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Neuron
Perspective

Stroke and the Connectome: How Connectivity Guides Therapeutic Intervention

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http://dx.doi.org/10.1016/j.neuron.2014.08.052

Connections between neurons are affected within 3 min of stroke onset by massive ischemic depolarization and then delayed cell death. Some connections can recover with prompt reperfusion; others associated with the dying infarct do not. Disruption in functional connectivity is due to direct tissue loss and indirect disconnections of remote areas known as diaschisis. Stroke is devastating, yet given the brain's redundant design, collateral surviving networks and their connections are well-positioned to compensate. Our perspective is that new treatments for stroke may involve a rational functional and structural connections-based approach. Surviving, affected, and at-risk networks can be identified and targeted with scenario-specific treatments. Strategies for recovery may include functional inhibition of the intact hemisphere, rerouting of connections, or setpoint-mediated network plasticity. These approaches may be guided by brain imaging and enabled by patient- and injury-specific brain stimulation, rehabilitation, and potential molecule-based strategies to enable new connections.

Introduction: Stroke as a Disease of Brain Connectivity

Stroke results when a loss of blood flow disables neurons and disconnects individuals from their ability to sense and act within the world around them. This loss of both afferent and efferent connectivity robs quality of life from individuals who may be otherwise healthy. Recently, through advances in both technology and analysis, we have begun to consider the possibility that a comprehensive wiring diagram of the connectome (all brain connections) is obtainable (Sporns et al., 2005). This schematic may provide greater understanding of the anatomical disruptions induced by the stroke, and the necessary instructions for future stroke treatments. With this in mind, stroke can be viewed as an acute disruption of an individual's connectome caused by a focal or widespread loss of blood flow. Here we will discuss workarounds and/or changes to the stroke-affected connectome that may form the basis of treatment or spontaneous recovery.

Most forms of stroke rehabilitation, whether intended or not, engage use-dependant structural and/or functional changes within remaining brain circuits to support recovery (Grefkes and Fink, 2014; Lohse et al., 2014). Recently, forms of noninvasive brain stimulation are being coupled with rehabilitation to enhance these key mechanisms of recovery (Brodie et al., 2014; Nouri and Cramer, 2011; Plow et al., 2014). Given that this approach relies on targeting brain areas that may contribute to recovery, detailed connectomic information may lead to principled forms of treatment. Current human imaging data strongly support linkages between connectivity and outcome after stroke (Carter et al., 2010a, 2012; Urbin et al., 2014), as functional deficits can extend to remote connected areas. In this review, we will outline how stroke and rehabilitation alter the connectome at various phases of initial damage and recovery, and also highlight the tools available for visualizing and modifying the connectome in both humans and animals. We will refer to connectomes on different structural and functional levels, corresponding to both widescale human tract level organization (corresponding to myelinated axons) and animal work, which can visualize regional as well as cellular-level connections (Figure 1). Our discussion will mainly focus on the motor system, for two reasons: first, the majority of animal studies on focal ischemia model sensorimotor deficits, and therefore the translational link is strongest in this domain; and second, the tools available for probing and interfering with brain circuitry in humans (such as transcranial magnetic stimulation) are most readily applied in the motor system. However, stroke and vascular dementia often have debilitating effects on memory and executive function (Black et al., 2009; Dacosta-Aguayo et al., 2014; Lim et al., 2014). It is quite possible that the rules we define for sensory-motor recovery will not be shared in these other cases, emphasizing the need for both animal models and imaging assessment tools for humans that are geared toward non-sensory-motor aspects of stroke.

The Brain's Connectome Assessed by Multiple Measures and Scales

The word connectome was coined by Olaf Sporns (Sporns et al., 2005) and refers to the complete set of structural connections between neurons of the brain. Currently, we speak about connectomes over three major spatial scales: microscopic (synapses, e.g., boutons and spines), mesoscopic (regional interactions, e.g., projections from sensory to motor cortex), and macroscopic (brainwide, between major subdivisions thalamus/cortex) (Bohland et al., 2009). To begin to appreciate stroke from the view of the connectome, we need to understand the progress and opportunity in this area. Beginning over 100 years ago, the fortuitous sparse cell labeling afforded by Golgi

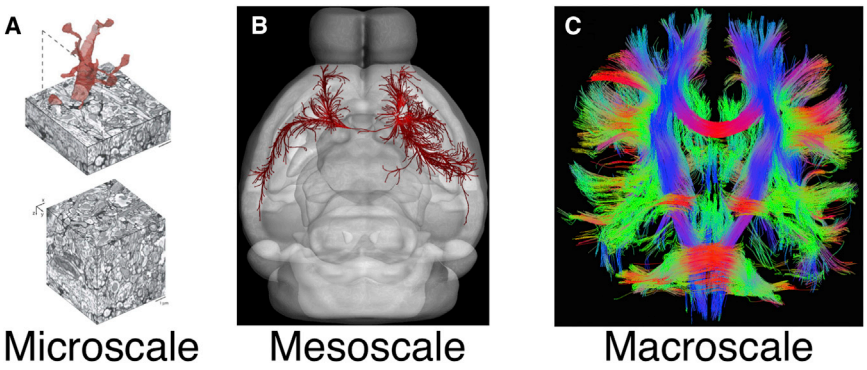

Figure 1. Scales over which Connectomes Are Assessed

(A) At the microscale level, serial blockface scanning electron microscopy has been used to reconstruct a volume of rodent brain tissue and manually trace a segment of spiny dendrite (shown in red) to visualize synaptic connections. (Briggman and Denk, 2006).

(B) Mesoscale connectomes of the rodent brain can be created by injecting anterograde tracers into cortical regions to follow projection targets to other brain regions (Oh et al., 2014; Allen Institute for Brain Science, http://connectivity.brain-map.org/). In the example shown, the injection targeted motor cortex in a Cux2-IRES-Cre mouse line where the reporter is expressed mainly in cortical layers 2–4.

(C) Advances in MRI techniques, such as diffusion tensor imaging, have facilitated the study of macroscale or whole-brain connectivity in the human brain (from http://www.humanconnectomeproject.org).

staining provided the first microscopic view of neuron-to-neuron connectivity (DeFelipe and Jones, 1991) and set the stage for generations of scientists (Figure 1). On the microscopic scale in the mouse brain, significant efforts have been put forth to reconstruct small regions of brain on the level of individual synaptic connections with ultrastructural resolution (Briggman and Denk, 2006; Lichtman et al., 2008). However, such exhaustive studies even in mouse only represent a small fraction of the whole brain or retina, on the order of 1 mm^3 (Helmstaedter et al., 2013). In mouse the determination of regional or mesoscopic neuronal structural connectivity is quite advanced and benefits from robust tracing and imaging methods that are implemented in the living brain (Matyas et al., 2010; Oh et al., 2014; Zingg et al., 2014), while tracing in human tissue is restricted to histological samples.

