Title
Molecular, cellular and functional events in axonal sprouting after stroke

Journal
Experimental Neurology, 287(Pt 3)

ISSN
0014-4886

Authors
Carmichael, S Thomas
Kathirvelu, Balachandar
Schweppe, Catherine A
et al.

Publication Date
2017

DOI
10.1016/j.expneurol.2016.02.007

Peer reviewed

HHS Public Access

Author manuscript

Exp Neurol. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Exp Neurol. 2017 January ; 287(Pt 3): 384–394. doi:10.1016/j.expneurol.2016.02.007.

Molecular, Cellular and Functional Events in Axonal Sprouting after Stroke

S. Thomas Carmichael, **Balachander Kathirvelu**, **Catherine A Schweppe**, and **Esther H Nie**

Departments of Neurology and of Neurobiology, David Geffen School of Medicine at UCLA, 710 Westwood Plaza, Los Angeles, CA 90095 USA

S. Thomas Carmichael: scarmichael@mednet.ucla.edu; Balachander Kathirvelu: bkathirvelu@ucla.edu; Catherine A Schweppe: caschweppe@ucla.edu; Esther H Nie: esthernie@mednet.ucla.edu

Abstract

Stroke is the leading cause of adult disability. Yet there is a limited degree of recovery in this disease. One of the mechanisms of recovery is the formation of new connections in the brain and spinal cord after stroke: post-stroke axonal sprouting. Studies indicate that post-stroke axonal sprouting occurs in mice, rats, primates and humans. Inducing post-stroke axonal sprouting in specific connections enhances recovery; blocking axonal sprouting impairs recovery. Behavioral activity patterns after stroke modify the axonal sprouting response. A unique regenerative molecular program mediates this aspect of tissue repair in the CNS. The types of connections that are formed after stroke indicate three patterns of axonal sprouting after stroke: Reactive, Reparative and Unbounded Axonal Sprouting. These differ in mechanism, location, relationship to behavioral recovery and, importantly, in their prospect for therapeutic manipulation to enhance tissue repair.

Keywords

Rehabilitation; regeneration; recovery; spinal cord; cortex; GDF10; TGFβ; astrocyte; behavior

Clinical Overview of Stroke

Stroke is the leading cause of adult disability. With over 800,000 new strokes a year, the limited degree of spontaneous recovery after stroke means a large personal and societal burden in lost productivity, lost independence and social withdrawal (Mozaffarian et al., 2015). In the presence of this large incidence, the clinical landscape in stroke is changing. Better acute stroke care means that the death rate in stroke is declining, with stroke sliding from the 3rd leading cause of death to the 5th in the past six years (Centers for Disease Control and Prevention). This is a welcome event, but a reduced death rate in stroke does mean a greater number of disabled survivors. The recent stent-retriever trials in acute stroke

Correspondence to: S. Thomas Carmichael, scarmichael@mednet.ucla.edu.

show reduced death and disability with the use of these devices (Pierot and Derdeyn, 2015). A key finding in these trials is that a larger percentage of patients with stent-retriever delivery have reduced disability compared with standard medical care. This was determined with the use of the modified Rankin Scale (mRS), and the stent-retrievers produce greater numbers of patients after stroke with a low or minimal disability level of 0–2 on this scale compared to medical therapy. In support of this stent/retriever effect, the mRS score at 3 months post-stroke is indeed predictive of long-term functional independence and of late stroke mortality (Huybrechts et al., 2008). However, an important limitation of the mRS in evaluating mild disability is that it has a floor effect, for example over two-thirds of patients that score in the no or minimal disability cutoff of the mRS (score of 0–2) actually report difficulties with hand use (Weisscher et al., 2008; Steward and Cramer, 2013). Thus, even with state of the art interventional device delivery and acute stroke unit care, stroke will remain a significant source of long term neurological disability.

The substantial burden of long term disability in stroke has prompted investigation into the mechanisms of neural repair. Stroke triggers a remarkable degree of plasticity in structural and functional connections. Neurons adjacent to stroke in peri-infarct cortex form new connections within motor, somatosensory and premotor areas in the hemisphere ipsilateral to the infarct. This has been observed in mice, rats and monkeys in experimental stroke models. These new connections in the hemisphere ipsilateral to the stroke can be local, within the damaged tissue very close to the infarct (Carmichael et al., 2001) or at longer distances in the hemisphere with the lesion, such as between areas in different lobes of the monkey (Dancause et al., 2005) or the mouse (Brown et al., 2009) (Fig. 1). In the hemisphere contralateral to the stroke, stroke induces new connections to form from frontal motor regions to parts of the brainstem or spinal cord that have lost their projection from the stroke site. Axonal sprouting from the cortex contralateral to stroke or similarly-sized cortical lesions occurs in the red nucleus (Seymour et al., 2006) and the cervical spinal cord (Benowitz and Carmichael, 2010; Lindau et al., 2014, Wahl et al., 2014). The occurrence of axonal sprouting, and its role in functional recovery, likely depends on the nature of the original stroke. In small to medium sized experimental strokes, axonal sprouting in peri-infarct and connected cortical areas is causally associated with motor recovery (Overman et al., 2012; Li et al., 2015). In large infarcts, in which most of the peri-infarct cortical hemisphere is damaged or lost in the stroke, axonal sprouting from contralateral cortex to the de-afferented side of the cervical spinal cord is causally associated with recovery (Bachmann et al., 2014; Wahl et al., 2014). The molecular and cellular mechanisms of these two axonal sprouting responses will be discussed in turn.

Peri-Infarct Axonal Sprouting in Stroke

Stroke triggers axonal sprouting in the cortical areas adjacent or connected to the infarct. This can be detected with anatomical mapping of cortical circuits as early as three weeks after stroke (Carmichael et al., 2001) and is robustly present one month after the stroke (Li et al., 2010; Li et al. 2015; Overman et al., 2012), and at several months after stroke (Dancause et al., 2005; Brown et al., 2009). The initiation phase for peri-infarct axonal sprouting is within the first week (Li et al., 2010). Axonal sprouting in peri-infarct cortex after stroke occurs in the tissue immediately bordering the infarct and in motor, somatosensory and

premotor areas distant to the infarct. Axonal sprouting is detected when the connections in motor or somatosensory are mapped in the control, non-stroke condition and compared to the same motor or sensory projections after stroke. In these kinds of studies, new projection neurons within, for example, adjacent somatosensory cortex 3 weeks after small strokes in the somatosensory cortex in the rat. These new projections alter the topography of cortical projections in the somatosensory system and statistically significantly shift the aggregate map of projections (Fig. 2) (Carmichael et al., 2001). In larger strokes in motor or somatosensory cortex, there is also a significant change in axonal projections from the motor cortex after stroke. This can be tested by quantitatively mapping the cortical connections after a tracer injection into forelimb motor cortex in control, non-stroke vs. stroke mice, and then using population statistics or polar statistics to test for differences in the location of connections in the cortex. There are significantly different, and new, connections from motor cortex to premotor cortex and primary and secondary somatosensory cortex one month after stroke in these mouse models of ischemia (Li et al., 2010; Li et al., 2015; Overman et al., 2012; Clarkson et al., 2013). Stroke in the monkey also produces a similar pattern of axonal sprouting as detected by anatomical labeling of projections between somatosensory and premotor cortex (Dancause et al., 2005). Stroke also produces visibly altered patterns of projections from sensorimotor cortex near the infarct site to parietal cortex (Brown et al., 2009). These patterns of axonal sprouting in mouse, rat and monkey overlap with changes in functional maps of motor control in humans in peri-infarct motor, premotor and somatosensory areas to indicate common areas of plasticity in the post-stroke brain (Fig. 1).

Contralateral Cortical Axonal Sprouting after Stroke

Stroke also triggers axonal sprouting from neurons in the cortex of the contralateral hemisphere. Corticospinal neurons in contralateral cortex sprout in their projections from the corticospinal tract and grow into the cervical spinal cord that has been denervated by the stroke, ipsilateral to the cortical origin of these projection neurons. This contralateral corticospinal axonal sprouting has been reported in the rat, mouse and non-human primate after stroke and other cortical lesions (Benowitz and Carmichael, 2010; Lindau et al., 2014; Morecraft et al., 2015) and is associated with remapping of motor representations of the ipsilateral limb in this motor cortex (Lindau et al., 2014). As noted, larger strokes are associated with greater contralateral cortical axonal sprouting. This is seen in rodent models of stroke, and was recently validated in the non-human primate, in which larger frontal lesions produced greater axonal sprouting from contralateral primary motor cortex into the denervated ventral horn of the cervical spinal cord (Morecraft et al., 2015). Interestingly, in this non-human primate study, axonal sprouting from the contralateral motor cortex was induced in strokes that involved motor cortex but suppressed if the lesion involved not just motor cortex, but also parietal somatosensory cortex (Morecraft et al., 2015). This may suggest a level of complexity in the post-stroke axonal sprouting response that has not been appreciated from rodent studies, related to network effects on neuronal activity levels, local trophic factor release from specific projection systems or possibly a behavioral activity interaction with axonal sprouting when somatosensory input is lost. Axonal sprouting is also seen from the cortex contralateral to the stroke into the striatum ipsilateral to the stroke (Szele et al., 1995; Carmichael and Chesselet, 2002; Riban and Chesselet, 2006). Finally,

axonal sprouting from cortex contralateral to stroke also occurs in the red nucleus in the brainstem, from the normal projection field into the opposite, denervated red nucleus. These cases of axonal sprouting in the contralesional corticospinal system are important to behavioral recovery. When the specific projections from cortex contralateral to the stroke to the ipsilateral cervical spinal cord are selectively inactivated, motor recovery is blocked (Wahl et al., 2014). Thus, axonal sprouting in contralateral corticospinal circuits is causally associated with recovery in these large volume stroke models.

While the intact, contralesional corticospinal tract in large volume strokes may sprout to denervated areas, other cortical circuits within the spared hemisphere after smaller strokes can actually form aberrant connectivity. For example, training of the nonparetic limb after unilateral stroke diminishes the outcome of rehabilitative training of the stroke-affected limb (Allred et al., 2005, 2010; Allred and Jones 2008). Moreover, overuse of the stroke-unaffected limb is correlated with diminished forelimb representation of the peri-infarct cortex, and aberrant synaptogenesis perhaps from transcallosal neurons (Kim et al. 2015). These implications are clinically important because the phenomenon of “learned nonuse,” or heightened reliance on the good limb after stroke, is a common sequelae to hemiparesis, but may hinder circuit repair and motor recovery. The effect of the contralateral cortex on recovery was also shown to be negative in blocking study: muscimol inactivation of the contralesional cortex for 3–14 days improves behavioral outcome (Mansoori et al, 2014). This data indicates that in different animal models of stroke, the contralateral (to the stroke) cortex has been shown to both mediate recovery and to impede recovery. In total, to truly dissect the role of contralateral cortex after stroke--whether it subserves or subverts recovery--necessitates an analysis of extent and location of the stroke along with relevant behavioral paradigms, both compensatory and rehabilitative.

