Takizawa S, Takanashi T, Morita Y, Fujita J, Fukuda K, et al. Administration of hematopoietic cytokines in the subacute phase after cerebral infarction is effective for functional recovery

facilitating proliferation of intrinsic neural stem/progenitor cells and transition of bone marrow-derived neuronal cells. Circulation. 2006;113(5):701–10. https://doi.org/10.1161/CIRCULATIONAHA.105.563668.

64. Zhao LR, Berra HH, Duan WM, Singhal S, Mehta J, Apkarian AV, et al. Beneficial effects of hematopoietic growth factor therapy in chronic ischemic stroke in rats. Stroke. 2007;38(10):2804–11. https://doi.org/10.1161/STROKEAHA.107.486217.
65. Liu XL, Zhang W, Tang SJ. Intracranial transplantation of human adipose-derived stem cells promotes the expression of neurotrophic factors and nerve repair in rats of cerebral ischemia-reperfusion injury. Int J Clin Exp Pathol. 2014;7(1):174–83.
66. Liu Y, Popescu M, Longo S, Gao M, Wang D, McGillis S, et al. Fibrinogen reduction and motor function improvement by hematopoietic growth factor treatment in chronic stroke in aged mice: a treatment frequency study. Cell Transplant. 2016;25(4):729–34. https://doi.org/10.3727/096368916X690791.
67. Cui L, Wang D, McGillis S, Kyle M, Zhao LR. Repairing the brain by SCF+G-CSF treatment at 6 months postexperimental stroke: mechanistic determination of the causal link between neurovascular regeneration and motor functional recovery. ASN Neuro. 2016;8(4):175909141665501. https://doi.org/10.1177/1759091416655010.
68. Zhou M, Greenhill S, Huang S, Silva TK, Sano Y, Wu S, et al. CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory. eLife. 2016;5. https://doi.org/10.7554/eLife.20985.
69. Joy MT, Ben Assayag E, Shabashov-Stone D, Liraz-Zaltsman S, Mazzitelli J, Arenas M, et al. CCR5 is a therapeutic target for recovery after stroke and traumatic brain injury. Cell. 2019;176(5):1143–57 e13. https://doi.org/10.1016/j.cell.2019.01.044.
70. Prentice H, Modi JP, Wu JY. Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases. Oxidative Med Cell Longev. 2015;2015:964518. https://doi.org/10.1155/2015/964518.
71. Jia M, Njapo SA, Rastogi V, Hedna VS. Taming glutamate excitotoxicity: strategic pathway modulation for neuroprotection. CNS Drugs. 2015;29(2):153–62. https://doi.org/10.1007/s40263-015-0225-3.
72. Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta Pharmacol Sin. 2009;30(4):379–87. https://doi.org/10.1038/aps.2009.24.
73. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. Ann Neurol. 2006;59(3):467–77. https://doi.org/10.1002/ana.20741.
74. Xu SY, Pan SY. The failure of animal models of neuroprotection in acute ischemic stroke to translate to clinical efficacy. Med Sci Monit Basic Res. 2013;19:37–45.
75. Hillis AE, Beh YY, Sebastian R, Breining B, Tippett DC, Wright A, et al. Predicting recovery in acute poststroke aphasia. Ann Neurol. 2018;83(3):612–22. https://doi.org/10.1002/ana.25184.
76. Byblow WD, Stinear CM, Barber PA, Petoe MA, Ackerley SJ. Proportional recovery after stroke depends on corticomotor integrity. Ann Neurol. 2015;78(6):848–59. https://doi.org/10.1002/ana.24472.
77. Puig J, Blasco G, Schlaug G, Stinear CM, Daunis IEP, Biarnes C, et al. Diffusion tensor imaging as a prognostic biomarker for motor recovery and rehabilitation after stroke. Neuroradiology. 2017;59(4):343–51. https://doi.org/10.1007/s00234-017-1816-0.