In the human brain the connectome could also loosely refer to a sequence of coactivated areas (presumably functionally connected areas), which are defined by fMRI resting-state signals and offer a more macroscopic view of stroke-related connectivity (Carter et al., 2012). The human macroscopic connectome can be defined by the specific array of white matter tracts determined by diffusion tensor imaging (DTI, Figure 1) (Ball et al., 2012; Hua et al., 2009; Wakana et al., 2004). The study of DTI-based tract signals is known as tractography. These tracts represent major information flow but may be such a gross representation that finer-scale functional rearrangements could be lost within them. By analogy to road networks, a map at the level of individual streets (axons dendrites) and the addresses of homes (synapses) would be preferable to one of just highways (white matter tracts). There are currently high-resolution tract-based DTI atlases of human brain (Ball et al., 2012; Hua et al., 2009; Wakana et al., 2004). The proliferation of connectivity data has spawned a working model of the brain termed "virtual brain" (Jirsa et al., 2010; Ritter et al., 2013), which may provide further insight into stroke damage, and perhaps suggest enlightened treatments through simulation of ischemic deficits (see Figure 2A; Alstott et al., 2009). Tract-level maps will be further supported by comprehensive projects such as the BRAIN Initiative, which is focused on higher-resolution connectome determination in both animal models and human brain (Devor et al., 2013).

Over the last 40 years, connectivity of axonal projections has been evaluated on a "need-to-know basis" through classic investigator-driven studies employing chemical anterograde and retrograde tracers (Felleman and Van Essen, 1990). Recently, the definition of the brain connectomes has proceeded in earnest. A database of mouse brain connectivity based on anterograde tracing (outward axonal projections) has been made available by the Allen Institute (Oh et al., 2014) and UCLA consortia (Zingg et al., 2014). Before initiating a new line of experimentation in mouse, it is now possible to visualize afferent and efferent axonal projections for literally hundreds of brain regions; see Figure 2B for an example of raw data (Oh et al., 2014). The Allen Institute's Mouse Connectivity Atlas and companion Brain Explorer allow one to easily find the output of a region using a "source" search or its inputs (or fibers passing through it) using a "target" search. These resources have made a single-axon level (meso-scale, middle-scale) view of the connections within the mouse brain available for researchers around the world. Voltage-sensitive dye imaging in mouse indicates that major patterns of intracortical structural connections defined by the Allen Connectivity Atlas are represented in spontaneous, sensation-evoked, and ChR2-stimulated intracortical activity (Mohajerani et al., 2013). Other rodent resources include matrices and connectivity diagrams showing the effect of Channelrhodopsin-2 (ChR2) point stimulation on cortical functional connectivity (Lim et al., 2012; Figure 2C). Such rodent databases can be used to hypothesize possible structural routes for recovery after stroke in mice and hopefully provide insight for translation to human stroke treatment.

Angiome: The Brain's Plumbing Schematic

The focus of this review is the neuronal connectome; however, the brain's angiome or distribution of vascular elements can also be considered a connectome of sorts. In contrast to the brain's electrical circuit diagram, which is far from complete in any mammalian model organism, recent breakthroughs in imaging and sectioning technology have resulted in high-resolution capillary-level reconstructions of the rodent brain angiome (Blinder et al., 2013; Mayerich et al., 2011; Pathak et al., 2011; Tsai et al., 2009; Xue et al., 2014). Tracing blood vessels is

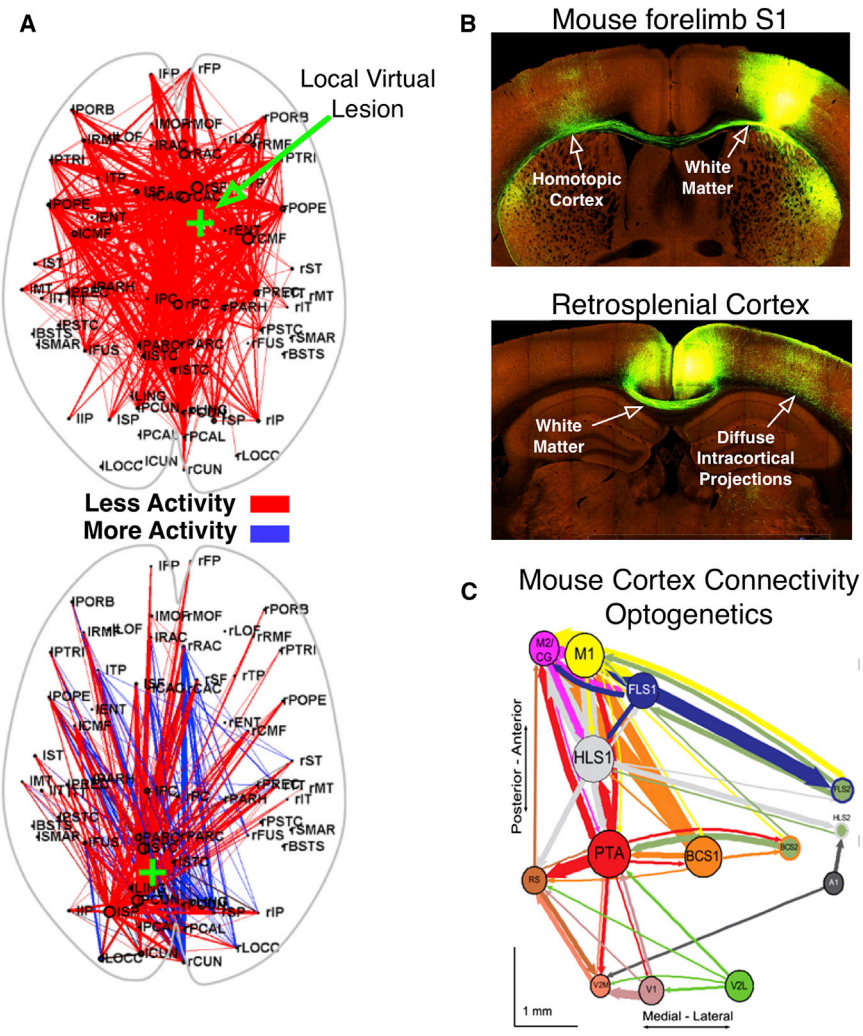

Figure 2. Graph Analysis of Human Brain Connectivity during Simulated Stroke and Mouse Brain Structural/Functional Connectivity Determination

(A) Model of human brain connectivity identifies two medial highly interconnected hub regions (upper, cingulate; lower, posterior parietal). Each line represents an identified pathway, and the color of the line indicates the effect of a virtual stroke affecting only 5% of cortex placed at the location of the green +. Red indicates weakened coupling, while blue indicates strengthened coupling after the virtual stroke (Alstott et al., 2009). Unilateral stroke weakens connections throughout both hemispheres when present within midline hub regions.

(B) Injection of an anterograde tracer into mouse forelimb primary somatosensory cortex (upper) reveals axonal terminations in homotopic cortex (traveling through the corpus callosum white matter) as well as the lateral striatum. Projecting axons through white matter are particularly sensitive to the effects of ischemia, and their loss can have a large impact on function, given that multiple systems may traverse the same tracts. The more medial retrosplenial cortex is analogous to human midline hub networks and also makes homotopic connections (lower), but also diffuse intrahemispheric connections with many cortical regions, making it have a potentially large impact if lost to focal or diffuse vascular injury (Oh et al., 2014). Raw data are from Allen Institute for Brain Science Mouse Connectivity Atlas (http://connectivity.brain-map.org/), experiments 100148142–RSP and 126909424–SSp-ul. Homotopic regions (mirrored in other hemisphere) are a site of interhemispheric remapping.