Reactive, Reparative and Unbounded Axonal Sprouting after Stroke

The data in post-stroke axonal sprouting indicate three patterns in this process, with distinct cellular mechanisms. The local axonal sprouting into brain tissue directly adjacent to the infarct suggests a reactive process that is part of the tissue reorganization and scar formation of the stroke itself, termed Reactive Axonal Sprouting (Fig. 2). This local axonal sprouting is present even when glial growth inhibitor signaling is enhanced, such as Ephrin signaling (Overman et al., 2012). Reactive axonal sprouting is present around the stroke in several different stroke models and across species (Carmichael et al., 2001; Carmichael and Chesselet, 2002; Li et al., 2010, Overman et al., 2012; Li et al., 2015; Omura et al., 2015). In the axonal sprouting from the contralateral sensorimotor cortex from the stroke into the ipsilateral cervical spinal cord, stroke also induces a very modest axonal sprouting response (Chen et al., 2002; Zai et al., 2009; Benowitz and Carmichael, 2010). This occurs into areas of the cervical spinal cord that experience reactive astrocytosis and microglial responses because of axonal degeneration from the projections from the stroke site. This pattern of local or reactive axonal sprouting is seen in other types of brain lesions in which axons are acutely damaged, such as in the hippocampus or entorhinal cortex (Kelley and Steward 1997; McKinney et al., 1997). The general axonal reaction to injury is one of activation of a local growth program and local sprouting (Dickson et al., 2007; Lang et al., 2012; Allegra-Mascara et al., 2013) in many if not most neurons. Reactive axonal sprouting is thus part of

scarring and endogenous brain tissue remodeling in stroke. Though Reactive Axonal Sprouting appears to be an automatic cellular response to neuronal injury, it may still be part of the limited recovery seen after stroke. For example, the region of Reactive Axonal Sprouting is within the areas of motor and somatosensory physiological remapping during recovery (Harrison et al., 2013). A limited and partially predictable amount of recovery occurs in humans with stroke (Winters et al., 2014; Krakauer and Marshall, 2015) and Reactive Axonal Sprouting may play a role in this spontaneous recovery process.

Longer distance axonal sprouting after stroke can be stimulated by blocking glial growth inhibitors or inducing a neuronal growth program after stroke and is clearly associated with behavioral recovery (Fig. 3). This can be termed Reparative Axonal Sprouting and is distinct from Reactive Axonal Sprouting. Reparative Axonal Sprouting is seen with NgR1 signaling antagonists, EphrinA5 signaling blockade or GDF10 or inosine delivery in peri-infarct cortical axonal sprouting and in spinal cord axonal sprouting after stroke (Chen et al., 2002; Li et al., 2004; Li et al., 2010; Wahl et al., 2014; Li et al., 2015). This axonal sprouting pattern occurs over a longer distance than Reactive Axonal Sprouting, and links functionally related brain areas. In peri-infarct cortex, blockade of NgR1 or EphrinA5, or delivery of the newly described brain growth factor GDF10, induces axonal sprouting largely within motor-premotor-somatosensory areas—in other words within the same functional domain (Li et al., 2010; Li et al., 2015; Overman et al., 2012). In the cervical spinal cord, Nogo antagonists or inosine treatment or both (Chen et al., 2002; Zai et al., 2009, 2011; Lindau et al., 2013) induce a more robust and longer distance axonal sprouting than stroke alone, but this sprouting occurs within motor laminae of the spinal cord. In both cases of Reparative Axonal Sprouting in peri-infarct cortex or spinal cord, blocking this response interferes with recovery (Overman et al., 2012; Li et al., 2015; Wahl et al., 2015), hence the term Reparative Axonal Sprouting.

Reactive Axonal Sprouting and Reparative Axonal Sprouting after stroke occur within circumscribed areas of the CNS: the immediate vicinity of the infarct (reactive) or sensorimotor areas of the ipsilateral hemisphere or the contralateral spinal cord (reparative). This pattern indicates that molecular or cellular limits exist within the sprouting response after stroke: axonal spouting does not occur in some random direction. If this were the case, then axonal sprouting in peri-infarct cortex might be expected to produce new projections in brain areas that are simply the closest to the stroke, or the closest to the neuroanatomical tracer injection site that is used to label these projections. Instead, new projections occur in non-random and spatially distinct patterns. This is most striking in quantitative connectional maps of peri-infarct cortex motor system connections. The forelimb motor cortex always projects to adjacent motor cortex, somatosensory cortex and premotor cortex when axonal sprouting is stimulated by the growth factor GDF10 (Li et al., 2015), by blockage of glial growth inhibitors NgR1 (Li et al., 2010) or EphrinA5 (Overman et al., 2012) or when axonal sprouting is mapped in a genetic strain of mice with natural, robust axonal growth after injury (Omura et al., 2015).

What would happen if post-stroke axonal sprouting did not occur in functionally related brain or spinal cord areas? This is the case with Unbounded Axonal Sprouting after stroke, which occurs when two conditions intervene: glial growth inhibitors are blocked at the same

time that the activity of the injured motor connections are increased (Fig. 4). To date, there are two examples of this. With stroke in the motor cortex, when the glial growth inhibitor EphrinA5 is blocked in peri-infarct cortex, and the animal is then forced to overuse its affected forelimb, the motor cortex sprouts new connections into virtually every region of the ipsilateral cortical hemisphere (Overman et al., 2012). This means that the forelimb motor cortex now connects with prefrontal, orbitofrontal, insular, parietal and temporal areas —a shockingly divergent pattern of connections. Simultaneous blockade of astrocyte growth inhibition and enhanced behavioral activity of the motor system will enable the motor system to connect in a pattern that is not bounded by functional domains—connections form far afield of the sensorimotor system or the premotor areas and extend to executive, attentional, memory and higher order associational areas of the brain.

In addition to Unbounded Axonal Sprouting in peri-infarct cortex, this process also occurs after stroke in similar conditions of blockage of a glial growth inhibitor and enhanced behavioral activity within the spinal cord. When Nogo signaling is blocked and the animal put into intensive, daily skilled reach training, axonal sprouting and functional recovery is enhanced if the Nogo blockage and the reach training are done sequentially, one after the other (blockade then training). However, when these two are done simultaneously, then axonal sprouting is increased and occurs into aberrant and functionally unrelated parts of the cervical spinal cord, such as the far dorsal horn. Behavioral performance does not improve, and in fact is worse than control stroke conditions (Wahl et al., 2014).

It should be noted that the results are not yet in on whether Unbounded Axonal Sprouting is universally maladaptive. It was in the spinal cord study (Wahl et al., 14) but there was no behavior in the study in cortex (Overman et al., ’12). It is possible that some forms of this very exuberant form of axonal sprouting may promote recovery on some level. At this time it is most appropriate to remain agnostic as to the functional implications of this form of axonal sprouting, and utilize a term that describes its occurrence across functional boundaries in the CNS.

These studies in post-stroke axonal sprouting open up a new chapter in our understanding of brain connections, neurorehabilitative therapy and enhanced functional recovery. The adult brain has a very limited capacity to form new connections after stroke, and this is induced by local cues in the vicinity of the scar in a process that resembles the formation of the scar itself—Reactive Axonal Sprouting. This limited capacity can be increased by blocking glial growth inhibitors or stimulating a neuronal growth program, and this enhances motor recovery by inducing new patterns of motor, somatosensory and premotor connections in cortex or new motor corticospinal projections in the cervical spinal cord—Reparative Axonal Sprouting. Manipulating behavioral activity, through constraint-induced movement patterns or intensive skilled reach training, while blocking glial growth inhibitors releases growing connections from the control of tissue boundaries and these connections can extend into vastly divergent functional areas—Unbounded Axonal Sprouting. This process can degrade behavioral recovery. There may be a clinical correlate for this. In trials of intensive neurorehabilitation, when this constraint-induced movement therapy is begun earlier, patients recover less well (Dromerick et al., 2009). There are many possible reasons for reduced recovery in early intensive neurorehabilitation. It may be that the brain damage is

too close to the time of the stroke and over-use causes modest subtotal increases in damage, not detectable in MRI. But it may be that the cues for axonal sprouting, which are stronger earlier after stroke (Biernaskie et al., 2004), are combining with intensive training in patients to induce Unbounded Post-Stroke Axonal Sprouting. It is possible that the preponderance of Unbounded Axonal Sprouting captured in these tracing studies can still be pruned or shaped with the right factors and the right timing into functionally meaningful circuits. Future studies in this field will need to establish behavior/circuit interactions to develop the optimal approach for novel axonal growth therapies and neurorehabilitation paradigms to promote recovery.

Demonstration of Axonal Sprouting after Stroke

An important element to these studies of axonal sprouting is their precise mapping. Cortical connections of course form a projection network. This network links brain regions into a functional system that plays a role in mediating recovery—for example linking motor, somatosensory and premotor areas in a new way after stroke (Overman et al., 2012, Li et al., 2015). Changes in the network of connections after stroke lead to the behavioral deficits in this disease, and also axonal sprouting or recovery in these affected circuits leads to recovery (Silasi and Murphy, 2014; Corbetta et al., 2015). In essence, stroke is a disease of neuronal networks. Many studies in the field of neural repair have simply measured immunohistochemical staining patterns of axonal proteins, such as neurofilaments or synapse-associated proteins, and presumed that this means that axonal sprouting has taken place. Such studies lack three elements in their ability to allow us to move forward in the field. First, they do not unequivocally demonstrate axonal sprouting. It is not clear that immunohistochemical staining of an axonal protein means that there are more axons, as immunohistochemical staining is not quantitative and in fact not linear in its intensity appearance. It may be that protein half-life, axonal transport, epitope presentation or other aspects or protein or immunohistochemical staining are the reason for an increase in an axonal protein stain after stroke or a stroke treatment. Second, even if there were a 1:1 relationship of immunohistochemical staining increases for say, neurofilament or MAP2 or synapsin and an increase in axonal projections in an area, this tells us nothing of the origin and termination of the connections. Stroke recovery lies in the new system of neuronal connections or the new properties of neurons in a recovering area. A greater brown reaction product for a neurofilament stain after stroke provides no information to help in an understanding of the circuit that is reorganizing after stroke. Third, simple stains of axonal proteins do not tell us if the axonal changes are reactive, reparative or unbounded forms of post-stroke axonal sprouting. These three types have very different meanings for stroke tissue repair. Undoubtedly, technical advancements in the long-term imaging of injured and sprouting neurons on a cell-by-cell basis will help resolve some of the open questions left by immunostaining methods.

Dendritic Spine Changes in Stroke

Stroke induces a change in dendritic morphology in the regions of axonal sprouting in peri-infarct cortex. Dendritic spines form the synaptic contacts of cortical pyramidal neurons. These are mostly stable in the adult cortex (Grutzendler et al., 2002; Trachtenberg et al.,

2002) although they remodel in response to loss of afferent inputs or learning (Cheetham et al., 2008; Jaskinka et al., 2010; Fu and Zuo, 2011). After stroke, there is initially a net loss of dendritic spines in peri-infarct cortex within 24 hours after photothrombotic stroke (Brown et al., 2007, 2008) and in the first week after middle cerebral artery occlusion (Mostany et al., 2010). The absolute tissue distances that define peri-infarct cortex vary between stroke models, but overall these body of studies are complementary- that peri-infarct cortex sees remarkable spine remodeling. Importantly, this occurs in regions with normal blood flow (Mostany et al., 2010), indicating that subacute spine loss is due to neuronal network damage from loss of axonal connections and not due to partial ischemia in peri-infarct regions. After this loss of connections, peri-infarct cortex within one millimeter of the infarct in the mouse recovers synaptic connections back to baseline (Brown et al., 2008; Mostany et al., 2010), whereas neurons in regions 2–3mm away from the infarct gain synaptic connections compared to control (Mostany et al., 2010), indicating these neurons may have formed new connections after stroke. This can be seen also as supernumerary axons exiting the neuronal cell body (Hinman et al., 2013). The actual branches of dendrites also remodel after stroke, with retraction and growth that is maximal two weeks after the infarct and occurs most prominently within 200μm of the infarct core (Brown et al., 2010).. Such post-injury synaptogenesis could potentially occur between remodeling dendrites and spared or sprouting neurons.