78.•• Lyden P, Pryor KE, Coffey CS, Cudkowicz M, Conwit R, Jadhav A, et al. Final results of the RHAPSODY trial: a multi-center, phase 2 trial using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein C, in combination with tissue plasminogen activator, mechanical thrombectomy or both in moderate to severe acute ischemic stroke. Ann Neurol. 2018;85:125–36. https://doi.org/10.1002/ana.25383 **This was the final result of phase 2 clnical trial of first neuroprotectant for acute ischemic stroke designed to assess the safety and tolerability of 3K3A-APC in combination with tPA, thrombectomy or both. This study showed a trend towards lowering the heamorrhage rate**.
79. Amar AP, Sagare AP, Zhao Z, Wang Y, Nelson AR, Griffin JH, et al. Can adjunctive therapies augment the efficacy of endovascular thrombolysis? A potential role for activated protein C. Neuropharmacology. 2018;134(Pt B):293–301. https://doi.org/10.1016/j.neuropharm.2017.09.021.
80. Griffin JH, Fernandez JA, Lyden PD, Zlokovic BV. Activated protein C promotes neuroprotection: mechanisms and translation to the clinic. Thromb Res. 2016;141(Suppl 2):S62–4. https://doi.org/10.1016/S0049-3848(16)30368-1.
81. Albensi BC, Igoechi C, Janigro D, Ilkanich E. Why do many NMDA antagonists fail, while others are safe and effective at blocking excitotoxicity associated with dementia and acute injury? Am J Alzheimers Dis Other Dement. 2004;19(5):269–74. https://doi.org/10.1177/153331750401900502.
82. Yuan H, Myers SJ, Wells G, Nicholson KL, Swanger SA, Lyuboslavsky P, et al. Context-dependent GluN2B-selective inhibitors of NMDA receptor function are neuroprotective with minimal side effects. Neuron. 2015;85(6):1305–18. https://doi.org/10.1016/j.neuron.2015.02.008.
83. Chen HS, Wang YF, Rayudu PV, Edgecomb P, Neill JC, Segal MM, et al. Neuroprotective concentrations of the N-methyl-D-aspartate open-channel blocker memantine are effective without cytoplasmic vacuolation following post-ischemic administration and do not block maze learning or long-term potentiation. Neuroscience. 1998;86(4):1121–32.
84. Chen HS, Lipton SA. Mechanism of memantine block of NMDA-activated channels in rat retinal ganglion cells: uncompetitive antagonism. J Physiol. 1997;499(**Pt 1**):27–46.
85. Trotman M, Vermehren P, Gibson CL, Fern R. The dichotomy of memantine treatment for ischemic stroke: dose-dependent protective and detrimental effects. J Cereb Blood Flow Metab. 2015;35(2):230–9. https://doi.org/10.1038/jcbfm.2014.188.
86. Aarts M, Liu Y, Liu L, Besshoh S, Arundine M, Gurd JW, et al. Treatment of ischemic brain damage by perturbing NMDA receptor- PSD-95 protein interactions. Science. 2002;298(5594):846–50. https://doi.org/10.1126/science.1072873.
87. Zhou L, Li F, Xu HB, Luo CX, Wu HY, Zhu MM, et al. Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. Nat Med. 2010;16(12):1439–43. https://doi.org/10.1038/nm.2245.
88. Bach A, Clausen BH, Moller M, Vestergaard B, Chi CN, Round A, et al. A high-affinity, dimeric inhibitor of PSD-95 bivalently interacts with PDZ1-2 and protects against ischemic brain damage. Proc Natl Acad Sci U S A. 2012;109(9):3317–22. https://doi.org/10.1073/pnas.1113761109.
89. Hill MD, Martin RH, Mikulis D, Wong JH, Silver FL, Terbrugge KG, et al. Safety and efficacy of NA-1 in patients with iatrogenic stroke after endovascular aneurysm repair (ENACT): a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2012;11(11):942–50. https://doi.org/10.1016/S1474-4422(12)70225-9.
90.•• Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. N Engl J Med. 2018;378(1):11–21. https://doi.org/10.1056/NEJMoa1706442 **The DAWN trial showed the efficacy of thrombectemy up to 24h after**

