Livin' on the Edge: Imaging Dendritic Spine Turnover in the Peri-Infarct Zone during Ischemic Stroke and Recovery

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Livin' on the Edge: Imaging Dendritic Spine Turnover in the Peri-Infarct Zone during Ischemic Stroke and Recovery

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The spontaneous recovery of sensory, motor, and cognitive functions after stroke is thought to be mediated primarily through the reorganization and rewiring of surviving brain circuits. Given that dendritic spine turnover underlies rewiring during normal development and plasticity, this process is likely to play a key role in mediating functional changes that occur during and after stroke. Recently, a new approach has been taken using two-photon microscopy to monitor, in real time, the temporal and spatial progression of dendritic plasticity in the living animal, both while it is experiencing the initial ischemic episode as well as during long-term recovery from stroke damage. Here, we highlight recent evidence showing that stroke can trigger extensive changes in the relatively hardwired adult brain. For example, when dendrites are challenged by acute ischemia, they can disintegrate within minutes of ischemia and rapidly reassemble during reperfusion. Over longer time scales, dendrites in the surviving peri-infarct zone show heightened levels of spine turnover for many weeks after stroke, thereby raising the possibility that future stroke therapies may be able to facilitate or optimize dendritic rewiring to improve functional recovery. NEUROSCIENTIST 14(2):139–146, 2008. DOI: 10.1177/1073858407309854

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Stroke is defined as a sudden loss of blood flow to the brain, making it a disorder of vascular plumbing. However, it is apparent that the stroke-plumbing problem evolves to affect the brain's electrical system through the ischemia-induced loss of synapses and circuits (Dirnagl and others 1999). Recently, our lab and others have taken the approach of using in vivo two-photon microscopy to monitor real-time changes in the brain's fine synaptic wiring during the initial ischemic episode (within the first hours) and during the days to weeks of stroke recovery (Brown and others 2007; Zhang, Boyd, and others 2005; Zhang and Murphy, 2007; Zhang, Zhang, and others 2005). Originally developed by Winfred Denk and Watt Webb (Denk and others 1990), two-photon microscopy uses pulsed infrared light to excite fluorophores by the combined power of two long-wavelength photons (that are nearly simultaneously absorbed). One of the key advantages of this approach is that it allows one to image structures deep within thick biological specimens while achieving micron-level resolution. Combining this imaging technique with transgenic mice engineered to express fluorescent proteins within a subset of neurons (Feng and others 2000) has enabled investigators to track changes in neuronal structures in living animals over days, weeks, and even months (Grutzendler and others 2002; Trachtenberg and others 2002). Here, we describe recent results using this approach to examine the acute vulnerability of synaptic circuits to stroke as well as the remarkable degree of plasticity in dendritic spines in regions adjacent to tissues lost to stroke damage (i.e., the peri-infarct zone). We then relate these new imaging results to previous histological and functional studies to suggest that dendritic remodeling in peri-infarct regions plays an integral role in the process of recovery from stroke damage.

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Dendritic Plasticity as a Potential Mechanism of Stroke Recovery

Dendrites play a fundamental role in cell-to-cell communication in the brain, as they are the postsynaptic targets of most synapses in the brain. A large percentage of dendrites are decorated with tiny protrusions known as dendritic spines, which usually possess at least one excitatory synapse (Arellano and others 2007). Dendritic spines are heterogeneous structures in nature, configured in a variety of shapes (often referred to as stubby, mushroom, or thin) and ranging in volume from 0.01 μm^3 to 0.8 μm^3 (Harris 1999). Although the majority of dendritic spines in the adult brain tend to be stable (Grutzendler and others 2002; Holtmaat and others 2005), emerging evidence suggests that under certain conditions, spines can become remarkably dynamic structures

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that likely play an active role in shaping synaptic signaling (Fischer and others 1998). For example, spine turnover rates can be augmented by manipulations of sensory experience (Holtmaat and others 2006; Zuo and others 2005), neuronal excitability (Mizrahi and others 2004), long-term potentiation or depression (Matsuzaki and others 2004; Zhou and others 2004), and neuropathological conditions (Fiala and others 2002; Rensing and others 2005). Furthermore, recent data have shown that the size of excitatory receptor-mediated currents, conduction of postsynaptic potentials, and diffusion kinetics of intracellular calcium from the spine head to the parent dendrite are all related to the particular geometry of the spine (Araya and others 2006; Bloodgood and Sabatini 2005; Majewska and others 2000). These data suggest that dendritic spines are well positioned to play a critical role in mediating brain plasticity that underlies recovery after stroke.