Axonal sprouting and dendritic morphogenesis overlap in similar regions of peri-infarct cortex. This should be expected because changes in dendritic structure would be the expected synaptic parallel to the changes in axonal structure that occur in axonal sprouting. As mentioned, axons that have formed new connections after stroke contain pre-synaptic markers that are closely paired with post-synaptic markers, strongly suggesting that these new connections form synaptic innervation (Li et al., 2015). However, at this moment in neuroscience, experimental observations of axonal sprouting and those of dendritic spine morphology involve distinct methodologies. Axonal sprouting is demonstrated most convincingly when new patterns of connections are demonstrated between brain maps after stroke vs. control (Brown et al., 2009; Li et al., 2010; Li et al., 2015; Clarkson et al., 2011, 2015; Overman et al., 2012). This approach allows a connectome, such as that of forelimb motor cortex, to be quantified and tested as distinct between stroke and control. However, dendritic spine changes are most convincingly demonstrated after stroke by in vivo observation over time in the same animal and in the same neuron, for example using two photon imaging in a very small window of cortex. Thus axonal sprouting studies measure hemispheric connectional maps, with scales of millimeters to tens of microns in populations of animals, and dendritic spine studies measure single cell morphology, at scales of the single micron in the individual cell. A future goal is to achieve pre- and post-synaptic mapping of new connection formation after stroke in the same study.

Triggers for Post-Stroke Axonal Sprouting

Axonal sprouting after stroke indicates that there are triggers from the infarct itself which initiate this process. Such a trigger will be relayed from the stroke site, which means that that initial cell death leads to secondary injury cascades that signal to surrounding tissue. These cascades involve reperfusion injury in damaged cells, which causes free radical

production, the activation of astrocytes and white blood cells, which release cytokines and free radicals, and the production of synchronized neuronal activity. Cytokines activate astrocytes, promote angiogenesis and induce axonal sprouting (Gleichman and Carmichael, 2014; Brumm and Carmichael, 2012; Gertz et al., 2012) and synchronized neuronal activity induces axonal sprouting and the formation of new connections (Carmichael and Chesselet, 2002).

Inflammatory cytokines are released by activated microglia (early), invading neutrophils (early) and macrophages (later) (Iadecola and Anrather, 2011; Benakis et al., 2015). These can directly stimulate aspects of neural repair (Ekdahl et al., 2009), and also induce an activated state in brain endothelial cells and astrocytes. Activated astrocytes and endothelial cells then further produce molecules that induce or alter the brain's reparative response (Zhao and Rempe, 2010; Brumm and Carmichael, 2012; Gleichman and Carmichael, 2013). Activation of astrocytes in peri-infarct cortex, in part through inflammatory cytokines, induces molecules that block axonal sprouting, such as chondroitin sulfate proteoglycans (Carmichael et al., 2005; Deguchi et al., 2005; Shen et al., 2008) or Ephrin-A5 (Overman et al., 2012). The transforming growth factor superfamily of cytokines contains several molecules that stimulate post-stroke axonal sprouting. Bone morphogenic protein 7 stimulates dendritic growth in neurons (Withers et al., 2000) and promotes behavioral recovery after stroke (Ren et al., 2000). The TGFβ family member GDF10 is induced in peri-infarct cortex after stroke in humans, rodents and primates and is a potent stimulant for axonal sprouting and functional recovery (Li et al., 2015). In mice with a genetic propensity for greater axonal sprouting after injury, including stroke, there is a selective enhancement in another TGFβ signaling pathway, the Activin system (Omura et al., 2015). Both GDF10 and Activin signal through their respective cell surface receptors to the transcription factor Smad2 (Li et al., 2015; Omura et al., 2015). Thus, specific peri-infarct inflammatory signals and cytokines, induced at the time of stroke, control both the induction of axonal sprouting and the induction of axonal growth inhibitors that block axonal sprouting.

In the early stages after stroke, peri-infarct tissue exhibits synchronized electrical activity. Within the first several days after stroke, synchronized low frequency neuronal discharges sweep across peri-infarct cortex, occurring at <0.1 Hz (Carmichael and Chesselet, 2002; Gulati et al., 2015). This synchronized low frequency neuronal activity is a trigger for axonal sprouting and the formation of new connections after stroke (Carmichael and Chesselet, 2002). This was shown in the axonal sprouting from contralateral cortex into the ipsilateral striatum after an ischemic lesion. When synchronized neuronal activity after stroke is blocked axonal sprouting from contralateral cortex into ipsilateral striatum does not occur. In serving as a trigger, this synchronized neuronal activity in peri-infarct cortex after stroke resembles similar activity patterns in the formation of brain connections during development in the retina, hippocampus and cortex (Katz and Shatz, 1996; Stellwagon and Shatz, 2002; Egorov and Draguhn, 2013). Synchronized neuronal activity may activate a downstream molecular program for neuronal growth, or may stimulate the co-activation of many synapses in a particular region of neurons, stimulating a Hebbian plasticity and synaptic sprouting after stroke, as it does in the developing brain.

Molecular Growth Program in Post-Stroke Axonal Sprouting

The process of axonal sprouting after stroke is a profound biological event for the adult brain. In a brain region that normally does not form substantial new connections (cortex), a process is triggered in which local and long distance projections are formed. This event means that the neurons in the adult cortex are induced into a molecular growth program after stroke. The molecular pathways in such a program may provide targets for novel drugs to stimulate recovery after stroke. There are several additional issues that relate to a molecular growth program after stroke. Does it differ by age? Axonal sprouting in other systems in the adult brain, such as in the entorhinal/hippocampal projections, is reduced with age. Further, stroke recovery is reduced in aged patients, yet stroke itself is more common in aged patients. It is important to determine how aging affects the molecular components of neural repair. Does the molecular growth program after stroke provide insights into the limited recovery in this disease? In other words, are there hallmarks of a functional limitation or perhaps a ceiling in the axonal sprouting response after stroke in its molecular underpinnings? Does the process of axonal sprouting after stroke resemble that of the original process of axonal sprouting in neurodevelopment? Both processes involve local and long distance axonal growth and synaptogenesis. Does regeneration recapitulate development?

Studies of the gene expression profile of sprouting neurons in stroke indicate that stroke activates a molecular program that has a coordinated pattern of signaling from the extracellular environment into the cell (Zai et al., 2009; Li et al., 2010; Li et al., 2015). In contralateral cortex to the stroke site, corticospinal neurons that sprout a connection into the contralateral (denervated) spinal cord, when stimulated by the pro-growth drug inosine, show increases in the growth associated genes galectin-3, metallothionen and a coordinated upregulation of complement genes (Zai et al., 2009), which have been associated with synaptic remodeling (Stephan et al., 2012). In peri-infarct cortex, isolation and gene profiling specifically of neurons that engage in axonal sprouting after stroke shows that these neurons activate specific growth factors and cytokines, cell surface receptors, intermediate cytoplasmic cascades and transcriptional and epigenetic control points. The molecular program of axonal sprouting differs between aged and young adult brains. Surprisingly, when the genes that are differentially regulated during axonal sprouting in the young adult are compared to those in the aged adult, there is little overlap in the two transcriptomes (Li et al., 2010). When these two data sets are directly compared, only 79 genes are commonly regulated by stroke in both young adult and aged sprouting neurons (Li et al., 2010). This indicates that in terms of tissue regeneration, stroke in the aged brain represents a very distinct, coordinated biological event. Moreover, the axonal sprouting transcriptome changes over time after stroke. There is an early upregulation of a molecular induction program for axonal sprouting within the first week after stroke, and then a later maintenance program in axonal sprouting at three weeks after stroke (Li et al., 2010). These findings indicate that a trigger for post-stroke axonal sprouting is present within the first week after stroke, as indicated by 1346 differentially regulated genes in young sprouting neurons (and 621 genes in aged sprouting neurons). This distinction between an induction and maintenance program can be seen in the fact that extracellular signaling molecules, transcription factor and

epigenetic control molecules are activated in the induction phase, whereas cytoskeletal and synaptic proteins are more associated with the later, maintenance phase of axonal sprouting (Li et al., 2010).

The molecular control of axonal sprouting is different in aged versus young adults. In directly comparing the transcriptional profile of sprouting neurons after stroke during the induction period of this response, there is little overlap of the young adult and aged sprouting transcriptome. Specifically, after correcting these transcriptional data sets for statistical significance, 1346 genes are differentially up- or down-regulated in young adult sprouting neurons (neurons that form a new connection in two month old rats after stroke) during the inductive phase after stroke. 671 genes are up- or down-regulated in aged sprouting neurons (sprouting neurons in stroke in two-year old rats).

Gene products that are involved in DNA epigenetic or structural regulation are upregulated in both aged and young adult sprouting neurons, and differ by age. Epigenetic control of DNA structure and gene expression has a prominent role in brain development, neural stem cell differentiation, and activity-dependent plasticity (MacDonald and Rostrums, 2009; Malik and Vierbuchen, 2014) and may play a role in the phenotypic change characterized by post-stroke axonal sprouting. ATRX is one of the most highly induced genes in aged sprouting neurons after stroke, and is also upregulated in young adult sprouting neurons. ATRX is a SWI2/SNF2 DNA helicase/ATPase that plays a role in chromatin remodeling. Loss of ATRX function leads to neuronal cell death during brain development, and altered neuronal precursor migration (Berube et al., 2005; Seah et al., 2008), and in humans produces a mental retardation syndrome. ATRX also interacts with MECP2 and may have a function in methylation-dependent epigenetic DNA expression (Nan et al., 2007). ATRX siRNA knockdown significantly inhibits axonal outgrowth of DRG neurons and ATRX overexpression in adult rat DRG neurons promotes axonal outgrowth. Interestingly, knockdown of ATRX not only blocks post-stroke axonal sprouting, but also reduces the number of labeled axons below that seen in the normal brain with forelimb motor cortex BDA injections. This suggests that ATRX may function to maintain axonal connections in the un-manipulated or un-lesioned brain (Li et al., 2010).