suspected onset of symptoms in select patients with occlusion of proximal vessels in the anterior circulation. This study provided a strong evidance in favor of expanding the therapeutic window.

91.•• Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. N Engl J Med. 2018;378(8):708–18. https://doi.org/10.1056/NEJMoa1713973 **The DEFUSE-3 trial showed the efficacy of thrombectemy up to 16h after suspected onset of symptoms in select patients with occlusion of proximal vessels in the anterior circulation. This study also provided a strong evidance in favor of expanding the therapeutic window**.

92. Sun HS, Doucette TA, Liu Y, Fang Y, Teves L, Aarts M, et al. Effectiveness of PSD95 inhibitors in permanent and transient focal ischemia in the rat. Stroke. 2008;39(9):2544–53. https://doi.org/10.1161/STROKEAHA.107.506048.

93. Zhou HH, Tang Y, Zhang XY, Luo CX, Gao LY, Wu HY, et al. Delayed Administration of tat-HA-NR2B9c promotes recovery after stroke in rats. Stroke. 2015;46(5):1352–8. https://doi.org/10.1161/STROKEAHA.115.008886.

94. Guo X, Bu X, Jiang J, Cheng P, Yan Z. Enhanced neuroprotective effects of co-administration of G-CSF with simvastatin on intracerebral hemorrhage in rats. Turk Neurosurg. 2012;22(6):732–9. https://doi.org/10.5137/1019-5149.JTN.6177-12.1.

95. Lo AC, Guarino PD, Richards LG, Haselkorn JK, Wittenberg GF, Federman DG, et al. Robot-assisted therapy for long-term upper-limb impairment after stroke. N Engl J Med. 2010;362(19):1772–83. https://doi.org/10.1056/NEJMoa0911341.

96. Wolf SL, Thompson PA, Winstein CJ, Miller JP, Blanton SR, Nichols-Larsen DS, et al. The EXCITE stroke trial: comparing early and delayed constraint-induced movement therapy. Stroke. 2010;41(10):2309–15. https://doi.org/10.1161/STROKEAHA.110.588723.

97. Yang Y, Zhao Q, Zhang Y, Wu Q, Jiang X, Cheng G. Effect of mirror therapy on recovery of stroke survivors: a systematic review and network meta-analysis. Neuroscience. 2018;390:318–36. https://doi.org/10.1016/j.neuroscience.2018.06.044.

98. McCabe J, Monkiewicz M, Holcomb J, Pundik S, Daly JJ. Comparison of robotics, functional electrical stimulation, and motor learning methods for treatment of persistent upper extremity dysfunction after stroke: a randomized controlled trial. Arch Phys Med Rehabil. 2015;96(6):981–90. https://doi.org/10.1016/j.apmr.2014.10.022.

99. Ward NS, Brander F, Kelly K. Intensive upper limb neurorehabilitation in chronic stroke: outcomes from the queen square programme. J Neurol Neurosurg Psychiatry. 2019;90:498–506. https://doi.org/10.1136/jnnp-2018-319954.

100. Lorenzo Bozzelli P, Alaiyed S, Kim E, Villapol S, Conant K. Proteolytic remodeling of perineuronal nets: effects on synaptic plasticity and neuronal population dynamics. Neural Plast. 2018;2018:5735789. https://doi.org/10.1155/2018/5735789.

101. Romberg C, Yang S, Melani R, Andrews MR, Horner AE, Spillantini MG, et al. Depletion of perineuronal nets enhances recognition memory and long-term depression in the perirhinal cortex. J Neurosci. 2013;33(16):7057–65. https://doi.org/10.1523/JNEUROSCI.6267-11.2013.

102. Lindau NT, Banninger BJ, Gullo M, Good NA, Bachmann LC, Starkey ML, et al. Rewiring of the corticospinal tract in the adult rat after unilateral stroke and anti-Nogo-A therapy. Brain. 2014;137(Pt 3):739–56. https://doi.org/10.1093/brain/awt336.

103. Kumar P, Moon LD. Therapeutics targeting Nogo-A hold promise for stroke restoration. CNS Neurol Disord Drug Targets. 2013;12(2):200–8.

104. Feeney DM, Gonzalez A, Law WA. Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. Science. 1982;217(4562):855–7.

105. Masihuzzaman AM, Uddin MJ, Majumder S, Barman KK, Ullah MA. Effect of low dose levodopa on motor outcome of different types of stroke. Mymensingh Med J. 2011;20(4):689–93.

106. Walker-Batson D, Smith P, Curtis S, Unwin H, Greenlee R. Amphetamine paired with physical therapy accelerates motor recovery after stroke. Further evidence. Stroke. 1995;26(12):2254–9.