Acute Changes to Dendritic Structure during Ischemia

When dendrites become ischemic, they undergo a relatively stereotyped pattern of degeneration; the most salient morphological feature is the appearance of varicosities or beads along the shaft of the dendrite (Hasbani and others 2001; Park and others 1996). Initially, these swellings can be very subtle, but as the severity or time of ischemia progresses, they become more pronounced and often appear like beads on a string (Obeidat and others 2000). These ischemia-induced changes in morphology have been described with a number of different cytoplasmic and membrane labeling methods (carbocyanine DiI, yellow/green fluorescent protein, and Golgi-Cox staining) and therefore reflect changes in neuronal morphology rather than artifacts of the labeling method (Brown and others in press; Hasbani and others 2001; Park and others 1996; Zhang, Boyd, and others 2005). Furthermore, blebbing of dendrites has also been observed in vivo after photothrombosis or middle cerebral artery occlusion, suggesting that it is also not specific to a particular model of ischemia (Enright and others 2007). Consequently, investigators have been able to use these telltale signs of dendritic ischemia, in tandem with live-cell imaging techniques, to elucidate the precise temporal and spatial progression of neuronal damage after various forms of ischemia as well as the various factors (i.e., neurotransmitters, electrolytes, etc.) that modulate these events.

One fundamental issue that has recently been addressed is to what degree dendrites can withstand the effects of ischemia before showing signs of degeneration. Using in vivo two-photon microscopy of transgenic mice expressing YFP-labeled cortical dendrites, Zhang, Boyd, and others (2005) tested the resiliency of cortical dendrites by exposing them to moderate and severe forms of ischemia. To model a moderate ischemic insult, they infused the vasoconstrictive peptide endothelin directly into the brain while repeatedly assessing blood flow and changes in dendritic structure. Rather unexpectedly, despite a 50% reduction in blood supply, endothelin infusions had little to no effect on dendritic structure or spine number during a five-hour period. However, if the brain were subject to more severe ischemia, such as during photothrombotic stroke in which blood flow is reduced by >90%, significant dendritic damage and spine loss occurred within 10 to 20 minutes (Fig. 1). Furthermore, the loss of dendritic structure occurred before any detectable signs of extravasation, suggesting that dendritic damage was related to the loss of oxygen and nutrients diffusing through the capillary wall rather than damaging elements from leaking blood plasma (Zhang, Boyd, and others 2005; Zhang and Murphy 2007).

As one might expect, ischemia-induced damage after focal stroke depends not only on the severity of blood restriction but also on the size of the ischemic territory. In the normal brain, it has been shown that on average, cortical dendrites are typically 13 μm from a flowing capillary (Zhang, Boyd, and others 2005). After an ischemic event such as photothrombosis, intact dendrites can be maintained within 80 μm of a flowing vessel, at least within the first several hours after stroke (Zhang and Murphy 2007). Based on this finding, one would predict that very small strokes, even ones that produce severe clotting, would not necessarily be accompanied by neuronal damage. Indeed, this appears to be the case, given that a stroke less than 0.1 mm^2 in diameter produces virtually no dendritic damage (during a five-hour period). This finding is consistent with recent work showing that the cerebrovascular network can rapidly redistribute blood supply after microvascular occlusion (Schaffer and others 2006). Therefore, the brain is resilient to moderate and potentially severe interruptions in blood flow, provided the spatial extent of ischemia is not too broad.