Axonal sprouting neurons in the aged brain paradoxically upregulate genes that block axonal growth. These include the genes EphA4 and Lingo-1 (Li et al., 2010). Neurons that are induced to form a new connection in the aged brain also carry the seeds of their own destruction—axonal sprouting is co-activated with axonal growth inhibition. This is not the case for sprouting neurons in the young adult and may account for differences in tissue plasticity and repair with age. EphA4 is a receptor for, among other proteins, EphrinA5 and Ephrin B3 (Coulthard et al., 2012). Ephrin A5 is expressed on reactive astrocytes, and is more induced in the aged brain after stroke then the young adult brain (Li and Carmichael, 2006; Overman et al., 2012): stroke in the aged brain activates both a receptor and a ligand for axonal growth inhibition. Functional tests of axonal sprouting and recovery in stroke bear this out. EphrinA5 expression in reactive astrocytes blocks axonal sprouting and functional recovery in several different experimental models of stroke (Overman et al., 2012). Genetically deleting the EphA4 receptor also improves recovery in stroke (Lemmens et al., 2013). EphA4 is a promiscuous receptor, and also binds Ephrin B3. Ephrin B3 is

expressed in myelin (Benson et al., 2005). Blocking EphA4 induces even greater axonal sprouting than blockade of EphrinA5—suggesting the EphA4 is funneling point in the molecular signaling for axonal growth inhibition for astrocyte and myelin inhibitors. Lingo-1 is part of the Nogo Receptor 1 (NgR1) signaling complex, which includes NgR1/p75 or TROY/Lingo-1 (Giger et al., 2010). Blockade of NgR1 signaling either directly by genetic knockout or pharmacologically with a Lingo-1 antagonist, also causes more robust axonal sprouting after stroke (Li et al., 2010). This data in peri-infarct cortex axonal sprouting, derived from studies of an axonal sprouting transcriptome in neurons in this brain region, follows the effect of NgR1 antagonists in promoting axonal sprouting after stroke in the spinal cord (Lee et al., 2004; Zai et al., 2011). In each of these cases of Lingo-1 or EphA4 blockade in peri-infarct cortex, axonal sprouting occurs within sensorimotor circuits and is associated with functional recovery and thus fits in the category of Reparative Axonal Sprouting.

An unexpected finding in the molecular control of axonal sprouting after stroke is the identification of a Dependency State in neurons in peri-infarct cortex. This is the case with the growth factor, insulin-like growth factor 1 (IGF-1). Many studies have shown that delivery of IGF-1 enhances brain events associated with recovery after stroke, particularly neurogenesis. Delivering IGF-1 by viral gene transfer, intra-nasal delivery or by natural release from microglia in the subventricular zone enhances the generation of new neurons after stroke (Wiltrout et al., 2007; Zhu et al., 2008; Thored et al., 2009). In the sprouting transcriptome after stroke, IGF-1 is uniquely induced in aged sprouting neurons compared to young adult sprouting neurons, along with downstream molecular pathways in IGF-1 signaling (Li et al., 2010). This sounds like it would be fairly straightforward: IGF-1 is induced by stroke in nearby neurons and causes axonal sprouting. Except, IGF-1 delivery does not cause axonal spouting after stroke. Instead, IGF-1 delivery does not alter cortical motor connections at all, but blocking the normal IGF-1 signaling that occurs after stroke causes neuronal death—even late, well after the stroke has occurred (7 days after stroke). Blocking IGF-1 signaling in the normal, non-stroke brain, does not cause neuronal death (Li et al., 2010). These findings show that plasticity in the adult brain after stroke comes at a cost. Neurons are indeed placed into a growth state but they also become growth factor dependent for survival. This Dependency State for a neuron on a growth factor resembles that of the developing brain. Neurons in the developing brain compete to form new connections and the winner, the neuron that has formed the appropriate connection, is then stabilized by retrograde delivery of a growth factor (such as NGF) from its synaptic partner. This, the classic neurotrophic hypothesis (Clarke, 1985; Barde, 1989), is how functional circuits are shaped from initial spontaneous activity during development. However, adult neurons are no longer dependent on growth factor delivery for survival—they age out of this stage (Clarke, 1985; Barde, 1989; Li et al., 2010). It appears that stroke may induce a similar degree of plasticity to development in the ability to form a new connection, and a similar type of dependency, in the need for IGF-1 signaling to sustain survival. Thus, reparative axonal sprouting relies on non-cell autonomous cues for survival and growth.

The finding of a Dependency State and the overall superficial similarity of axonal sprouting after stroke to that seen in neurodevelopment has led to an open question in the neural repair field: does “regeneration recapitulates development”? Based on the similarity of brain events

during recovery after stroke and those initially seen in the developing brain, it has been suggested that regeneration recapitulates development (Cramer and Chopp, 2000). These similarities include the Dependency State and that in both conditions the brain is forming new connections and there are similar alterations in the brain environment occur in regeneration and sprouting (such as reduced peri-neuronal nets) (Carmichael et al., 2005, Karetko-Sysa et al., 2011). Despite these superficial similarities in cellular phenotype (axonal sprouting, peri-neuronal nets) the molecular programs that underlie post-stroke axonal sprouting are unique tissue regeneration events (Li et al., 2010; Li et al., 2015). When the transcriptional profile of sprouting neurons after stroke is directly compared to that of neurons during development, there is a substantial statistical difference between these two molecular programs. In fact, if unsupervised genome-wide association analysis is applied to 180 different transcriptomes from the literature, from neurodevelopment, to learning and memory paradigms to spinal cord trauma and other injuries, the greatest distinction is between neurons that have been induced into an axonal sprouting state after stroke and early post-natal neurons that are still forming new connections (Li et al., 2015). These studies indicate that neural repair does not strictly follow neural development on a molecular level, but instead triggers a unique post-stroke transcriptional landscape.

The process of axonal sprouting after stroke is a profound biological event for the adult brain. In a brain region that normally does not form such substantial new connections, a process is triggered in which local and long distance projections are formed. This implies that there is a molecular trigger for this event. Working with candidate signaling molecules that are present in the aged neuron post-stroke sprouting transcriptome, such a trigger was recently identified. The TGFβ family member GDF10, which previously did not have a known role in brain function, was found to trigger axonal sprouting. GDF10 is induced in peri-infarct cortex in mouse, rat, non-human primate and monkey. GDF10 promotes axonal outgrowth in vitro in many types of neurons, and in vivo after stroke, and enhances motor recovery. GDF10 is thus a novel brain growth factor and stroke-induced trigger for recovery (Li et al., 2015). Interestingly, when endogenous levels of GDF10 are reduced, axonal sprouting after stroke is prevented, and motor recovery is reduced (Li et al., 2015). GDF10 signals through TGFβRI and II and Smad 2/3, to activate PI3 Kinase gene systems and to inhibit PTEN and SOCS3 signaling. These gene systems mediate axonal sprouting in other contexts in the adult, such as in optic nerve and spinal cord injury (Sun et al., 2011; Lu et al., 2014; Danilov and Steward, 2015). These data indicate that GDF10 is one molecular trigger after stroke and coordinately activates parallel growth promotion cascades.

Future Directions

The basic science of axonal sprouting after stroke may take the field of stroke neural repair toward 1) clinically relevant markers for this process, 2) movement of axonal sprouting drugs into clinical trials, and 3) formal consideration of the interaction of a clinical neural repair treatment with rehabilitative activity. At present there are no biomarkers for axonal sprouting, even in well-defined animal models of stroke. On a molecular level, there currently is no singular molecular marker of a “regenerating” axon that can be used in experimental models of stroke. Candidate regeneration-associated or axonal sprouting genes, such as GAP43 are often expressed in dendrites or in non-neuronal cells such as astrocytes

and oligodendrocytes (Vitkovic et al., 1988; Deloulme et al., 1990; Fox et al., 2006). On a connectional level, there is not a truly unique pattern of connections after stroke that can be identified in a high-throughput or non-invasive way as characteristic of, or a biomarker for, axonal sprouting. Future work will need to define biomarkers for this process. With present technology, it is possible that in well-defined and common strokes, such as subcortical middle cerebral artery strokes (40% of all stroke), a characteristic pattern of correlated brain activity can be demonstrated in resting state MRI, that can then be used to benchmark new brain networks that develop with a drug or cell therapy that stimulates axonal sprouting.

The application of drugs or biologics that stimulate axonal sprouting to clinical stroke remains an important future direction. There are two current biologics that may work in this regard. An anti-NogoA antibody chimera has been tested in human spinal cord injury, and has a wealth of evidence in pre-clinical stroke models (Lindau et al., ’14; Wahl et al., 2014). An anti-myelin associated glycoprotein antibody chimera also has been taken into Phase 1 studies in humans (Cramer et al., 2013) and has shown promise in pre-clinical models (Barbay et al., 2015). An important question of these kinds of biologics is that, as large proteins, do they penetrate the blood brain barrier to enter the post-stroke brain in sufficient concentration? Other drugs or molecules that have been associated with axonal sprouting also stimulate other aspects of neural repair, such as angiogenesis and neurogenesis, and include erythropoietin or G-CSF. These are in a sense “first generation” neural repair drugs, as they are directly involved in injury-induced neural signaling and have been co-opted into a therapy with no modification of the natural biological molecule. A limitation for this pathway to a therapeutic has been that these molecules are powerful, pleiotrophic cytokines or growth factors with specific effects in non-CNS tissue that cause side effects. Future therapeutics for axonal sprouting in stroke will likely need to derive from molecular systems that are differentially active in axonal sprouting in the brain and utilize small molecule targeting (Carmichael, 2016).

A third issue for future development of the post-stroke axonal sprouting field is timing: timing of delivery of the axonal sprouting therapeutic and timing of neurorehabilitative therapy. As noted above, early (within the first week of stroke) and simultaneous delivery of increased behavioral activity and an axonal sprouting therapeutic caused Unbounded Axonal Sprouting and behavioral deterioration. On the other hand, Schwab and colleagues found that sequential administration of first an axonal sprouting therapeutic (anti-NogoA, started at the time of the stroke) and then rehabilitative training produced substantial functional recovery (Wahl et al., 2014). Much research needs to be done to define timing of these two approaches, and the timing might differ according to the specific mechanism of action of a candidate axonal sprouting therapy. For example, a therapy that stimulates a neuronal axonal growth program (Li et al. 2015) might have a different optimal time window and interaction with neurorehabilitation than an anti-growth inhibitor approach (Overman et al., 2012; Wahl et al., 2014; Barbay et al., 2015).

Conclusions

Stroke triggers axonal sprouting in local and long distance sites that are connected to the area of damage. Axonal sprouting is initiated within the first week after stroke and one

identified trigger for growth is the TGFβ family member GDF10. This induces a molecular growth program that involves coordinated regulation of cell surface, intracellular and nuclear transcriptional events. PTEN and SOCS3 signaling are downregulated, the epigenetic modifying protein ATRX is induced and other mechanistic players in the neuronal growth response are activated. In this early induction period after stroke, aged neurons paradoxically activate growth inhibitory proteins, such as Lingo-1 and EphA4. In the case of EphA4, one of is cognate ligands is also activated by stroke in nearby reactive astrocytes, especially in the aged brain. Ephrin A5/EphA4 is a unique, age-associated axonal growth inhibitory system. By three weeks after stroke, neurons enter a maintenance mode of axonal sprouting, with a distinct and less substantial transcriptional program. Axonal sprouting after stroke can be classified into three patterns, reactive axonal sprouting near the stroke site, reparative axonal sprouting over longer distances within ipsilesional sensorimotor/premotor areas and contralesional spinal cord, and unbounded axonal sprouting when glial growth inhibitors are blocked and behavioral activity of the injured motors system is potentiated. Reparative axonal sprouting is associated with recovery but unbounded axonal sprouting after stroke occurs across functional CNS networks and can reduce motor recovery after stroke. Post-stroke axonal sprouting is a powerful cellular process to harness for neurological recovery, but will require careful development with neurological rehabilitative therapies.

