

Phase-specific manipulation of neuronal activity: a promising stroke therapy approach

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Introduction: Ischemic stroke accounts for ~87% of all stroke cases (Virani et al., 2020). It is a leading cause of death and long-term disability worldwide, and constitutes a major burden for families and healthcare systems alike. Although medical treatment can help prevent stroke, post-stroke treatment is limited to either pharmacologic (e.g., tissue plasminogen activator - tPA) or mechanical (e.g., thrombectomy) reperfusion. During the past few decades, many neuroprotective and neurorestorative strategies have been tested in hopes of discovering improved treatment options for stroke patients, particularly patients who are not eligible for reperfusion therapy. Sadly, such hopes have not yet been fulfilled, and thus, patients are still in dire need of new stroke therapies as well as increased vigilance for amelioration of risk factors.

Development of new effective treatments for stroke requires a precise understanding of stroke pathophysiology. Importantly, it has become increasingly clear that this knowledge can only be obtained within the context of specific stroke phases, and the brain regions and different cell types involved. There are two main temporal phases of stroke progression: the acute/subacute phase and the recovery phase. During the acute/subacute phase, which lasts for days after stroke onset, the stroke dynamically evolves into a mature infarct, often expanding with progressive cellular damage, edema, and changes in collateral blood flow. During the recovery phase, circuits and cells try to overcome the stroke-induced damage through a variety of reparative processes and resolution of diaschisis, or functional disconnection between brain regions. Neurons are critically involved in both phases, as their activity intrinsically defines both stroke progression and recovery. Thus, an improved understanding of the roles of neuronal activity in specific phases and brain regions after stroke is central to developing novel effective approaches to acute/subacute neuroprotection and chronic neurorestoration. Here, we concisely discuss the therapeutic potential of phase-specific manipulation of neuronal activity in ischemic stroke (**Figure 1**), with a focus on recent discoveries through chemogenetics technology.

Acute/subacute stroke phase: reperfusion and neuroprotection: Ischemic stroke rapidly activates pathologic processes that lead to acute brain cell death in the ischemic core, followed by progressive recruitment of surrounding regions (i.e., the penumbra) to form a consolidated infarct. Since brain tissue in the penumbra is metabolically compromised but still salvageable, reperfusion therapy that enhances blood flow is the most effective immediate approach for treating ischemic stroke. However, only a fraction of ischemic stroke patients with large vessel occlusion are eligible for this treatment, and even among these treated stroke patients, many still do not regain functional independence. Therefore, tremendous effort has been invested in the search for neuroprotective treatments to 1) decelerate conversion of the penumbra into infarcted tissue during ischemia and thus, extend the therapeutic window for reperfusion, and/or 2) to protect the brain against

ischemia/reperfusion injury after reperfusion and thus, improve long-term functional recovery (**Figure 1**).

Abnormal neuronal activity has long been considered a key player in defining brain damage during the acute/subacute phase, thus providing a promising therapeutic target. However, current clinical trials that target neuronal activity have largely failed. Despite this disappointing progress, this line of research should be continued, in our opinion, especially in light of new technologic and scientific advances in this field. In the acute stroke phase, brain ischemia causes metabolic supply-demand mismatch, dysfunction in ion channels, and release of neurotransmitters. These changes disrupt neuronal homeostasis leading to excitotoxicity and peri-infarct spreading depolarizations (SDs), both of which contribute to acute brain damage. Notably, accumulating data indicate that glutamate excitotoxicity is tightly coupled with SD occurrence. SDs are electrophysiologic events that manifest as cortical waves of severe neuronal and glial depolarization, in response to brain irritation or ischemia. Importantly, SDs have been increasingly recognized as key pathologic events that occur spontaneously and contribute to secondary brain injury. Indeed, peri-infarct SDs (or PIDs) play a crucial role in edema and infarct expansion after stroke (Hartings et al., 2017; Mestre et al., 2020). Thus, prevention and suppression of PIDs could be a promising target in stroke therapy during the acute/subacute phase. However, the mechanistic understanding of PIDs remains largely unknown, and consequently, specific and effective approaches to interfere with stroke-induced PIDs are not yet available. Notably, recent discoveries shed light on the primary role of excitatory neuronal activity in SD occurrence (Sugimoto et al., 2020; Wang et al., 2020), an exciting advance in the field, indicating the potential of targeting neuronal activity to alleviate the detrimental effects of stroke-induced SD/PIDs.