Even the appearance of severely damaged dendrites does not necessarily mean that all hope is lost. This fact was elegantly pointed out several years ago by Goldberg and others (Hasbani and others 2001; Jourdain and others 2002; Park and others 1996), who showed in vitro that severely blebbed and dysmorphic cortical dendrites subjected to oxygen–glucose deprivation or glutamate receptor agonists could show complete recovery of structure within two hours of insult. These reversible effects on dendritic structure were subsequently demonstrated by Kirov and others (2004) in hippocampal slices exposed to chilling and rewarming. More recently, our lab has shown that the resurrection of damaged dendrites could also occur in the living brain in certain instances in which significant reperfusion occurred after photothrombotic stroke (Fig. 1; Zhang, Boyd, and others 2005). We first noticed this phenomenon in a subset of animals in which ischemic, damaged dendrites would re-emerge in certain regions where clots would spontaneously break up over a time scale of tens of minutes after the induction of stroke (Fig. 1). Remarkably, the majority of dendritic spines that re-emerged after ischemic challenge returned to the exact same site from which they disappeared, perhaps owing to the stability of presynaptic elements during stroke (Hasbani and others 2001; Zhang, Boyd, and others 2005). Furthermore, recovered spines appear to be functional, considering that synaptic responses can be elicited from revived hippocampal slices (Kirov and others 2004) and sensory-evoked responses can be mapped in the cortex after transient global ischemia–induced loss and recovery