(C) Regional connectivity of mouse cortex (right hemisphere) determined from point stimulation of ChR2 and imaged using voltage-sensitive dyes. Circles represent functional nodes, and the size of the connecting arrows indicates the strength of functional coactivation between the interconnected regions. Graph theory analysis shows that the parietal association area (PTA) is highly connected with other cortical regions and serves as a hub region; for all abbreviations, see Lim et al. (2012).

more straightforward compared to much finer and more numerous neuronal connections. A synapse can be found within nearly every cubic micrometer of brain, while capillary densities are more than 100 times lower (Tsai et al., 2009; Zhang et al., 2005). Furthermore, the connections between vascular elements can be modeled as simple tubes, in contrast to neuronal connectivity, where the rules for inferring synaptic communication from structural information are not yet complete.

The first comprehensive analysis of angiomes at the level of brain capillaries was put forward by Kleinfeld and colleagues in mouse (Tsai et al., 2009). Optimization of histological and sectioning techniques in combination with classic methods such as India ink perfusion has resulted in remarkably complete brain-wide capillary-level data sets for mice (Mayerich et al., 2011; Xue et al., 2014). Such data may be used to predict local vulnerability to vascular disruptions, but insight from the data with respect to stroke is still in progress. It is conceivable that interactive computer models could be made of the effects of clots in animal models to validate experiments (Blinder et al., 2010; Schaffer et al., 2006). Future work will be guided by seminal quantitative studies that have focused on rat barrel cortex (Blinder et al., 2013). Furthermore, the determination of fine-scale angiomes in humans, combined with vascular simulation studies, may provide more-accurate predictions of outcome from stroke in patients.

An exciting possibility would be to combine databases of functional connectivity with structural connections, as well as vascular flow to predict functional disruptions when specific elements of the angiome are compromised. Based on work in animal models, penetrating arterioles that supply the cortical surface represent a key bottleneck in cerebral blood flow and a site of vulnerability (Black et al., 2009; Shih et al., 2013). Rodents appear to have particular redundancies in their vasculature, where anastomoses can be recruited to salvage ischemic tissue through activity-dependent neuroprotection (Lay et al., 2010). It is unclear whether humans benefit from such extensive vascular redundancy; however, the possibility of engaging collateral circulation by restricting flow to the periphery has been explored as a potential stroke intervention (Shuaib et al., 2011; Winship et al., 2014). Human studies also indicate positive effects of a

single bout of aerobic exercise on the perfusion of potentially vulnerable white matter tracts measured 40 min later, further supporting proposals that activity can be used to alter perfusion (MacIntosh et al., 2014). While there is obvious power in angiome determination and insight from its modeling, establishing consensus angiome structures may be more difficult than gross axonal projection structure. Recent work shows that the angiome does not necessarily follow neuronanatomical boundaries, such as the columnar organization of rodent cortex (Blinder et al., 2013), and the arrangement of major brain vessels differs within inbred mouse strains (Beckmann, 2000) and even between hemispheres. The angiome is not a static network; it is capable of both rapid changes, such as activity-dependent vasodilation and constriction, and longer-term changes, such as experience-induced angiogenesis (Hermann and Chopp, 2012). Work in rat cortex shows active dilation of penetrating arterioles that help normalize perfusion following stroke (Shih et al., 2009). Recent work shows that vascular branching and density can be modulated by sensory experience early in postnatal development (Lacoste et al., 2014). Given links between recovery from injury and development, similar activity-dependent plasticity may be recapitulated during stroke recovery. The angiome represents a relatively well-defined structural network where flow can be understood, thus making the modeling of functional deficits due to ischemia an obtainable goal.

How Stroke Changes the Connectome

Movies of the First Minutes of a Stroke Indicate that the Connectome Is Vulnerable

Advances in mouse in vivo imaging (Holtmaat et al., 2009) have enabled a relatively comprehensive description of the initiation of experimental stroke and the effect of reperfusion on structural connections (Zhang et al., 2005) that complement previous histology (Colbourne et al., 1999; Garcia et al., 1993). The use of in vivo two-photon imaging, involving the assessment of layer 5 neuron GFP-labeled dendrites and axons, has provided a new means of longitudinal circuit assessment. A series of in vivo studies that were focused on cortex (Zhang et al., 2005) demonstrated dynamic changes to dendrites in layer 1 during the onset of ischemic depolarization (Murphy et al., 2008; Risher et al., 2010; Takano et al., 2007). Within 60–120 s of the induction of forebrain ischemia, energy supplies rapidly dwindle, and ischemic depolarization occurs (Murphy and Corbett, 2009). This massive ischemic depolarization leads to the loss of membrane potential from neurons and spreading waves of damage (Joshi and Andrew, 2001). Individual dendrites were rapidly swollen and beaded within 3 min (Murphy et al., 2008). In addition to regular dendritic swellings, there was a loss of dendritic spines (Murphy et al., 2008), as well as swelling of glial elements (Risher et al., 2009, 2012). These studies are consistent with stroke having rapid fulminant effects on cortical connections in both focal and global ischemia models (Li and Murphy, 2008; Murphy et al., 2008; Risher et al., 2010). An important implication of these experiments is that effects on the brain connectome are unavoidable consequences of stroke, as they occur within minutes of onset. Interestingly, if reperfusion is promptly initiated within 60 min of stroke onset, most of the spines can recover (Li and Murphy, 2008; Murphy et al., 2008; Risher et al., 2010). Although cellular imaging and histology indicate that the connectome is rapidly damaged by stroke, network-wide functional effects have been best studied using human resting-state activity imaging.

Using Resting-State Activity to Assess the Poststroke Connectome

Activity when the brain is not engaged in any particular task has been termed resting state (Han et al., 2008; Shmuel and Leopold, 2008; Zhang and Raichle, 2010). The flow of synaptic events through resting-state networks is constrained by underlying structural connectivity, and this activity provides a continuous sample of functional connectivity (Carter et al., 2010a; Mohajerani et al., 2013; Vincent et al., 2007). Increasingly, study of this relatively poorly defined form of activity can nonetheless reflect the function and integrity of circuits both in the healthy brain and after disease such as stroke. Resting-state functional connectivity has been assessed in a large number of human stroke patients (Carter et al., 2010a, 2012; Grefkes and Ward, 2014; He et al., 2007; Tuladhar et al., 2013). Brain-wide connections based on resting-state data in humans were used to simulate the effects of a virtual stroke. Here, a surprisingly small virtual lesion (5% of area) had network-wide bihemispheric effects when placed unilaterally within putative cortical midline hub regions (Alstott et al., 2009; Figure 2A). Perhaps the pervasive use of resting-state assessment is attributed to the ease with which this data set can be obtained. Resting-state data do not require much in the way of patient compliance and are more likely to be standardized across centers and different scanning modalities than event-driven signals. These studies have primarily relied on using correlation to establish coactivated networks (Fox and Raichle, 2007); however, the parcellation and analysis of this data are still evolving. Although resting state can be assessed using electrophysiological measures such as EEG or magnetoencephalography, it is most commonly determined using fMRI BOLD signals in humans. Resting-state signals can be routinely measured, although the methodology has very low time resolution, on the order of 0.1 Hz. The signals also carry caveats about being indirect, since they primarily reflect hemodynamic changes.

Recently imaging of intrinsic signals (reflect hemodynamics) was used to define networks in mouse that are affected by stroke damage (Bauer et al., 2014). The study was similar to human (Grefkes and Fink, 2014) and rat work (van Meer et al., 2010, 2012) and demonstrated a loss of homotopic connectivity in most severely affected animals (Bauer et al., 2014). There was also a behavioral component, which demonstrated links between intact midline retrosplenial hub networks and recovery in a sensorimotor task (Bauer et al., 2014). Overall, the mouse resting-state data were best at mapping deficits after stroke, and were less sensitive at revealing broad changes in spontaneous activation patterns that were associated with unique networks for recovery.