Chronic stroke phase: recovery and restoration: During the chronic phase, most stroke patients exhibit partial, spontaneous functional recovery, strongly indicating that the brain has endogenous restorative mechanisms (Jones, 2017; Micera et al., 2020). These mechanisms, although largely undefined, form the basis for neurorestorative therapies (e.g., brain stimulation), with the potential to enhance recovery compared to rehabilitation and spontaneous recovery alone (**Figure 1**). Because effective neurorestorative therapies hold great promise for reducing long-term disability and improving quality of life in chronic stroke patients (Cramer et al., 2017), identifying intrinsic restorative mechanisms is of high clinical significance. It is believed that restorative mechanisms involve re-organization of structure and function of the damaged brain via brain plasticity, and improvement in functional connection between regions as activity increases, resolving diaschisis (Jones, 2017). To enhance brain plasticity, non-specific brain stimulation has been tested in stroke as this treatment may improve axonal plasticity as well as collateral blood flow in the peri-infarct area. However, results from clinical stroke studies on brain stimulation have been

inconsistent (Micera et al., 2020), highlighting our deficiency in understanding specific cell activity, especially neuronal activity, in neurorestoration after stroke.

The current notion is that recovery of neurologic function occurs due to activation of endogenous restorative processes not only in peri-infarct regions but also in auxiliary and contralesional areas. In addition, appropriate neuronal activity in both ipsilateral and contralateral regions is intrinsically interleaved with brain plasticity and stroke outcome. For example, increased neuronal activity may enhance sprouting and development of new axonal pathways as well as re-activation and strengthening of unused, latent circuits, to “work around” the damaged regions, particularly if coupled with rehabilitation and conscious intent to use these alternative pathways. To gain deep insights into these restorative processes, future work may require a combination of advanced techniques, such as *in vivo* gene delivery, temporal and spatial manipulation of neuronal activity, and tracing of neural connections.

Phase-specific modulation of neuronal activity as a promising approach to improve stroke outcome:

As discussed above, modulating neuronal activity in the brain is a very promising strategy in stroke therapy, particularly when engaging critical (but differing) needs during each phase. Thus, we must have a better understanding of how neuronal activity in each specific phase and across regions affects stroke pathology. However, filling this knowledge gap was a daunting task until the advent of two new technologies in neuroscience: optogenetics and more recently, chemogenetics. In particular, the Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)-based chemogenetics approach has emerged as a highly useful tool in stroke research. DREADDs belong to a class of chemogenetically engineered proteins that are based on G protein-coupled receptors (GPCRs) (Roth, 2016). GPCRs play key roles in neuronal signaling in the brain, with activation of Gq or Gi GPCRs leading to neuronal activation or inhibition, respectively. These engineered GPCRs (i.e., DREADDs) work similarly to endogenous GPCRs but can only be activated by exogenous designed small molecule actuators. The most frequently used actuator is clozapine-N-oxide (CNO), due to its excellent blood-brain barrier permeability.

Currently, 2 DREADDs – hM3Dq (Gq GPCR) and hM4Di (Gi GPCR) – have been well characterized (Roth, 2016). Activation of hM3Dq leads to increased intracellular Ca²⁺, thereby exciting neurons, while inhibition of neuronal activity by hM4Di activation is believed to involve two mechanisms: 1) induction of post-synaptic membrane hyperpolarization, and 2) inhibition of the pre-synaptic release of neuronal transmitters. There are clear advantages to using a DREADD-based chemogenetic approach to manipulate neuronal activity in stroke research. First, DREADD expression within brain cells across regions in a cell-specific manner can be achieved virally or transgenically, and its activity can be non-invasively controlled by a simple timed intraperitoneal injection of CNO. Second, CNO has a rapid and long-lasting effect (hours) on target cells, which may be required to produce notable effects on stroke outcome. Finally, activity of targeted cells can be modulated in a large brain area by controlling spatial expression of DREADDs. Therefore, by fully capitalizing on the power of this novel technology, answers to many fundamental stroke questions that are related to neuronal activity specific to each phase now come within reach.

Using the DREADD chemogenetic approach, we recently discovered, for the first time, that

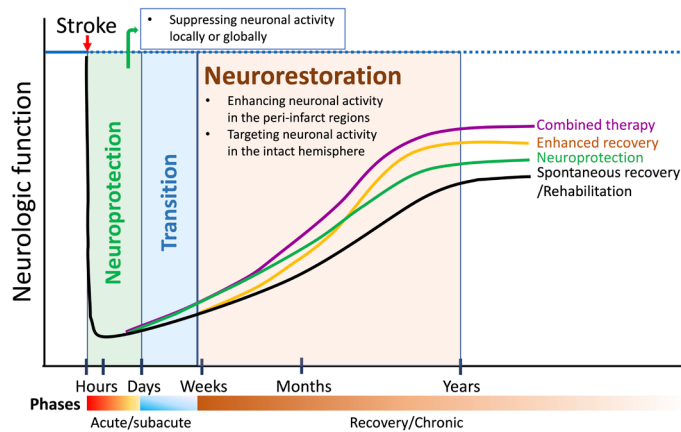


Figure 1 | Conceptual framework for phase-specific manipulation of neuronal activity in stroke therapy. This figure depicts the relative time frame for stroke therapies. Initially suppressing neuronal activity may be beneficial. Then, there is a transition period as the brain prepares the environment for recovery. Finally, during the neurorestoration phase, modulation of neuronal activity is expected to facilitate structural and functional recovery of cerebral vasculature and neural network. Therapy targeting both early and later phases may be synergistic in improving outcome, as shown by the recovery trajectory line (purple).