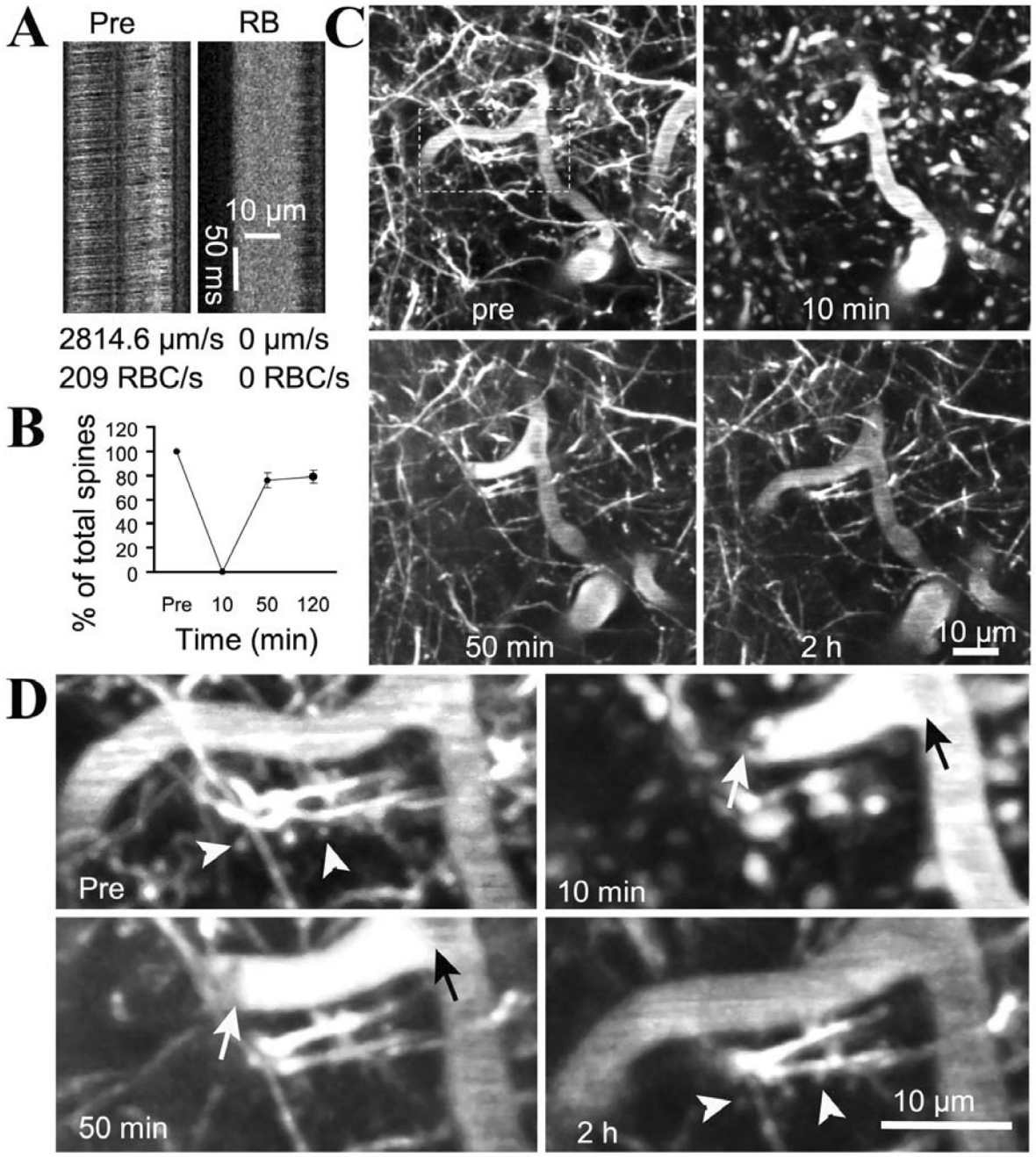

Fig. 1. Rapid, reversible changes to dendritic spine structure during ischemia and reperfusion. (*A*) Line scan showing blood flow through a mouse capillary (shown in *C* and *D*) before (pre) and 10 minutes after photothrombosis. Note that blood flow has completely stopped 10 minutes after stroke. (*B*) Quantification of spine number expressed as a percentage of prestroke values (from YFP fluorescence). Note the complete loss of spines 10 minutes after stroke when blood flow has ceased. (*C*) In vivo images showing the rapid degeneration of dendrites within 10 minutes of ischemia (note the severe beading) and subsequent recovery of structure with reperfusion at 50 and 120 minutes. Note that in this experiment, both the vessels and dendrites were detected as green fluorescence and are shown as grayscale images. (*D*) Higher magnification images of the boxed region in *C*, showing spine loss with severe ischemia and recovery with reperfusion (white arrowheads). The arrows indicate clots in a section of capillary that resolved spontaneously during the experiment. Modified, with permission, from Zhang, Boyd, and others (2005), and images taken from the somatosensory cortex of a urethane anaesthetized adult mouse. Copyright by the Society for Neuroscience, 2005.

of spines (unpublished observations, Murphy and others). These results suggest that the brain is capable of recovering its structure and function, even when severely damaged, if sufficient blood flow is restored in time. However, this optimism is tempered by the fact that we do not know how long dendrites can sustain damage before irreversible changes take place and thus close the window of opportunity for effective recovery.

Synaptic Plasticity Underlies Long-term Recovery from Stroke Damage

The adult brain is not static, but rather, continuously modifies its functional organization to cope with everyday experiences and events. Therefore, it should come as no surprise that in the weeks and months after stroke, compensatory changes take place throughout the brain. It is believed that many of these changes, induced intrinsically via changes in synaptic activity, gene expression, or inflammation or through modifications in the behavior or experience of the organism, participate in the recovery of functions initially lost to stroke (for review, see Carmichael 2006). In human patients, imaging studies (fMRI, PET) have shown that good functional recovery of paretic limbs was associated with remapping of functional activation patterns in sensory-motor areas in the stroke-affected hemisphere (Cramer 2004; Ward 2004). Similar findings have been described in several animal models of stroke. Glees and Cole (1950) were the first to show that functional representations of the cortex can reorganize and re-emerge in regions adjacent to the site of damage. More recent work from Nudo and colleagues (1996) has shown that rehabilitative strategies that promote recovery of skilled movements after focal stroke were associated with significant changes in cortical motor representations (assessed with intracortical microstimulation) and functionally related regions of the damaged hemisphere. Collectively, these observations suggest that the adult brain is capable of making profound functional adaptations in response to injury. Given that functional plasticity must be supported by changes in the structure of neuronal circuits, we now discuss some recent developments in the search for anatomical substrates of stroke recovery—in particular, the role of dendrites and spines.