Acknowledgments

Supported by NS085019, NS081055, NS077521, NS071481, American Stroke Association/Bugher Foundation UCLA Stroke Collaborative Research Center, the Richard Merkin Foundation for Neural Repair at UCLA, and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

References

Allegra Mascaro AL, Cesare P, Sacconi L, Grasselli G, Mandolesi G, Maco B, Knott GW, Huang L, De Paola V, Strata P, Pavone FS. In vivo single branch axotomy induces GAP-43-dependent sprouting and synaptic remodeling in cerebellar cortex. Proc Natl Acad Sci U S A. 2013; 110:10824–9. [PubMed: 23754371]

Allred RP, Jones TA. Maladaptive effects of learning with the less-affected forelimb after focal cortical infarcts in rats. Exp Neurol. 2008; 210:172–181. [PubMed: 18054917]

Allred RP, Maldonado MA, Hsu JE, Jones TA. Training the “less-affected” forelimb after unilateral cortical infarcts interferes with functional recovery of the impaired forelimb in rats. Restor Neurol Neurosci. 2005; 23:297–302. [PubMed: 16477091]

Allred RP, Cappellini CH, Jones TA. The “good” limb makes the “bad” limb worse: experience-dependent interhemispheric disruption of functional outcome after cortical infarcts in rats. Behav Neurosci. 2010; 124:124–132. [PubMed: 20141287]

Bachmann LC, Lindau NT, Felder P, Schwab ME. Sprouting of brainstem-spinal tracts in response to unilateral motor cortex stroke in mice. J Neurosci. 2014 Feb 26; 34(9):3378–89. [PubMed: 24573294]

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 Jun; 46(6):1620–5. [PubMed: 25931462]

Barde YA. Trophic factors and neuronal survival. Neuron. 1989 Jun; 2(6):1525–34. [PubMed: 2697237]

Benakis C1, Garcia-Bonilla L1, Iadecola C1, Anrather J. The role of microglia and myeloid immune cells in acute cerebral ischemia. Front Cell Neurosci. 2015 Jan 14.8:461. [PubMed: 25642168]

Benowitz LI, Carmichael ST. Promoting axonal rewiring to improve outcome after stroke. Neurobiol Dis. 2010 Feb; 37(2):259–66. [PubMed: 19931616]

Benson MD, Romero MI, Lush ME, Lu QR, Henkemeyer M, Parada LF. Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. Proc Natl Acad Sci U S A. 2005 Jul 26; 102(30):10694–9. [PubMed: 16020529]

Bérubé NG, Mangelsdorf M, Jagla M, Vanderluit J, Garrick D, Gibbons RJ, Higgs DR, Slack RS, Picketts DJ. The chromatin-remodeling protein ATRX is critical for neuronal survival during corticogenesis. J Clin Invest. 2005; 115:258–267. [PubMed: 15668733]

Biernaskie J, Chernenko G, Corbett D. Efficacy of rehabilitative experience declines with time after focal ischemic brain injury. J Neurosci. 2004 Feb 4; 24(5):1245–54. [PubMed: 14762143]

Brown CE, Li P, Boyd JD, Delaney KR, Murphy TH. Extensive turnover of dendritic spines and vascular remodeling in cortical tissues recovering from stroke. J Neurosci. 2007 Apr 11; 27(15): 4104–9.

Brown CE, Wong C, Murphy TH. Rapid morphologic plasticity of peri-infarct dendritic spines after focal ischemic stroke. Stroke. 2008 Apr; 39(4):1286–91. [PubMed: 18323506]

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 Feb 11; 29(6):1719–34. [PubMed: 19211879]

Brown CE, Boyd JD, Murphy TH. Longitudinal in vivo imaging reveals balanced and branch-specific remodeling of mature cortical pyramidal dendritic arbors after stroke. J Cereb Blood Flow Metab. 2010 Apr; 30(4):783–91. [PubMed: 19920846]

Brumm AJ, Carmichael ST. Not just a rush of blood to the head. Nat Med. 2012 Nov; 18(11):1609–10. [PubMed: 23135507]

Buma FE, Lindeman E, Ramsey NF, Kwakkel G. Functional neuroimaging studies of early upper limb recovery after stroke: a systematic review of the literature. Neurorehabil Neural Repair. 2010 Sep; 24(7):589–608. [PubMed: 20439501]

Carmichael ST, Chesselet MF. Synchronous neuronal activity is a signal for axonal sprouting after cortical lesions in the adult. J Neurosci. 2002 Jul 15; 22(14):6062–70. [PubMed: 12122067]

Carmichael ST, Wei L, Rovainen CM, Woolsey TA. New patterns of intracortical projections after focal cortical stroke. Neurobiol Dis. 2001 Oct; 8(5):910–22. [PubMed: 11592858]

Carmichael ST, Archibeque I, Luke L, Nolan T, Momiy J, Li S. Growth-associated gene expression after stroke: evidence for a growth-promoting region in peri-infarct cortex. Exp Neurol. 2005; 193:291–311. [PubMed: 15869933]

Carmichael ST. Emergent properties of neural repair: elemental biology to therapeutic concepts. Annals Neurol. 2016 In press.

Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/data/databriefs/db178.htm

Cheetham CE, Hammond MS, McFarlane R, Finnerty GT. Altered sensory experience induces targeted rewiring of local excitatory connections in mature neocortex. J Neurosci. 2008 Sep 10; 28(37): 9249–60. [PubMed: 18784305]

Chen P, Goldberg DE, Kolb B, Lanser M, Benowitz LI. Inosine induces axonal rewiring and improves behavioral outcome after stroke. Proc Natl Acad Sci U S A. 2002 Jun 25; 99(13):9031–6. [PubMed: 12084941]

Clarke PHG. Neuronal death in the development of the nervous system. Trends Neurosci. 1985; 8:345–9.

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 Mar 9; 31(10):3766–75. [PubMed: 21389231]

Clarkson AN, López-Valdés HE, Overman JJ, Charles AC, Brennan KC, Thomas Carmichael S. Multimodal examination of structural and functional remapping in the mouse photothrombotic stroke model. J Cereb Blood Flow Metab. 2013 May; 33(5):716–23. [PubMed: 23385201]

Corbetta M, Ramsey L, Callejas A, Baldassarre A, Hacker CD, Siegel JS, Astafiev SV, Rengachary J, Zinn K, Lang CE, Connor LT, Fucetola R, Strube M, Carter AR, Shulman GL. Common behavioral clusters and subcortical anatomy in stroke. Neuron. 2015; 85:927–41. [PubMed: 25741721]

Coulthard MG, Morgan M, Woodruff TM, Arumugam TV, Taylor SM, Carpenter TC, Lackmann M, Boyd AW. Eph/Ephrin signaling in injury and inflammation. Am J Pathol. 2012 Nov; 181(5): 1493–503. [PubMed: 23021982]

Cramer SC, Chopp M. Recovery recapitulates ontogeny. Trends Neurosci. 2000; 23:265–71. [PubMed: 10838596]

Cramer SC, Abila B, Scott NE, Simeoni M, Enney LA. MAG111539 Study Investigators. Safety, pharmacokinetics, and pharmacodynamics of escalating repeat doses of GSK249320 in patients with stroke. Stroke. 2013 May; 44(5):1337–42. [PubMed: 23471268]

Danilov CA, Steward O. Conditional genetic deletion of PTEN after a spinal cord injury enhances regenerative growth of CST axons and motor function recovery in mice. Exp Neurol. 2015 Apr. 266:147–60. [PubMed: 25704959]

Deguchi K, Takaishi M, Hayashi T, Oohira A, Nagotani S, Li F, Jin G, Nagano I, Shoji M, Miyazaki M, Abe K, Huh NH. Expression of neurocan after transient middle cerebral artery occlusion in adult rat brain. Brain Res. 2005 Mar 10; 1037(1–2):194–9. [PubMed: 15777769]

Deloulme JC, Janet T, Au D, Storm DR, Sensenbrenner M, et al. Neuromodulin (GAP43): a neuronal protein kinase C substrate is also present in 0–2A glial cell lineage. Characterization of neuromodulin in secondary cultures of oligodendrocytes and comparison with the neuronal antigen. J Cell Biol. 1990; 111:1559–1569. [PubMed: 2170423]

Dickson TC, Chung RS, McCormack GH, Staal JA, Vickers JC. Acute reactive and regenerative changes in mature cortical axons following injury. Neuroreport. 2007 Feb 12; 18(3):283–8. [PubMed: 17314672]

Dromerick AW, Lang CE, Birkenmeier RL, Wagner JM, Miller JP, Videen TO, Powers WJ, Wolf SL, Edwards DF, Huybrechts KF, Caro JJ, Xenakis JJ, Vemmos KN. The prognostic value of the modified Rankin Scale score for long-term survival after first-ever stroke. Cerebrovasc Dis. 2008; 26:381–387. [PubMed: 18753743]

Ekdahl CT, Kokaia Z, Lindvall O. Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience. 2009 Feb 6; 158(3):1021–9. [PubMed: 18662748]

Egorov AV, Draguhn A. Development of coherent neuronal activity patterns in mammalian cortical networks: common principles and local hetereogeneity. Mech Dev. 2013 Jun-Aug;130(6–8):412–23. [PubMed: 23032193]

Fox MA, Afshari FS, Alexander JK, Colello RJ, Fuss B. Growth conelike sensorimotor structures are characteristic features of postmigratory, premyelinating oligodendrocytes. Glia. 2006 Apr 1; 53(5): 563–6. [PubMed: 16355369]

Fu M, Zuo Y. Experience-dependent structural plasticity in the cortex. Trends Neurosci. 2011; 34:177–87. [PubMed: 21397343]

Gertz K, Kronenberg G, Kälin RE, Baldinger T, Werner C, Balkaya M, Eom GD, Hellmann-Regen J, Kröber J, Miller KR, Lindauer U, Laufs U, Dirnagl U, Heppner FL, Endres M. Essential role of interleukin-6 in post-stroke angiogenesis. Brain. 2012 Jun; 135(Pt 6):1964–80. [PubMed: 22492561]

Gleichman AJ, Carmichael ST. Astrocytic therapies for neuronal repair in stroke. Neurosci Lett. 2014 Apr 17.565:47–52. [PubMed: 24184876]

Giger RJ, Hollis ER 2nd, Tuszynski MH. Guidance molecules in axon regeneration. Cold Spring Harb Perspect Biol. 2010 Jul.2(7):a001867. [PubMed: 20519341]

Grefkes C, Ward NS. Cortical reorganization after stroke: how much and how functional? Neuroscientist. 2014; 20(1):56–70. [PubMed: 23774218]

Grutzendler J, Kasthuri N, Gan WB. Long-term dendritic spine stability in the adult cortex. Nature. 2002; 420:812–6. [PubMed: 12490949]

Gulati T, Won SJ, Ramanathan DS, Wong CC, Bodepudi A, Swanson RA, Ganguly K. Robust neuroprosthetic control from the stroke perilesional cortex. J Neurosci. 2015 Jun 3; 35(22):8653–61. [PubMed: 26041930]

Harrison TC, Silasi G, Boyd JD, Murphy TH. Displacement of sensory maps and disorganization of motor cortex after targeted stroke in mice. Stroke. 2013; 44:2300–6. [PubMed: 23743973]

Hinman JD, Rasband MN, Carmichael ST. Remodeling of the axon initial segment after focal cortical and white matter stroke. Stroke. 2013 Jan; 44(1):182–9. [PubMed: 23233385]