Imaging approaches have obvious advantages for spatially resolving infarcts versus surviving and potentially altered networks. Although human and previous rodent studies were limited to the relatively low time resolution and indirect nature of circuit assessment through hemodynamically driven signals, animal studies do offer the ability to produce resting-state activity

data using higher temporal resolution physiological measures such as membrane potential (Mohajerani et al., 2010, 2013). Higher temporal resolution direct measures are desirable, although imaging of hemodynamics signals with low time resolution (Bauer et al., 2014) can lead to similar macroscopic cortical parcellation as obtained with voltage sensors (Mohajerani et al., 2010, 2013).

Conceivably, future work employing voltage-sensitive fluorescent proteins or other recombinant indicators of activity could provide a longitudinal means of resting-state assessment in animal models (Daniel et al., 2014). These studies will be important for validating possible patient-based activity assessments, but they have obvious limitations: it is only possible to conclude that networks are coactivated, and one cannot directly infer degree of activation, causality, or direction (driver versus follower). Directionality can be inferred using optogenetic intracortical microstimulation (Lim et al., 2013; Figure 2C). With these caveats in mind, assessment of functional connectivity based on spontaneous events still has merits for showing which circuits are still activated and potentially able to aid recovery in the poststroke brain. Whether resting-state activations can be used to tease apart potential nascent connections from strong activation attributed to major routes of information flow will await better imaging analysis and acquisition methods in both animal models and patients.

Resting-state physiology can potentially impact treatment. Previous studies find better patient outcome if disruptions in connectivity due to stroke are only unilateral, and do not extend to the other hemisphere (Carter et al., 2012). However, it is also conceivable that these cases represent patients with less severe damage. Perhaps the most effective use of resting-state data may be comparisons of the resting-state activation pattern of a stroke-affected individual to a large database of other patients that contains resting-state features, as well as outcomes for interventions such as rehabilitation. These comparisons could be used to discern which type of rehabilitation is generally effective for patients with a particular pattern of resting-state activation. Such work is likely underway, but challenges exist, since functional imaging data will need to be classified and compared to varied streams of clinical metadata that describe outcomes for specific interventions. Thus, by combining databases of resting-state connectivity with physical rehabilitation parameters, it may be possible to choose a specific course of action based on the presence of certain resting-state features indicative of surviving plastic circuits (Table 1).

Local and Remote Structural-Functional Changes to Connectomes after Stroke

The area of tissue bordering the stroke core exhibits partially reduced blood flow and is termed the penumbra. Over time, as the core stroke-affected tissues die, this becomes the peri-infarct zone and a site for possible plasticity (Hossmann, 2006; Murphy and Corbett, 2009; Zhang and Murphy, 2007). Surviving neurons in the peri-infarct cortex undergo active structural and functional remodeling, which is associated with a remapping of lost function (Brown et al., 2009; Mostany et al., 2010). Longitudinal two-photon imaging approaches have demonstrated compensatory dendritic spine production in recovering circuits in the weeks after stroke that was specific to the peri-infract zone (Brown et al., 2009; Johnston et al., 2013; Mostany et al., 2010). Although turnover was specific to the peri-infarct zone, it is possible that these studies were not sufficiently sensitive to resolve structural rearrangements involving a relatively small fraction of the total inputs to a distant area in the background of many unchanged connections. Recent data in noninjured animals support the proposal that motor learning is achieved through long-lasting changes in dendritic spine turnover and the fine-tuning of individual neuron responsiveness in motor cortex (Peters et al., 2014; Xu et al., 2009). It is conceivable that physiological factors known to regulate structural plasticity during motor learning in normal animals, such as sleep (Yang et al., 2014), may provide insight into how to modulate recovery within the poststroke brain. Although the structural connectome can recover with reperfusion, the functional recovery of cortex in animal models lags (Chen et al., 2012). Changes in excitation-inhibition balance (Wang, 2003) and reduced motor thresholds (Volz et al., 2014) have been reported poststroke, although the major characteristic of the stroke-affected brain is profound and maintained inhibition of synaptic and sensory processing (Krakauer et al., 2012).

In both clinical cases and animal models, the sites mediating recovery may depend mainly on the size and location of the initial infarct, and on the extent of the secondary degeneration. Incomplete lesions of the motor cortex in animal models produce lasting behavioral impairments, with residual function likely driven by spared peri-infarct connections providing weak sensory or motor signals that can be enhanced through plasticity over weeks in the poststroke brain (Brown et al., 2009; Plow et al., 2014). In such cases, recovery involves spared peri-infarct tissue with function and connectivity that is similar to the infarct (Biernaskie et al., 2005; Brown et al., 2009). In contrast, after a large stroke, tissue with similar function may only be found at more distant sites, such as the premotor cortex in the injured hemisphere (for motor cortex stroke) (Plautz et al., 2003; Zeiler et al., 2013). After effective rehabilitation, the human premotor cortex develops increased functional connectivity with ipsilesional M1 (Grefkes and Fink, 2014), thus potentially serving as an effective target for brain stimulation-based therapies (Dancause, 2006; Plow et al., 2014). The presence of structural reorganization in the contralesional hemisphere has been less clear, as some animal work suggests homotopic regions (Figure 2B) to the infarct can support recovery (Biernaskie et al., 2004) and undergo structural remodeling (Takatsuru et al., 2009), while other mouse longitudinal imaging work indicates no poststroke structural reorganization (Johnston et al., 2013). Conceivably, these differences may be related to the subsets of regions assessed, or the possibility that apparent contralateral cortex remapping may involve non-structure-based rearrangements in function. Although investigators have focused on the peri-infarct areas, the brain is rife with alternative pathways. Perhaps the most obvious area of redundancy is ipsilateral or noncrossed sensorimotor processing, which could serve as a potential backup system following stroke.

The majority of cortical projections terminate on the contralateral spinal cord, while only 10%–15% project ipsilaterally. Given the relatively small number of ipsilaterally projecting fibers, a