Dendritic Plasticity in Peri-infarct Regions and Beyond: Implications for Recovery

Surrounding the death and destruction of the ischemic core is a region of hypoperfused tissue known as the peri-infarct zone (Hossmann 2006). Currently, there is some debate as to what exactly constitutes the "peri-infarct zone" or how large this area extends. For the sake of simplicity, we will define it as the region of surviving tissue immediately surrounding the core of the infarct, which, in a rodent, could be less than a millimeter, whereas in humans, this region may be several orders of magnitude larger (Carmichael 2005). Indeed, the ambiguity of this distinction arises from the fact that the type of experimental stroke and the angio-architecture of the animal studied will affect the rate of blood perfusion, metabolism, and cell death within the peri-infarct zone and how long after the initial insult (Carmichael 2005; Dirnagl and others 1999). In the case of the photothrombotic model of focal ischemic stroke in rodents (Watson and others 1985), histological studies have shown that much of the ischemic damage (assessed by infarct volume) occurs within the first 24 hours after stroke (Van Hoecke and others 2005). By examining the structural integrity of fluorescently labeled cortical dendrites in transgenic mice, we found that the lateral spread of dendritic damage stabilized within six hours after stroke (Enright and others 2007). Comparably, repeating imaging of dendritic structure in vivo indicated that the transition between damaged and structurally intact dendrites stabilized within a couple of hours after stroke and was accompanied by a very abrupt border between dying and intact structures, occurring over just tens of microns (Zhang and Murphy 2007). However, despite the presence of intact-looking dendrites at the border (as revealed with YFP in vivo or Golgi-Cox staining in fixed tissue), more subtle changes were occurring at the level of individual spines, which became progressively fewer in number during the first few hours and days after stroke (Akulinin and others 1997; Brown and others in press; Ito and others 2006; Zhang and Murphy 2007). Consistent with in vitro studies of mildly ischemic neuronal dendrites (Hasbani and others 2001; Jourdain and others 2002; Kirov and others 2004), surviving peri-infarct spines after focal stroke in vivo became significantly longer in length (from spine head to shaft) during the first 24 hours after stroke (Brown and others in press). Furthermore, these changes were spatially limited, as spine number and length in more distant regions (~800 μm away) were unchanged. Although the functional consequences of these acute changes are uncertain, longer dendritic spines in vulnerable peri-infarct neurons may help to prevent the effects of potentially damaging signaling events at the spine head (i.e., where synapses are typically found) from triggering the death of the entire cell, given that longer dendritic spines can limit potentially damaging ionic, biochemical, and electrical signaling from the spine head to the dendritic shaft.

In the days to weeks following an ischemic insult, accumulating data suggest that the peri-infarct region is a hot spot for neuronal plasticity that subserves functional recovery from stroke (Witte 1998). In particular, peri-infarct zones undergo significant changes in the expression of growth-promoting and growth-inhibitory factors that are essential for neuronal rewiring, angiogenesis, and neurogenesis (Carmichael 2006). Furthermore, anatomical studies have shown that for several weeks after focal stroke, peri-infarct regions show increased synaptogenesis, are enriched with histochemical markers of axonal sprouting, such as GAP-43, and receive new intracortical projections (Dancause and others 2005; Ito and others 2006; Stroemer and others 1995). As one might suspect, changes

in presynaptic connectivity are accompanied by postsynaptic modifications of the dendrite. Postmortem examinations of dendritic structure in rats showing some, albeit limited, functional recovery from middle cerebral artery occlusion displayed a significant decrease in basilar dendritic branch length and complexity in the ipsilesional cingulate cortex (Gonzalez and Kolb 2003). Interestingly, the atrophy or simplification of ipsilesional dendritic arbors and loss of dendritic spines could be prevented by infusions of nerve-growth factor (Kolb and others 1997) or nicotine (Gonzalez and others 2006), which correlated with a greater improvement in behavioral recovery.