Huybrechts KF, Caro JJ, Xenakis JJ, Vemmos KN. The prognostic value of the modified Rankin Scale score for long-term survival after first-ever stroke. Results from the Athens Stroke Registry. Cerebrovasc Dis. 2008; 26(4):381–7. [PubMed: 18753743]

Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med. 2011 Jul 7; 17(7):796–808. [PubMed: 21738161]

Jasinska M, Siucinska E, Cybulska-Klosowicz A, Pyza E, Furness DN, Kossut M, Glazewski S. Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J Neurosci. 2010; 30:1176–84. [PubMed: 20089926]

Kantak SS, Stinear JW, Buch ER, Cohen LG. Rewiring the brain: potential role of the premotor cortex in motor control, learning, and recovery of function following brain injury. Neurorehabil Neural Repair. 2012; 6:282–92. [PubMed: 21926382]

Karetko-Sysa M, Skangiel-Kramska J, Nowicka D. Disturbance of perineuronal nets in the perilesional area after photothrombosis is not associated with neuronal death. Exp Neurol. 231:113–26. [PubMed: 21683696]

Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. Science. 1996 Nov 15; 274(5290):1133–8. [PubMed: 8895456]

Kelley MS, Steward O. Injury-induced physiological events that may modulate gene expression in neurons and glia. Rev Neurosci. 1997; 8:147–77. [PubMed: 9548230]

Kim SY, Allred RP, Adkins DL, Tennant KA, Donlan NA, Kleim JA, Jones TA. Experience with the “good” limb induces aberrant synaptic plasticity in the perilesion cortex after stroke. J Neurosci. 2015 Jun 3; 35(22):8604–10. [PubMed: 26041926]

Krakauer JW, Marshall RS. The proportional recovery rule for stroke revisited. Ann Neurol. 2015 Dec; 78(6):845–7. [PubMed: 26435166]

Lang C, Guo X, Kerschensteiner M, Bareyre FM. Single collateral reconstructions reveal distinct phases of corticospinal remodeling after spinal cord injury. PLoS One. 2012; 7(1):e30461. [PubMed: 22291960]

Lee JK, Kim JE, Sivula M, Strittmatter SM. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. J Neurosci. 2004 Jul 7; 24(27):6209–17. [PubMed: 15240813]

Lemmens R, Jaspers T, Robberecht W, Thijs VN. Modifying expression of EphA4 and its downstream targets improves functional recovery after stroke. Hum Mol Genet. 2013 Jun 1; 22(11):2214–20. [PubMed: 23418304]

Li S, Overman JJ, Katsman D, Kozlov SV, Donnelly CJ, Twiss JL, Giger RJ, Coppola G, Geschwind DH, Carmichael ST. An age-related sprouting transcriptome provides molecular control of axonal sprouting after stroke. Nat Neurosci. 2010 Dec; 13(12):1496–504. [PubMed: 21057507]

Li S, Nie EH, Yin Y, Benowitz LI, Tung S, Vinters HV, Bahjat FR, Stenzel-Poore MP, Kawaguchi R, Coppola G, Carmichael ST. GDF10 Is a Signal for Axonal Sprouting and Functional Recovery after Stroke. Nat Neurosci. 2015 In press.

Lindau NT, Bänninger BJ, Gullo M, Good NA, Bachmann LC, Starkey ML, Schwab ME. Rewiring of the corticospinal tract in the adult rat after unilateral stroke and anti-Nogo-A therapy. Brain. 2014 Mar; 137(Pt 3):739–56. [PubMed: 24355710]

Lu Y, Belin S, He Z. Signaling regulations of neuronal regenerative ability. Curr Opin Neurobiol. 2014 Aug.27:135–42. [PubMed: 24727245]

MacDonald JL, Roskams AJ. Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. Prog Neurobiol. 2009; 88:170–183. [PubMed: 19554713]

Malik AN, Vierbuchen T, Hemberg M, Rubin AA, Ling E, Couch CH, Stroud H, Spiegel I, Farh KK, Harmin DA, Greenberg ME. Genome-wide identification and characterization of functional neuronal activity-dependent enhancers. Nat Neurosci. 2014 Oct; 17(10):1330–9. [PubMed: 25195102]

Mansoori BK, Jean-Charles L, Touvykine B, Liu A, Quessy S, Dancause N. Acute inactivation of the contralesional hemisphere for longer durations improves recovery after cortical injury. Exp Neurol. 2014 Apr.254:18–28. [PubMed: 24447424]

McKinney RA, Debanne D, Gähwiler BH, Thompson SM. Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of posttraumatic epilepsy. Nat Med. 1997 Sep; 3(9):990–6. [PubMed: 9288725]

Mostany R, Chowdhury TG, Johnston DG, Portonovo SA, Carmichael ST, Portera-Cailliau C. Local hemodynamics dictate long-term dendritic plasticity in peri-infarct cortex. J Neurosci. 2010 Oct 20; 30(42):14116–26. [PubMed: 20962232]

Morecraft RJ, Ge J, Stilwell-Morecraft KS, McNeal DW, Hynes SM, Pizzimenti MA, Rotella DL, Darling WG. Frontal and frontoparietal injury differentially affect the ipsilateral corticospinal projection from the nonlesioned hemisphere in monkey (Macaca mulatta). J Comp Neurol. 2015 Jul 30.doi: 10.1002/cne.23861

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation. 2015 Jan 27; 131(4):e29–322. [PubMed: 25520374]

Nan X, et al. Interaction between chromatin proteins MECP2 and ATRX is disrupted by mutations that cause inherited mental retardation. Proc Natl Acad Sci U S A. 2007; 104:2709–2714. [PubMed: 17296936]

Omura T, Omura K, Tedeschi A, Riva P, Painter MW, Rojas L, Martin J, Lisi V, Huebner EA, Latremoliere A, Yin Y, Barrett LB, Singh B, Lee S, Crisman T, Gao F, Li S, Kapur K, Geschwind DH, Kosik KS, Coppola G, He Z, Carmichael ST, Benowitz LI, Costigan M, Woolf CJ. Robust Axonal Regeneration Occurs in the Injured CAST/Ei Mouse CNS. Neuron. 2015 Jun 3; 86(5): 1215–27. [PubMed: 26004914]

Overman JJ, Clarkson AN, Wanner IB, Overman WT, Eckstein I, Maguire JL, Dinov ID, Toga AW, Carmichael ST. A role for ephrin-A5 in axonal sprouting, recovery, and activity-dependent plasticity after stroke. Proc Natl Acad Sci U S A. 2012 Aug 14; 109(33):E2230–9. [PubMed: 22837401]

Overman JJ, Carmichael ST. Plasticity in the injured brain: more than molecules matter. Neuroscientist. 2014 Feb; 20(1):15–28. [PubMed: 23757300]

Pierot L, Derdeyn C. Interventionalist perspective on the new endovascular trials. Stroke. 2015 Jun; 46(6):1440–6. [PubMed: 25944327]

Ren J, Kaplan PL, Charette MF, Speller H, Finklestein SP. Time window of intracisternal osteogenic protein-1 in enhancing functional recovery after stroke. Neuropharmacology. 2000 Mar 3; 39(5): 860–5. [PubMed: 10699451]

Riban V, Chesselet MF. Region-specific sprouting of crossed corticofugal fibers after unilateral cortical lesions in adult mice. Exp Neurol. 2006 Feb; 197(2):451–7. [PubMed: 16321385]

Seah C, et al. Neuronal death resulting from targeted disruption of the Snf2 protein ATRX is mediated by p53. J Neurosci. 2008; 28:12570–12580. [PubMed: 19020049]

Seymour AB, Andrews EM, Tsai SY, Markus TM, Bollnow MR, Brenneman MM, O20Brien TE, Castro AJ, Schwab ME, Kartje GL. Delayed treatment with monoclonal antibody IN-1 1 week after stroke results in recovery of function and corticorubral plasticity in adult rats. J Cereb Blood Flow Metab. 2005 Oct; 25(10):1366–75. [PubMed: 15889044]

Shen LH, Li Y, Gao Q, Savant-Bhonsale S, Chopp M. Down-regulation of neurocan expression in reactive astrocytes promotes axonal regeneration and facilitates the neurorestorative effects of bone marrow stromal cells in the ischemic rat brain. Glia. 2008 Dec; 56(16):1747–54. [PubMed: 18618668]

Silasi G, Murphy TH. Stroke and the connectome: how connectivity guides therapeutic intervention. Neuron. 2014 Sep 17; 83(6):1354–68. [PubMed: 25233317]

Stellwagen D, Shatz CJ. An instructive role for retinal waves in the development of retinogeniculate connectivity. Neuron. 2002 Jan 31; 33(3):357–67. [PubMed: 11832224]

Stephan AH, Barres BA, Stevens B. The complement system: an unexpected role in synaptic pruning during development and disease. Annu Rev Neurosci. 2012; 35:369–89. [PubMed: 22715882]

Stewart JC, Cramer SC. Patient-reported measures provide unique insights into motor function after stroke. Stroke. 2013 Apr; 44(4):1111–6. [PubMed: 23422082]

Sun F, Park KK, Belin S, Wang D, Lu T, Chen G, Zhang K, Yeung C, Feng G, Yankner BA, He Z. Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature. 2011 Nov 6; 480(7377):372–5. [PubMed: 22056987]

Szele FG, Alexander C, Chesselet MF. Expression of molecules associated with neuronal plasticity in the striatum after aspiration and thermocoagulatory lesions of the cerebral cortex in adult rats. J Neurosci. 1995 Jun; 15(6):4429–48. [PubMed: 7790918]

Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J, Nygren JM, Jacobsen SE, Ekdahl CT, Kokaia Z, Lindvall O. Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. Glia. 2009 Jun; 57(8):835–49. [PubMed: 19053043]

Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature. 2002 Dec 19–26; 420(6917):788–94. [PubMed: 12490942]

Vitkovic L, Steisslinger HW, Aloyo VJ, Mersel M. The 43-kDa neuronal growth-associated protein (GAP-43) is present in plasma membranes of rat astrocytes. Proc Natl Acad Sci U S A. 1988; 85:8296–8300. [PubMed: 3054883]

Wahl AS, Omlor W, Rubio JC, Chen JL, Zheng H, Schröter A, Gullo M, Weinmann O, Kobayashi K, Helmchen F, Ommer B, Schwab ME. Neuronal repair. Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke. Science. 2014 Jun 13; 344(6189): 1250–5. [PubMed: 24926013]

Weisscher N, Vermeulen M, Roos YB, de Haan RJ. What should be defined as good outcome in stroke trials; a modified Rankin score of 0–1 or 0–2? J Neurol. 2008 Jun; 255(6):867–74. [PubMed: 18338195]

Wiltrout C, Lang B, Yan Y, Dempsey RJ, Vemuganti R. Repairing brain after stroke: a review on post-ischemic neurogenesis. Neurochem Int. 2007 Jun; 50(7–8):1028–41. [PubMed: 17531349]

Winters C, van Wegen EE, Daffertshofer A, Kwakkel G. Generalizability of the Proportional Recovery Model for the Upper Extremity After an Ischemic Stroke. Neurorehabil Neural Repair. 2015 Aug; 29(7):614–22. [PubMed: 25505223]

Withers GS, Higgins D, Charette M, Banker G. Bone morphogenetic protein-7 enhances dendritic growth and receptivity to innervation in cultured hippocampal neurons. Eur J Neurosci. 2000 Jan; 12(1):106–16. [PubMed: 10651865]

Zai L, Ferrari C, Subbaiah S, Havton LA, Coppola G, Strittmatter S, Irwin N, Geschwind D, Benowitz LI. Inosine alters gene expression and axonal projections in neurons contralateral to a cortical infarct and improves skilled use of the impaired limb. J Neurosci. 2009 Jun 24; 29(25):8187–97. [PubMed: 19553458]

Zhao Y, Rempe DA. Targeting astrocytes for stroke therapy. Neurotherapeutics. 2010 Oct; 7(4):439–51. [PubMed: 20880507]

Zhu W, Fan Y, Frenzel T, Gasmi M, Bartus RT, Young WL, Yang GY, Chen Y. Insulin growth factor-1 gene transfer enhances neurovascular remodeling and improves long-term stroke outcome in mice. Stroke. 2008 Apr; 39(4):1254–61. [PubMed: 18309153]

Highlights

- Stroke induces the formation of new connections in brain and spinal cord.
- These mediate some aspects of motor recovery.
- A unique molecular program, a regenerative transcriptome, underlies post-stroke axonal sprouting
- Axonal sprouting occurs in three different patterns: Reactive, Reparative and Unbounded.
- Each pattern of post-stroke axonal sprouting has unique relationships to behavioral activity and molecular control points.