Table 1. Poststroke Interventions and Relationships to Functional Connectivity

Poststroke Intervention	Best Application	Enablers	Barriers	Mechanism	Clinical Status
Rehabilitation (Carter et al., 2010b; Krakauer et al., 2012; Liepert et al., 2000; Lohse et al., 2014)	Focal stroke with some residual function	Specialized stroke units, outpatient facilities, constrained induced movement therapy (CIMT)	Patient compliance, intensity/duration may be inadequate, cost and availability	Plasticity in peri-infarct cortex, spared hemisphere or spinal cord	Conventional rehabilitation practiced in most centers, CIMT not widely available
Virtual reality, robotic movement training, and enabling (Dukelow et al., 2010; Gustavo et al., 2010; McEwen et al., 2014; Scott and Dukelow, 2011)	Use with patient-specific rehabilitation; can be used at home with online oversight and coaching	Technology widely available for robots; commercially available gaming consoles can be used for VR; aging of tech-savvy generation will favor uptake	Severely impaired may not be able to participate	Plasticity in peri-infarct cortex, spared hemisphere, or spinal cord	Randomized clinical trials
Modulate excitability with repetitive TMS or TDCS (Dayan et al., 2013; Edwards et al., 2013; Reis et al., 2008)	Combination with rehab to improve efficacy may produce long-term changes	Relatively simple, to administer; ability to induce focal potentiation or depression in brain regions	Short duration of action, need for better understanding of physiological changes induced by applied current or magnetic field	Activity-dependent plasticity, although location or mechanism not clear; may determine impact with resting-state imaging	Phase 2 clinical trials
Rehabilitation guided by functional brain connectivity (Gillebert and Mantini, 2013; James et al., 2009)	Profile lesions based on functional brain imaging/ stimulation and tailor rehab based on known clinical outcomes from database of similar lesions	Large databases of functional imaging and clinical descriptors; probing spared circuits with TMS (Blicher et al., 2009; Brodie et al., 2014; Plow et al., 2014)	Need for large databases that classify heterogeneous lesion profiles and/or multiple lesions based on spared connectivity and clinical exam; standardization of imaging parameters across centers	Plasticity in peri-infarct cortex, spared hemisphere, or spinal cord	Experimental/under development
Anti-Nogo side switch spinal cord administration (Bachmann et al., 2014; Lindau et al., 2014; Wahl et al., 2014)	Large stroke in motor and sensory system	Paired rehabilitation based on rodent work must be applied	Involves intrathecal injection; need proof of concept in primate motor system	Side switching of connections from spared corticospinal tract to stroke affected tract through sprouting	Phase 2 clinical trial ongoing for ALS, strong efficacy in rodent work
Chondroitinase ABC local spinal cord administration (Soleman et al., 2012)	Large stroke in motor and sensory system	Paired rehabilitation based on rodent work with anti-Nogo may be beneficial (Bachmann et al., 2014; Lindau et al., 2014; Wahl et al., 2014)	Involves intrathecal injection; need proof of concept in primate motor system	Side switching of connections from spared corticospinal tract to stroke-affected tract through sprouting	Experimental positive work in aged rodent
CNS sprouting reconnections peri-infarct (Carmichael, 2006; Chen et al., 2002; Gherardini et al., 2013; Jeffers et al., 2014; Overman et al., 2012)	Peri-infarct sprouting and structural plasticity within affected hemisphere	Better definition of targets and connections, will benefit from human/rodent connectome, possible rational treatment basis	Invasive, human safety unknown	Ephrin-A5 blocker, EGF/EPO, inosine, chondroitinase ABC	Positive rodent work

Although rehabilitation is currently the only intervention used clinically, there are a number of potential interventions under various stages of investigation that may provide beneficial. Because of space limitations, we do not discuss pharmacological agents that directly modulate synaptic transmission (and thus information flow through connectomes) or regenerative cell therapies. Information on clinical status is from https://clinicaltrials.gov.

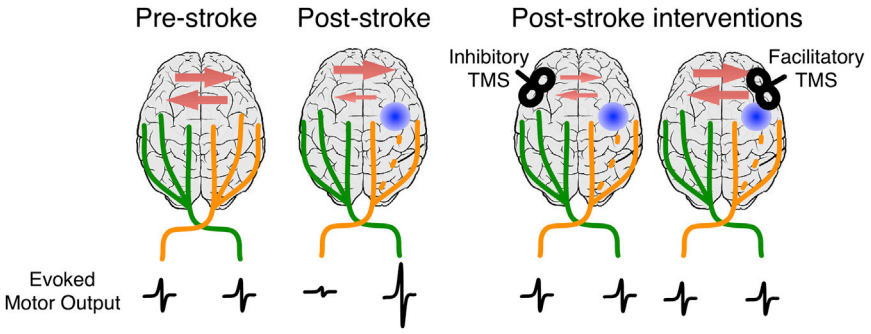

Figure 3. Interhemispheric Balance Is Altered after Stroke and Can Be Corrected with Brain Stimulation

Performing a motor task requires not only descending projections from the cortex to the spinal cord but also balanced interhemispheric inhibition of homotopic areas (red arrows). The loss of cortical tissue after stroke (blue circle) results in the disinhibition of the contralesional cortex. The intact hemisphere therefore has lower threshold for producing movement when stimulated with TMS (Volz et al., 2014), while motor output from the injured side is diminished (Escudero et al., 1998). Noninvasive brain stimulation, such as TMS, may be used to correct the interhemispheric imbalance by providing either inhibitory pulses to the intact hemisphere or facilitory pulses to the injured hemisphere. Such interventions may only be appropriate for a subclass of stroke patients, as variability in stroke location, size, and time since injury likely influence outcome (Nowak et al., 2009). Nonetheless, TMS may be used to search for treatment strategies that normalize motor-evoked potentials in patients recovering from stroke (cartoon based on synthesis from patient studies referenced above and in the text).

mechanism involving the intact hemisphere (e.g., right hemisphere controlling right limb) would at best control a small subset of muscles, or may be insufficient to reach threshold for muscle firing. Studies in both human and animal models also suggest that ipsilateral (noncrossed) task-specific activation within the intact hemisphere can actually be counterproductive, since it may provide long-range functional inhibition of the peri-infarct cortex (Takeuchi and Izumi, 2012). Consistent with this, a mainstay of experimental activity-dependent interventions is to inhibit the intact hemisphere to functionally disinhibit the peri-infract cortex (Nowak et al., 2008; Figure 3).

Viewing stroke from the perspective of the connectome, it is possible to conceptualize the recovery process as one that aims for efficiency in circuit utilization. During small strokes, an efficient design for recovery will incorporate proximal circuits in functionally related structures. In cases of large stroke, solutions involving proximal connections are abrogated, and more distally located, less-efficient connections are recruited to mediate recovery. Support for this concept may be attained from graph theory analysis of fMRI data (Figure 2A), where the brain is represented by nodes (anatomical brain regions) and edges (connections among regions) that are assigned based on the communication efficiency in the brain. Such methods are currently being employed to identify network disturbances after stoke (Alstott et al., 2009; Wang et al., 2010); however, a systematic analysis of the effect of lesion size and location is not yet available.

New Wiring for Recovery or Best Use of What Is Left?

Cortical remapping of function after stroke is associated with durable changes in structural connectivity due to axonal sprouting (Dancause et al., 2005; Overman et al., 2012) and dendritic spine turnover (Brown et al., 2009; Mostany et al., 2010). Long-lasting structural plasticity is at the heart of many models of stroke recovery (Carmichael, 2003b; Murphy and Corbett, 2009); however, most evidence is correlational, and it is unclear whether these new structural connections are necessary for recovery or whether they are maladaptive processes that contribute to impairments (Takeuchi and Izumi, 2012; Xerri et al., 2014). There is also evidence suggesting that functional cortical remapping is not solely due to structural changes. Some remapping of function can occur on very brief timescales that are inconsistent with sprouting and synaptogenesis (Jacobs and Donoghue, 1991). For example, intrahemisheric and even bihemispheric remapping of forelimb somatosensory responses was observed within minutes after acute stroke (Mohajerani et al., 2011). These findings are reminiscent of effects from studies in which brain regions are reversibly inactivated through cooling. Inactivating primary somatosensory cortex in one hemisphere causes immediate expansion of receptive fields in the other hemisphere (Clarey et al., 1996). Such changes are likely mediated by unmasking of latent functional connections (perhaps by disinhibition), and interestingly, with prolonged inactivation the receptive field properties return to baseline levels (Clarey et al., 1996). It is possible that similar homeostatic mechanisms will be observed on a more prolonged timescale during stroke recovery. In sum, these results suggest that the connectome may be able to reroute signals over rapid timescales if some surviving wiring is present.