One limitation of histological studies is that they provide only endpoint measures of plasticity, therefore yielding little information about the process by which peri-infarct dendrites change in real time in the living brain. To overcome this problem, we recently applied two-photon microscopy to examine in vivo changes in dendritic architecture and spine turnover in the cortex of YFP transgenic mice at different time points of recovery from photothrombotic stroke (Brown and others 2007). What was quite striking about the postischemic brain was that peri-infarct dendritic arbors and vasculature were organized in a completely different manner, relative to controls (Fig. 2*A*–2*F*). At six weeks' recovery, dendritic arbors lost their tortuous appearance and were aligned in one direction, radiating outward from the infarct border, which paralleled that of the cerebral vasculature. Intriguingly, growing evidence suggests that the molecular–genetic programs responsible for vessel formation can also influence neurite development and outgrowth (Carmeliet and Tessier-Lavigne 2005), thereby raising the possibility that dendritic remodeling after stroke may be predicated on peri-infarct angiogenesis. In tandem with these large-scale changes in dendritic arbor organization were robust changes in rates of dendritic spine turnover. By sampling levels of spine formation and elimination during a six-hour period at various points of recovery (for example, see Fig. 2*G*), we discovered that spine turnover rates were dramatically increased at one to two weeks after stroke and were still above control levels even six weeks after injury (Fig. 2*H*). In tandem with the elevation in spine turnover rates, we found that spine density levels in peri-infarct regions gradually recovered in six weeks' time (Fig. 2*I* and Fig. 3 for summary scheme). Notably, this protracted period of spinogenesis and recovery occurred within the same areas in which our lab (unpublished data) and others (Dijkhuizen and others 2001) have observed functional rearrangements in cortical response properties and during the same time period in which the brain is most amenable to therapeutic interventions (Biernaskie and others 2004) and when most recovery of function occurs.

The processing, coordination, and execution of cognitive, sensory, and motor programs involves multiple brain regions. Therefore, to recover from a stroke, the brain must recruit not only those regions close to the damage but also those distributed in more remote locales (Nudo 2006). One area that has sparked considerable interest in the stroke recovery field is the homotopic region of the hemisphere contralateral to the site of damage. Seminal work by Kolb, Jones, and Schallert demonstrated that unilateral lesions of the frontal or forelimb sensorimotor cortex were associated with an overgrowth of dendritic arbors and synapse formation in layer 2/3 and 5 pyramidal neurons in the contralateral hemisphere (Jones and Schallert 1992; Kolb and Gibb 1991). This exuberant dendritic growth was most pronounced during the first three weeks after damage, when the animals were overly reliant on the unimpaired paw, and returned to near-control levels (but not completely) as the animals began to recover the use of the affected paw (Jones and Schallert 1992). Furthermore, promoting contralesional growth by rehabilitative training (Biernaskie and others 2004) or blocking it by forelimb immobilization (Jones and Schallert 1994) was associated with an improvement or decrement, respectively, in recovery of the impaired forelimb. Collectively, these data suggest that dendritic remodeling in both damaged and intact hemispheres may play an active role in behavioral recovery.

Conclusions and Future Directions

There are now abundant data indicating that dendrites in the adult brain can change after stroke (summarized in Fig. 3). Because many of the changes reside within brain regions functionally related to those lost and take place during the first few weeks to months after stroke, when restoration of function is most likely to occur, there is good reason to suspect that dendritic remodeling is a fundamental mechanism of brain plasticity that mediates recovery. Despite this optimism, we still have much to learn about the resiliency of dendrites to different forms of ischemia as well as how adaptive these ischemia-related changes are. With the recent development of in vivo imaging techniques and transgenic mice expressing a variety of fluorescent markers, we can now examine questions regarding the precise nature and mechanisms through which dendrites change in the living animal while it is in the process of recovering from stroke. For example, how do recovering dendritic arbors within a single neuron grow or retract over time and space? Are there cell-type specific rules governing dendritic remodeling after stroke? Because recovery is highly variable, are certain changes in dendritic structure or within a particular brain region correlated with a favorable outcome or a particularly efficacious rehabilitative strategy? Can we promote "positive" changes in dendrites and spines with the application of pharmacological agents, gene therapy, or electrical stimulation? Providing answers to these long-standing questions will undoubtedly pave the way for future therapies seeking to optimize recovery from stroke.