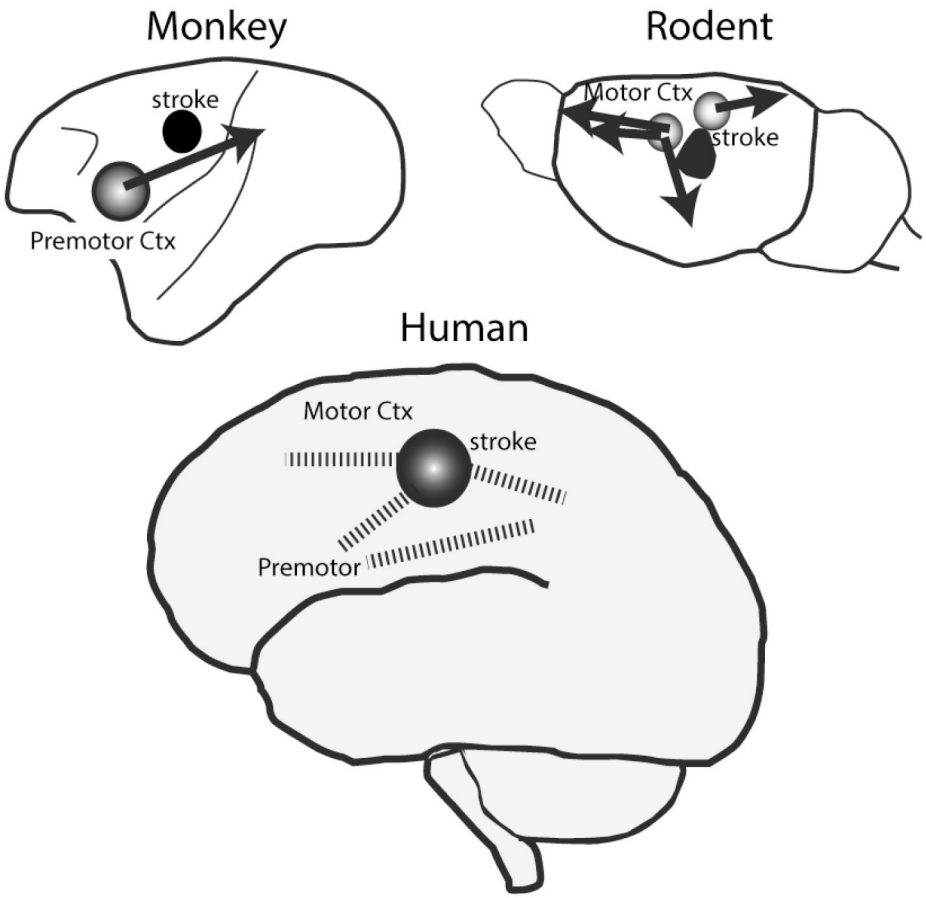

Figure 1. Patterns of Axonal Sprouting or Sensorimotor Map Plasticity in Peri-Infarct Cortex

(A) Axonal sprouting after stroke in the monkey occurs between premotor and somatosensory areas, establishing novel long-distance connections after stroke. This is a long distance axonal sprouting process, spanning a centimeter of tissue and occurring between frontal and parietal lobes (Dancause et al., 2005). (B) In rat and mouse models of stroke, axonal sprouting occurs in motor, premotor, somatosensory and posterior parietal areas after stroke (Brown et al., 2009; Li et al., 2010; Overman et al., 2012: Li, Nie et al., 2015). (C) Human motor and sensory maps reorganize after stroke into new representations in peri-infarct and connected cortical areas, in a process correlated with recovery (Buma et al., 2010; Kantak et al., 2012; Grefkes and Ward, 2014)

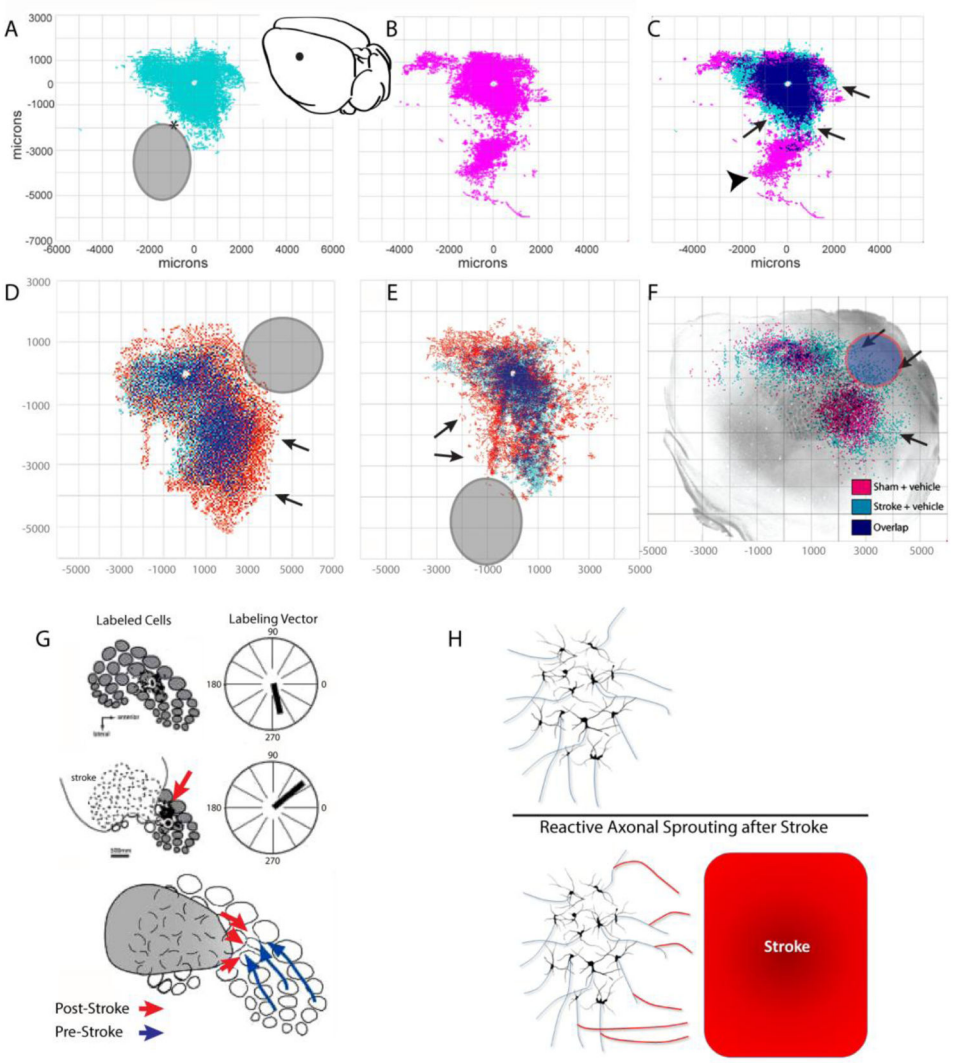

Figure 2. Reactive Axonal Sprouting after Stroke

(A–E). The images depict maps of the location of axons labeled from a BDA neuroanatomical tracer injection into the forelimb motor cortex of the mouse. Each map is the plot of 5–6 mice (separate brain maps). Thus each map shows the aggregate motor system connections in the cortical hemisphere. The inset between (A) and (B) shows a schematic of the location of the neuroanatomical tracer injection in the mouse brain. (A) is a map of the forelimb motor system connection after stroke. The stroke is schematically shown as the grey ellipse. This is one type of stroke model, as distal middle cerebral artery stroke. (B) shows the location of motor system connections in the control (non-stroke) mouse. (C) shows the overlap of the two motor system networks in control (magenta) and stroke (blue). Note the loss of projections near the stroke (arrowhead) and the increase in projections near the stroke site and immediately outside of the control motor system connectional network (arrows). (D) shows a similar map from a different stroke model and different set of studies. This is a photothrombotic stroke in motor cortex (grey ellipse). The tracer injection (clear circle) is placed in surviving motor cortex anterior to the stroke. In

light blue the projections of motor cortex in the control (non-stroke) brain are shown. In red, the projections of the motor cortex region after stroke are shown. The dark blue shows the region of dense overlap of the stroke and non-stroke projection. The arrows show the increase in motor system projections around the stroke site. (E) shows another set of studies with a tracer injection into forelimb motor cortex in control (blue) and stroke (red) in a distal middle cerebral artery stroke model (grey ellipse). Axons that project from forelimb motor cortex after stroke (red) can be seen near the stroke site at a site that has only sparse projection in the control brain (blue). (F) shows maps of cell bodies in control (magenta) and stroke cases (blue). The previous figures mapped the axonal projections. In these two maps of control (sham+vehicle) and stroke (stroke+vehicle) the motor system projections after stroke come from a population of neurons near the stroke site that are not present in the control motor system network (arrows). The stroke is schematically depicted in the ellipse. In this map the quantitative plots of cortical projections have been warped back onto a tissue section of the brain, to localize the projections within the sensorimotor cortex (see Li et al., 2010; Overman et al., 2012). (G) shows maps of the location of neurons labeled from tracer injections into the somatosensory cortex, in the representation of the facial vibrissae (or the “barrel field”). In this model of stroke in the rat, small strokes are placed in the barrel cortex. In control (non-stroke) most of the cells project in a posterior-medial direction, establishing a direction vector for the population of projections After stroke, a new population of neurons projects within the somatosensory cortex (red arrow). These are located near the stroke. This new population establishes a different vector for the connections within the somatosensory cortex. The summary of this process of post-stroke axonal sprouting in somatosensory cortex of the rat is that new projections are formed adjacent to the stroke site. (H) Summary of the process of reactive axonal sprouting after stroke. The control state is shown at the top. At the bottom, stroke induces axonal sprouting and new projections (red) toward the stroke site. (A–C) are modified from Overman et al., 12). (D) is from Omura et al., 2015. (E) is from Li et al., 2010. (F) is unpublished data from Andrew Clarkson and S. Thomas Carmichael. (G) is from Carmichael et al., 2001.