acute suppression of neuronal activity limited to excitatory neurons is sufficient to improve stroke outcome (Wang et al., 2020). In this study, we generated hM4Di-DREADD;Emx1-Cre (hM4Di-TG^{Neuron}) mice in which expression of the inhibitory hM4Di DREADD is primarily restricted to forebrain excitatory neurons. hM4Di-TG^{Neuron} mice that were treated with CNO either before stroke or after reperfusion exhibited significantly improved outcome compared to CNO-treated control mice. Further, using a potassium-mediated *in vivo* SD model, we provided the first indirect evidence that the beneficial effect is likely attributed to suppressive effects of the hM4Di DREADD on the incidence of ischemia-induced SD/PIDs. Of note, although PIDs and potassium-induced SDs have many similar properties, there are critical differences. For example, with stroke-induced PIDs, there is decreased blood flow (due to lack of collateral into the penumbra region of the stroke), whereas with potassium-induced SDs, there is typically increased compensatory blood flow. Thus, further clarification of the role of neuronal activity in stroke-induced PIDs using DREADD-specific mice is warranted.

Another recent study tested the DREADD chemogenetic approach in a relatively late stroke phase (Hu et al., 2019). The authors used viral gene delivery to express the excitatory hM3Dq DREADD in glutamatergic neurons in the primary motor cortex bilaterally in rats before stroke. Two days after ischemic stroke, these rats were dosed with CNO to activate the hM3Dq-expressing neurons for 10 consecutive days. They found that this manipulation of neuronal activity improves performance in several behavioral tests, including even a memory test. However, the underlying mechanisms responsible for these improved neurologic outcomes observed were not dissected in this study.

These novel, proof-of-concept studies not only confirm the feasibility of phase-specific modulation of neuronal activity to improve stroke outcome, but also underscore that application of the DREADD approach in experimental stroke research will help answer critical questions. For example, since phase-sensitive alterations in neuronal activity (likely in opposing directions) are required to improve functional stroke outcome, a defined time window for such intervention is critical. What is the best time frame for acute or chronic modulation of neuronal activity to be effective in improving stroke outcome? More specifically, what is the transition time point between acute and chronic phases in order to

apply varying interventions to either suppress or enhance neuronal activity (Figure 1)? Further, is a combination of both early and late time interventions in a synergistic fashion crucial to enhance overall outcome? Which neuronal modulation approach (e.g., global, peri-infarct, or contralateral targeting) is more effective in promoting stroke recovery? How do these modulations influence structure and function of vasculature as well as neural network remodeling in the post-stroke period? Clearly, more detailed, well-designed, and especially mechanism-centered research is required, for which the incorporation of DREADD technology should be valuable.

Future stroke therapy: The translation of phase-specific neuronal modulation strategy to clinical stroke treatment will require considerably more investigation of underlying mechanisms firstly at the cellular and circuit levels, and development of innovative approaches. Optogenetic and chemogenetic techniques will certainly allow us to gain deeper mechanistic insights into the role of phase-specific neuronal activity in stroke pathophysiology. However, current technology has not yet paved a clear path whereby new stroke therapeutic strategies based on specific manipulation of neuronal activity can be translated into the clinic. We may envision the use of gene or cell therapy approaches to achieve phase- and region-specific manipulation of neuronal activity after stroke. For example, it may be possible to perform virus-mediated *in vivo* gene delivery after a stroke, similar to many gene therapy trials for Parkinson's and Alzheimer's diseases, or cellular transplantation. However, these approaches will not work for acute stroke treatment for which new physical devices or pharmacologic tools need to become available. It is our great hope that this line of research in pursuit of novel stroke therapies will lead to innovative treatments that will ensure a better quality of life for stroke patients.

The present work was supported by NIH NS103468, NS111093, and NS040984-11 (to DAT), and NIH NS099590 and AHA 18CSA34080277 (to WY).

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Date of submission: July 8, 2020
Date of decision: September 7, 2020
Date of acceptance: October 15, 2020
Date of web publication: December 12, 2020

<https://doi.org/10.4103/1673-5374.301005>
How to cite this article: Turner DA, Yang W (2021) Phase-specific manipulation of neuronal activity: a promising stroke therapy approach. *Neural Regen Res* 16(7):1425-1426.

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C-Editors: Zhao M, Qiu Y; T-Editor: Jia Y