Small Strokes Fell Great Oaks: Small Vessel Disease and Diffuse Network Damage

In contrast to focal ischemia, the relatively inclusive term "small vessel disease" describes a form of silent stroke in which there are many diffuse interruptions in blood flow leading to multiple sites of network impairment, and potentially different rules for circuit recovery. Recently, strong links have been identified between the occurrence of small vessel disease and Alzheimer's disease pathology (Black et al., 2009). Furthermore, animal models have been created which expand upon the Alzheimer's phenotype, or employ methods to occlude individual small vessels (Dorr et al., 2012; Jiwa et al., 2010; Shih et al., 2013; Shin et al., 2007). High-field strength human imaging studies are able to visualize small (1 mm diameter) microinfarcts (Smith et al., 2012); however, most cases are identified during postmortem analysis, making it difficult to assess the functional impact of these lesions. One key observation from functional imaging data is that disruption of cholinergic pathways and activity hubs, such as the default mode network, or executive function networks might contribute to dementia after acute ischemic stroke (Behl et al., 2007; Lim et al., 2014). Indeed, blocking cholinergic neuromodulator input to cortex impairs posttraining plastic reorganization in the healthy brain (Conner et al., 2010).

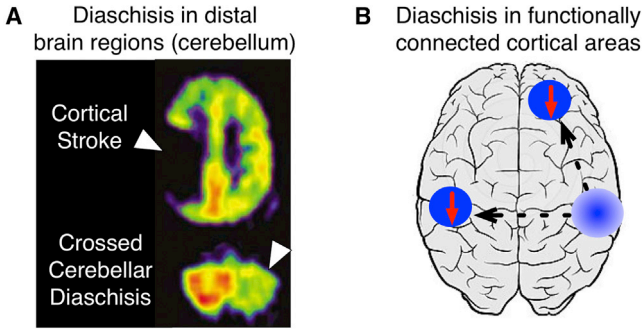

Figure 4. Diaschisis Is Defined as a Depression of Functionally Connected Brain Regions following Stroke

(A) Examples of crossed cerebellar diaschisis can be visualized with PET imaging in human patients as a decrease in blood flow in the cerebellar hemisphere contralateral to a cortical stroke (adapted from Sobesky et al., 2005).

(B) Functionally connected regions in the neocortex may also undergo diaschisis due to a loss of afferent connections after a focal stroke.

We anticipate that small vessel diseases will have very different consequences for network function than a focal targeted attack. In contrast to focal ischemia, patients with small vessel disease have diffuse injuries throughout the brain, and may require connectome-wide interventions that promote widespread remodeling or activity enhancement. Due to their small size, in many cases microinfarcts are not detected with standard imaging tools; therefore regional abnormalities in brain structures are assumed to reflect sites of damage, while actual affected networks may be more widely distributed. Nonetheless, there is evidence that regions such as the hippocampus and white matter tracts may accumulate more hyperintense damaged regions compared to the rest of the brain (van Veluw et al., 2013). Animal models of microinfarction can be used to systematically identify sites of damage and also reflect the trend for a greater number of infarcts in vulnerable brain regions (Wang et al., 2012). However, given that the microinfarcts can occur in white matter, which forms the conduit for multiple systems (Figure 2B), there may be little in the way of unaffected connectivity. Thus, widespread impairment due to small vessel disease could be in some ways less forgiving than focal infarctions. The wide incidence of small vessel disease and the fact that many patients also develop focal infarcts (Smith et al., 2012) represent a serious health problem and future challenge for stroke scientists. A key to progress will be animal models (Yoshizaki et al., 2008) in which treatments can be piloted in the manner that reflects the slow clinical course of the human disease (Jiwa et al., 2010). Whether these aspects can be accurately replicated within mouse is currently unclear, but this nonetheless remains an area for future development. With regard to therapeutics, use-dependent blockers of NMDA receptors, such as memantine, may be a key to reducing the spread of injury following blockage of penetrating arterioles in rat models (Shih et al., 2013). Other agents shown to have activity against vascular dementia in animal models include inositol that may function to normalize blood flow (Dorr et al., 2012).

Major Themes of Circuit Disruption and Recovery: Diaschisis and Vicariation

Our understanding of the poststroke recovery process is heavily influenced by the concepts of diaschisis and vicariance, which can be used to conceptualize many of the human and animal circuit-level findings described above (Carrera and Tononi, 2014). The term "diaschesis" was first introduced by von Monakow to describe the phenomenon of a transient depression of brain function at a site distal from the injury (Carmichael et al., 2004; Feeney and Baron, 1986; Von Monakow, 1969). A common form of diaschisis observed in patients is crossed cerebellar diaschisis, caused by the disruption of the corticopontocerebellar pathways after a cortical lesion (Figure 4) (Gold and Lauritzen, 2002; Sobesky et al., 2005). Resting-state fMRI experiments in patients with heterogeneous lesions have also demonstrated that cortical dysfunction extends beyond the stroke area but remains within the bounds of existing interconnected networks that are functionally correlated (Nomura et al., 2010). Animal models provide further evidence that focal cortical injury can induce a temporary depression in intact tissue, producing functional impairments (Carmichael et al., 2004; Feeney and Baron, 1986). For example, digit representation in the primate brain is organized into functional clusters interconnected with strong horizontal projections of layer 5 output neurons. A focal stroke injuring part of the digit representation results in remaining digit areas shrinking in size and the emergence of functional impairments in digit use (Nudo and Milliken, 1996). It is hypothesized that the loss of synaptic input from the site of injury causes a functional depression of connected noninjured sites (see Figure 2A for simulation example). Restoration of function is thought to occur, at least in part, through a process that normalizes activity at the areas with lost input. One way of achieving this is through rehabilitative training, as repeated forced use of the impaired hand prevents the secondary depression of areas functionally connected to the stroke (Nudo, 1997); however, the exact mechanism(s) mediating this effect remains unknown.

In the case of focal ischemia, functionally connected (but distal) sites from the stroke may also suffer from diaschesis in the short term, followed by more prolonged degeneration due to the loss of afferent synaptic input from distally connected regions (Kuceyeski et al., 2014). Databases of tractograms from healthy individuals can be used to predict the sites of distal degeneration in patients with focal ischemia (Kuceyeski et al., 2014) and may open the door for therapeutic interventions aimed at preventing distal degeneration. Developing targeted neuroprotective or rehabilitative strategies, based on connectomic information to prevent such delayed degeneration, could potentially impact a greater number of stroke patients than acute penumbral neuroprotective strategies that have a relatively short therapeutic window.