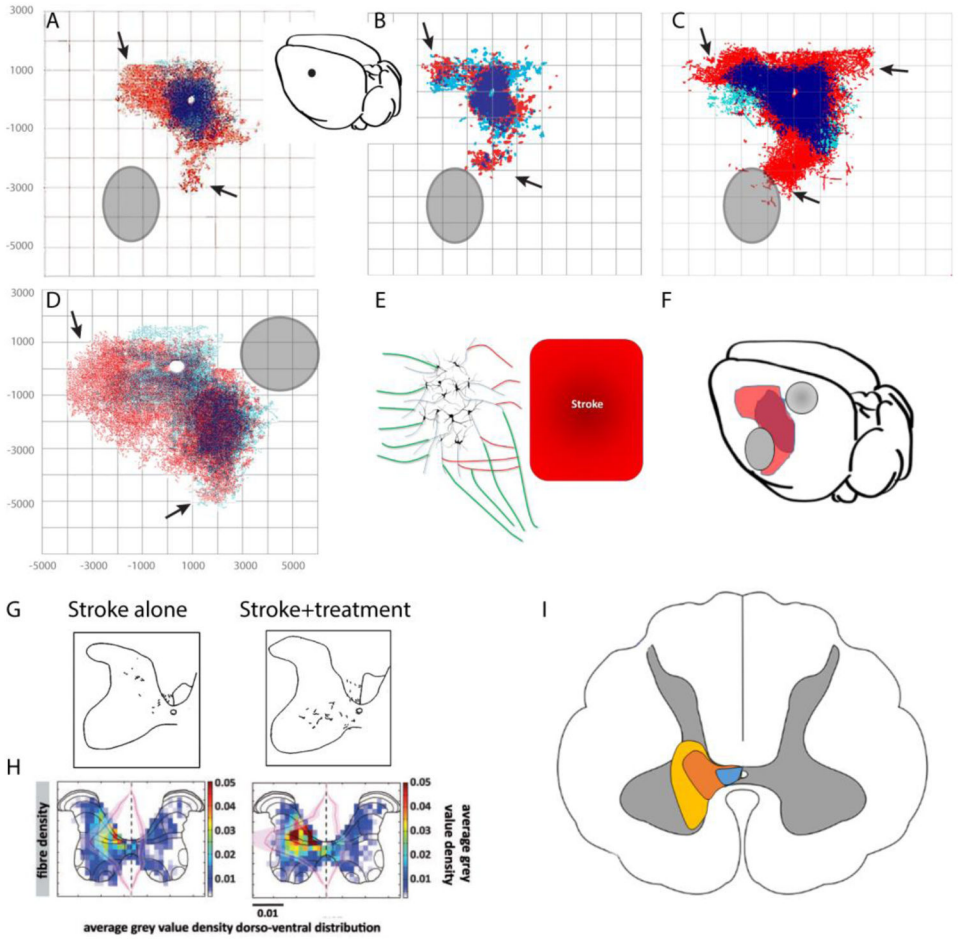

Figure 3. Reparative Axonal Sprouting after Stroke

(A–D). The cortical mapping conventions are the same in this figure as in the previous figure. Each map shows the quantitative plot of axonal projections from forelimb motor cortex in 5–6 mice per condition. In this map, all conditions are stroke (light blue) or stroke +growth promoting agent (red). Dark blue is an area of dense overlap of the two projections. (A) Anti-Lingo-1 antibody treatment in stroke (red) induces a significant increase in motor system connections to prefrontal cortex and to the second somatosensory cortex (SII) (arrows) compared to the motor system connections of stroke-alone (blue). (B) Blockade of EphrinA signaling (EphA5-Fc delivery) causes shifts of motor system axonal projection to premotor cortex and an increase in motor projections to SII. (C) Blockade of EphA4 signaling (EphA4-Fc) (red) produces a substantial axonal sprouting from motor cortex to premotor cortex, SII and SI (arrows). As noted in the text this substantial axonal sprouting response is likely because blocking EphA4 blocks astrocyte EphrinA signaling and also myelin inhibition through Ephrin B3. (D) Delivery of the stroke-induced brain growth factor GDF10 induces motor system axonal sprouting into premotor cortex and SII (arrows) compared to the stroke-alone condition (blue). In all of these maps, axonal sprouting is stimulated by blockade of an axonal growth inhibitor or stimulation of an axonal growth program after stroke but remains confine to the sensorimotor system. (E) Schematic of reparative axonal sprouting after stroke. In reparative axonal sprouting stimulation of the

post-stroke brain produces new patterns of connections within the sensorimotor system (green) that are associated with recovery, on top of the normal post-stroke reactive axonal sprouting (red axons). (F) Schematic view of the mouse brain with the normal motor system projection (blue) and the reparative post-stroke axonal projection (red). The location of two stroke sites in the models used in these studies is shown in the grey ellipses.
(G–I) Axonal sprouting in the cervical spinal cord in normal (untreated or unstimulated) stroke and after stimulation of axonal sprouting. (G) Drawings of one half of the cervical spinal cord grey matter showing the half of the spinal cord that has lost its projection from a stroke in the sensorimotor cortex. The axons projecting from the hemisphere contralateral to the stroke are traced in black. In stroke-only, these axons are sparse and located near the midline. After stroke plus treatment with the axonal growth stimulating molecule inosine, there is an increase in axonal projections that extend further into the spinal cord and toward the ventral horn (motor spinal cord). (H) Maps of the same region of cervical spinal cord in a different set of studies in a large stroke has been placed in the sensorimotor cortex and the projection of the contralateral corticospinal neurons is mapped. The color coding represents density of axonal projections (red = high, blue = low). In stroke alone (left image) there is a sparse projection into contralateral spinal cord that is close to the midline. In stroke plus treatment with a Nogo antagonist, there is an increase in contralateral axonal projections, seen as a greater density and an extension in all directions into the ventral, lateral and dorsal laminae of the spinal cord. In the case of both inosine and anti-Nogo treatments, functional recovery is enhanced in association with these patterns of cervical spinal cord axonal sprouting. (I) Schematic view of the cervical spinal cord with representative zones of axonal projections in the region of the cord that is contralateral to the normal corticospinal projection. In control (blue) there are few axonal projections to the contralateral spinal cord. After stroke (dark orange) there is a small increase in axonal projections into the contralateral spinal cord near the central canal and sparsely into the middle layers of the spinal cord (reactive axonal sprouting). In stroke plus treatment, in this case inosine or with Nogo blockade, there is a further increase in axonal sprouting into the ventral horn and partially into the dorsal horn (reparative axonal sprouting).
(A) is from Li et al., 2010. (B, C) are from Overman et al., 2012. (D) is from Li, Nie et al., 2015. (G) is from Zai et al., 2009. (H) is from Lindau et al., 2014.

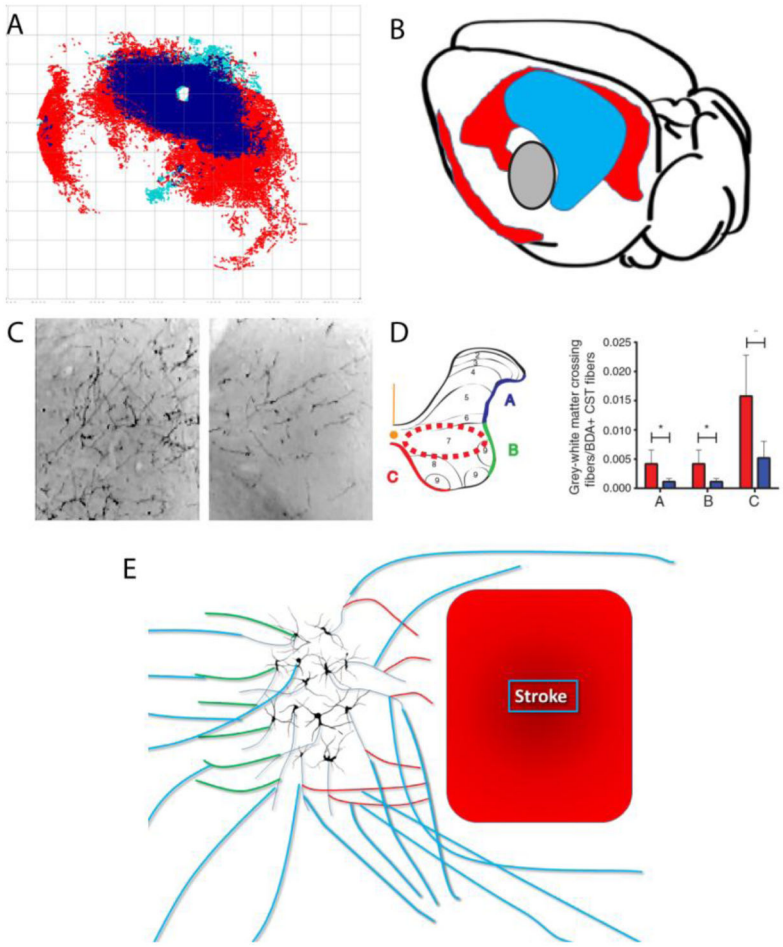

Figure 4. Unbounded Axonal Sprouting after Stroke

When axonal growth is stimulated by blockade of a glial growth inhibitor at the same time that behavioral activity of the motor system is manipulated by neurorehabilitative activities, axonal sprouting can extend beyond the sensorimotor areas to widespread brain systems. (A) connectional map of motor system axonal projections in stroke with forced overuse of the affected forelimb. Compared to normal post-stroke axonal sprouting (Figure 3A) there is an increase in projections within the motor cortex itself (blue). With blockade of the astrocyte growth inhibitor EphrinA5, there is a massive increase in axonal projections to orbital prefrontal cortex, lateral prefrontal cortex and temporal and parietal areas (red). (B) schematic rendering of post-stroke axonal sprouting with behavioral overuse of the affected limb (blue) and with behavioral overuse of the affected limb and blockage of a glial growth inhibitor (red). (C, D) Studies from Dr. Martin Schwab of axonal sprouting the spinal cord from neurons in the contralateral hemisphere from the stroke. The left panel in (C) shows axons that project into the ipsilateral spinal cord when the glial growth inhibitor Nogo is blocked and simultaneously the animal uses its affected forelimb in a daily repetitive reach task. The right panel in (C) shows the axons in this same projection when Nogo is blocked first, and then skilled reach training is used. There is a substantial increase in axonal projections when behavioral activity is manipulated at the same time as axonal growth is molecularly stimulated. (D) Schematic of the spinal cord grey matter, with the laminae. Red

columns indicate simultaneous Nogo blockade and behavioral activity; blue columns indicate first Nogo blockade then behavioral activity. When Nogo is blocked and skilled reach training implemented simultaneously, there is axonal sprouting throughout the spinal cord grey matter, including to non-motor areas (such as the dorsal spinal cord, section “A”). The region of the photomicrographs in (C) is highlighted with a red dotted ellipse in (D). (E) Schematic of unbounded axonal sprouting after stroke (blue axons). In conditions of manipulation of behavioral activity, such as with repetitive skilled reach or forced overuse of the affected limb, plus blockade of a glial growth inhibitor, axonal sprouting occurs over a very widespread brain or spinal regions. This occurs on top of the reactive (red) and reparative (blue) axonal sprouting after stroke.