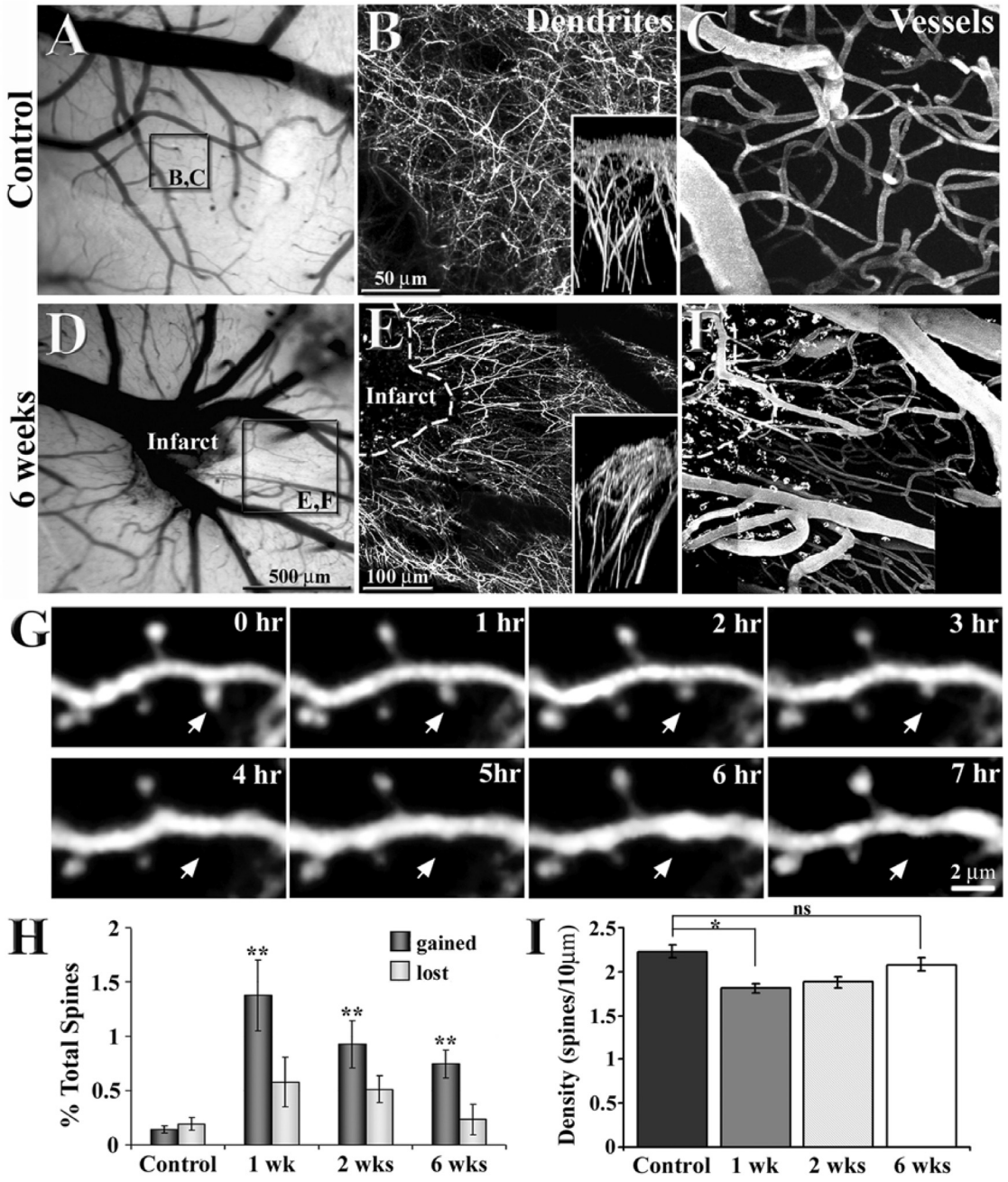

Fig. 2. Remodeling of apical dendrites, spines, and vasculature in the peri-infarct cortex. (*A*, *D*) Brightfield images showing the pattern of blood vessels on the brain's surface in a control adult mouse and one six weeks after photothrombotic stroke. (*B*, *C*, *E*, *F*) In vivo two-photon images (each is a maximal intensity projection of 80 optical sections, taken 2 µm apart) of YFP labeled cortical dendrites and flowing vasculature, labeled with Texas Red Dextran (each channel is shown separately as a grayscale image). In control mice (*B*, *C*), the apical dendritic tufts and vasculature have a tortuous appearance and are intertwined with one another. Six weeks after stroke (*E*, *F*), dendrites and blood vessels radiate outward from the infarct border in parallel with one another. To examine the effect of stroke on spine turnover rates, we repeatedly imaged dendrites over a six-hour to seven-hour period at one, two, and six weeks after stroke. (*G*) Time-lapse imaging showing the retraction of a dendritic spine. (*H*) Quantification of spine formation and elimination during time-lapse imaging experiments revealed that spine turnover rates in the peri-infarct cortex were significantly increased at one, two, and even six weeks after stroke, relative to controls, which showed low levels of spine turnover. (*I*) Spine densities in the peri-infarct zone are significantly reduced one week after stroke but gradually recover to control values by six weeks after stroke. $^{*}P < 0.05$, $^{**}P < 0.005$. Modified, with permission, from Brown and others (2007), and images taken from the sensorimotor cortex of a urethane anaesthetized adult mouse. Copyright by the Society for Neuroscience, 2007.

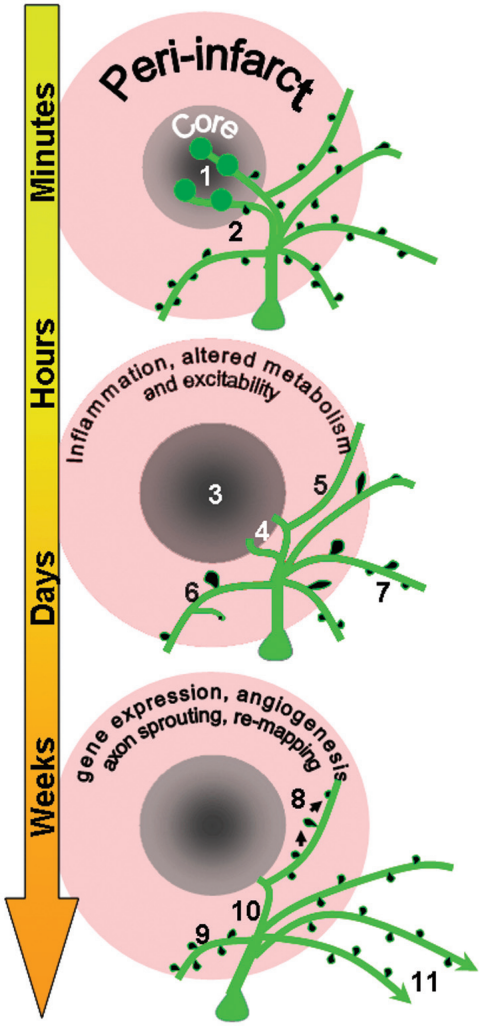