Another concept present in the literature, which is related to diaschisis, is "vicariation of function" (Dancause, 2006). During vicariation, neighboring tissues, which may have a different function, will take over the function lost by the stroke-affected tissue. A reasonable prediction is that areas with shared connectivity to the site of injury are more likely to facilitate vicariance than unrelated structures. These regions could include those that receive common thalamic input or send axonal projections to

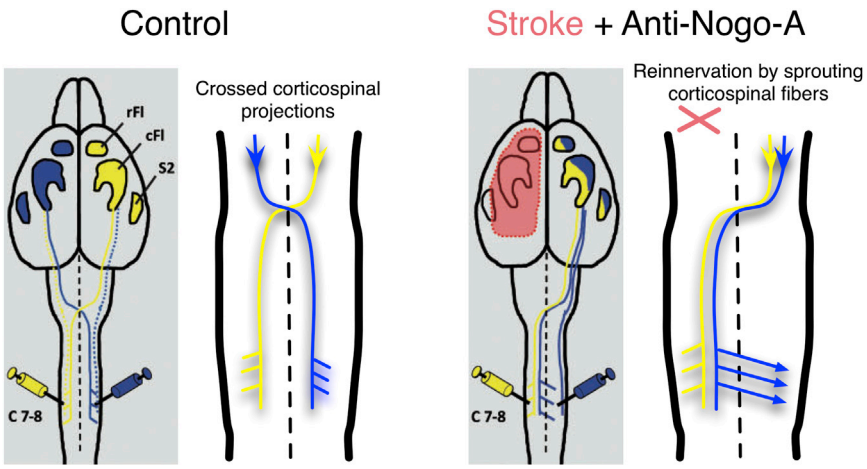

Figure 5. Anti-Nogo-A Treatment Causes Rewiring of the Spinal Cord to Mediate Recovery after Stroke

The descending cortical fibers mediating fine motor control are largely crossed, and therefore each hemisphere controls fine movements on the opposite side of the body. A focal stroke induces motor deficits on the contralesional side. Functional recovery may be induced by promoting sprouting of fibers from the intact hemisphere into the denervated side of the spinal cord through anti-Nogo-A administration; see blue arrows on far right adapted from (Lindau et al., 2014). In order for these newly formed fibers to be functionally beneficial, the animals need to engage in rehabilitative training after the growth promoting treatment is applied. Temporarily inactivating the crossed connections after recovery using pharmacogenetics reinstates the functional deficits (Wahl et al., 2014).

neighboring targets. For example, motor cortex, primary somatosensory cortex, and secondary somatosensory cortex all receive thalamic input (Hunnicutt et al., 2014; Oh et al., 2014), and function in secondary areas can be preserved after stroke to primary somatosensory areas (Sweetnam and Brown, 2013). In the rodent motor cortex, for example, the strongest intracortical connections are with the rest of the sensory-motor cortex in the same hemisphere, but strong interhemispheric connections also exist with homotopic areas (Bauer et al., 2014). A long-term goal for both basic and rehabilitative scientists is to predict where lost functions will remap on the cortex and to use this information to optimize treatments that may restore function following damage to the connectome.

How to Modify and Guide Connectome Changes Poststroke

Induction of Plasticity and Molecular Mediators

The plastic response of the poststroke brain can be shaped by both positive and negative factors. Seminal work from Tom Carmichael and others has defined the axonal sprouting response of the stroke-affected brain (Carmichael, 2003b; Carmichael et al., 2001; Overman et al., 2012; Overman and Carmichael, 2014). By studying the sprouting connectome, they have identified key regulatory molecules, such as the inhibitor of sprouting ephrin-A5 (Overman et al., 2012), which can be inhibited to promote reconnections within the stroke-affected brain.

Given that there have been excellent reviews on this subject (Carmichael, 2003a, 2003b; Overman and Carmichael, 2014), we only superficially address this area and direct readers to these resources.

At the level of spinal cord (Lindau et al., 2014), investigators have exploited removal of Nogo-A's inhibitory influences on the sprouting process leading to elaboration of corticofugal axon sprouts (Lee et al., 2004; Wahl et al., 2014). Alternatively, sprouting can also be directly enhanced by treatment with inosine (Chen et al., 2002), or by modulating the extracellular matrix with chondroitinase ABC treatment to rewire either the spinal cord (Soleman et al., 2012) or the peri-infarct cortex (Gherardini et al., 2013) (see Table 1 for overview). Following motor cortex stroke, a widescale reorganization of corticospinal axons from the intact cortex can support near-complete behavioral recovery if poststroke rehabilitative training is preceded by treatment with the growth-promoting Nogo-A antibody (Wahl et al., 2014). This effect is mediated by contralesional corticospinal tract neurons sprouting at the level of the cervical spinal cord to innervate motor pools that lost descending cortical input after the stroke (Figure 5). Although other pharmacological (Chen et al., 2002) and cell-transplant therapies have shown benefit through similar mechanisms (Hermann and Chopp, 2012), it is important to note that inducing a nonspecific increase of corticospinal tract sprouting by applying Nogo-A treatment during rehabilitative training can actually exacerbate poststroke motor impairments (Wahl et al., 2014). This result demonstrates the importance of restoring connectivity at functionally relevant sites, which may be distal from the injury (Table 1). Therefore under right conditions agents may promote plasticity at the level of spinal cord, which functions to reassign sensory and motor signals in the stroke-affected brain to make best use of the intact hemisphere (Figure 5).

Two well-known forms of activity-dependent plasticity are homeostatic and Hebbian mechanisms (Turrigiano and Nelson, 2004). Hebbian plasticity can be thought of as a synaptic strengthening system, which is dependent on coactivated synapses and enables relatively strong inputs to be further enhanced. In this manner, neurons that fire together wire together. In contrast, homeostatic mechanisms are involved in setting global network excitability and balance by adjusting global excitability and synaptic weights across the connectome. In animal models and cell culture systems, one can engage homeostatic or setpoint-mediated enhancement by using agents that depress activity to model the known depressive effects of stroke (Krakauer et al., 2012). Homeostatic mechanisms may be involved in recovery, as proposed in review articles (Lee et al., 2014; Murphy and Corbett, 2009; Nahmani and Turrigiano, 2014), by inducing compensatory increases in synaptic strength or excitability (Turrigiano and Nelson, 2004). It is possible that low levels of activity in peri-infarct zone engage homeostatic mechanisms that attempt to increase this region's excitability, similar to observations from the visual system following loss of retinal input (Keck et al., 2013).

Overall, when considering the potential influences of homeostatic plasticity on stroke recovery, one is left with a contrarian perspective. Here, it may be advantageous to further reduce activity within the peri-infarct zone, rather than increase it. Reduced activity would act as a stimulus for homeostatic plasticity and change the setpoint of the system. Although this is a hypothesis, it could have implications with respect to the onset or type of rehabilitation given. Interestingly, there are reports that the introduction of early rehabilitation in both animal models and human clinical data can be counterproductive in some cases (Kozlowski et al., 1996; Krakauer et al., 2012). However, there is also strong evidence from a phase II clinical trial that even early rehab intervention 24 hr after stroke may be beneficial (Cumming et al., 2011). We mention homeostatic plasticity as a powerful potentially understudied mechanism for circuit recovery. However, as mentioned in the introduction, traditional rehabilitation or principled sensory and motor practice are likely to be the most effective means of shaping the structural and functional connectome. Given space limitations, we have not focused on rehabilitation in this review.

Tools to Shape the Connectome: Animal Models

Optogenetics provides exquisite control over brain circuitry (Madisen et al., 2012). In the case of stroke, this technique provides (1) an effective means of probing brains of stroke-affected animals to understand the deficits associated with stroke (Anenberg et al., 2014; Chen et al., 2012; Xie et al., 2013), and (2) a platform to optimize effects of brain stimulation (Cheng et al., 2014), or inhibition on recovery that may be later attempted in humans using more clinically tractable means. To shape activity within the rodent connectome, investigators have a host of different optogenetic actuator and monitoring tools (Chen and Schlaug, 2013; Daniel et al., 2014; Madisen et al., 2012). For example, activity of individual neurons can be excited or inhibited using light-sensitive ion channels. This approach has been used in new studies of acute stroke to define windows of time when cortical neurons remain excitable when stimulated directly by light, but become unresponsive to peripheral sensory stimulation (Chen et al., 2012). More prolonged optogenetic stimulation protocols targeted to the injured hemisphere during the first 2 weeks of recovery were found to increase plasticity-related markers in the intact hemisphere and promote functional recovery (Cheng et al., 2014). We anticipate that future assessment of connectomes in stroke will employ extensive use of optogenetics to monitor and alter pathways. Other promising strategies include designer receptor exclusively activated by designer drugs (DREADDs), which allows for the modulation of neuronal function by expressing an engineered receptor in the brain and administering its ligand systemically at a desired time (Alexander et al., 2009; Conklin et al., 2008). New tools involving optogenetic agents that create reversible loss of function in recovered circuits (Conklin et al., 2008; Wahl et al., 2014) provide increased specificity compared to previous technologies (such as focal cooling, or pharmacological inactivation), and could be used to provide causal evidence for structure-based models of recovery after stroke. Future work using optogenetics to create brief focal, but nonischemic, inactivations (Carrera and Tononi, 2014; Madisen et al., 2012) will make it possible to evaluate the effects of a virtual stroke on the brain's functional connectome. These agents have been recently used to chemically inactivate specific cortical cell populations, providing much needed loss-of-function experiments that clearly demonstrate a role for the spared hemisphere in anti-NogoA stimulated recovery from stroke (Wahl et al., 2014). Other means to alter connectomes include the molecular strategies outlined earlier, which affect sprouting of connections through removing the effect of endogenous inhibitors (Bachmann et al., 2014; Overman et al., 2012; Overman and Carmichael, 2014; Soleman et al., 2012; Wahl et al., 2014). The tools for connectome assessment and manipulation are better developed in animal models. Future challenges will be to effectively translate insights from rodent work to similar or analogous systems present in humans. While optogenetics can be powerful and selective means of stimulation for animal work, it is generally not practical on a large scale in human brain. In this case it may be possible to find a unique transform between human applicable TMS and specific forms of animal optogenetics that can be used to optimize protocols. Such proposals are not impractical, given findings showing that TMS can be either excitatory or inhibitory based on the parameters used for stimulation (Hallett, 2007).

Shaping Functional Connectome in Human Patients

Conceivably, the connectome could be restored in a human stroke patient through the use of regenerative technology, such as stem cells (Savitz et al., 2014) or strategies that permit the delivery of proteins that affect neuronal sprouting (Bachmann et al., 2014; Overman et al., 2012; Overman and Carmichael, 2014; Soleman et al., 2012; Wahl et al., 2014). However, many of these approaches are invasive and difficult to justify in patients that have an otherwise stable condition (Table 1). Some recent measures, such as the injection of anti-NogoA, can be applied with less-invasive intrathecal spinal injections, making it a potentially more translatable option (Lindau et al., 2014).

Practical measures, such as rehabilitation (Krakauer et al., 2012; MacLellan et al., 2011), environmental enrichment (MacLellan et al., 2011), and TMS (Nowak et al., 2009; Plow et al., 2014), are less-invasive means of altering connectome strength. TMS is typically only applied transiently, so any lasting benefit for stroke recovery would be through engaging durable mechanisms of circuit-level plasticity (Brodie et al., 2014; Raffin and Siebner, 2014). Alternatively, transcranial direct current stimulation (TDCS) is another relatively safe and noninvasive means of altering peri-infarct function in human (Hummel et al., 2005), and may be used during physical therapy to promote the retraining of affected circuits (Dayan et al., 2013). Recent evidence indicates that scalp montage electrodes can be used to relatively selectively target TDCS to regions of human motor cortex (Edwards et al., 2013). In additional to noninvasive stimulation using TMS or TDCS (Figure 3), electrode-based brain stimulation can play a role in facilitating and inhibiting brain areas affected by stroke. However, the results of clinical trials in stroke using invasive brain stimulation have been mixed (Plow et al., 2009). Promising new applications of electrode-based stimulation tested in rats are closed-loop devices that can trigger stimulation of somatosensory cortex based on sensing of endogenous activity within premotor cortex. Premotor cortex activity then triggers stimulation to produce in-phase activation of somatosensory cortex that was functionally disconnected by

stroke or other injury (Guggenmos et al., 2013). However, these approaches are relatively invasive and require surgery to implement. Questions also remain as to whether the effect of stimulation is truly restorative, or if it functions as timing signal (a cortical metronome) around which endogenous mechanisms of plasticity are built. Nonetheless, functional data using a premotor to somatosensory cortex bridge to work around an injury to motor cortex have been very promising (Guggenmos et al., 2013).

Perhaps the most practical approach to altering connectome function will be through innovative rehabilitation strategies based on the patient's connectome outlook defined by resting-state fMRI imaging (Carter et al., 2010a). Therapeutic strategies may include virtual reality videogames which may engage select sensory and motor circuits (Gustavo et al., 2010; McEwen et al., 2014), or smart kinematic systems such as the KINARM exoskeleton to both assess and rehabilitate lost sensory motor function after stroke (Dukelow et al., 2010; Scott and Dukelow, 2011; Table 1). Given that insufficient participation in rehabilitative training is an unfortunate reality for many stroke patients, it is important to note that the uptake of gaming technology for rehab use will favor the emerging tech-savvy generation, which may already have experience with touch/gesture recognition devices. It is anticipated that these rehabilitation tools will indirectly affect the flow of information through brain connectomes and through circuit-level plasticity that may have a lasting and positive impact on stroke patients.

Potential Barriers and Future Translation

Questions will remain on whether structure predicts or dictates recovery of function after stroke. In guiding interventions, will it be better to focus on assessment of regional functional connectivity, using techniques like resting-state fMRI, or focus on the predictive value of structural connectivity, assessed using techniques such as DTI in humans. Ideally, future studies will use techniques to acquire both structural and functional connectivity, as well as information about regional blood flow to investigate relationships between structure and function during recovery from stroke. Application of graphical methods to large data sets of functional imaging data is already in place (Biswal et al., 2010). However, descriptors of these data that allow correlation with patient metadata on clinical parameters are still being developed. While resting-state functional MRI can be easily collected in terms of patient compliance, the analysis and interpretation of such data can be challenging and difficult to standardize across different research centers. Questions remain, such as whether data from individual patients can be compared to the consensus to guide treatment. Every person has their own unique connectome, and it remains to be seen how important this will be in developing optimal treatment and rehabilitation strategies. Stroke patients often have multiple strokes, and confounding factors such as age, gender, circadian rhythms, and homeostatic mechanisms all influence functional connectivity (Hodkinson et al., 2014). This raises the following questions: How we can interpret the data from existing connectome databases? And to what extent can we rely on these to shape treatments for patients? Regardless of these limitations, it is clear that the connectome controls all that we do, and knowledge of its interworkings will help reveal novel treatments for stroke.

ACKNOWLEDGMENTS

This work was supported by a Canadian Institutes of Health Research (CIHR) Operating Grant MOP-111009, a Heart and Stroke Foundation of Canada Grant in Aid, and a Canadian Partnership for Stroke Recovery expansion grant to T.H.M. We thank Dianna Lim for discussion and figure suggestions, and Lara Boyd and Angela Auriat for helpful comments.

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