Fig. 3. Schematic summarizing ischemia-induced changes to dendritic structure over time and space. Within minutes to hours after stroke, dendrites in the center of the severely ischemic core become fragmented and degenerate (*1*). Fortunately, this degeneration can be reversed, particularly in the outer edges of the core, if sufficient reperfusion occurs (Zhang, Boyd, and others 2005). (*2*) Surrounding the infarct core is a region of mildly ischemic tissue called the peri-infarct zone. Note that dendrites and spines remain intact in this zone and even inside the core (i.e., within 80 µm of the border) because of the diffusion of oxygen and nutrients from flowing vasculature (Zhang and Murphy 2007). During the next several hours to days, dendrites in the core are permanently lost (*3*; Brown and others 2007; Brown and others in press). The border between dead and surviving tissue becomes more distinct and stable (*4*). Peri-infarct dendrites lose many of their spines (*5*), whereas those that remain enlarge and extend in length (*6*; Brown and others in press). Dendritic spines in regions more distant from the infarct border appear to be relatively stable in both length and density (*7*). During the next several weeks to months after stroke, peri-infarct dendrites undergo a number of changes. For example, rates of spine formation and elimination are dramatically increased (*8*), with spine density levels' recovering to prestroke levels (*9*; Brown and others 2007). Dendritic arbors near the infarct border appear slanted because of mechanical forces around the lesion (*10*). Dendrites extend and possibly grow outward from the infarct border in alignment with new blood vessels (*11*; Brown and others 2007).

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