

Should the injured and intact hemispheres be treated differently during the early phases of physical restorative therapy in experimental stroke or parkinsonism?

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After unilateral focal damage to the sensorimotor cortex, rats preferentially use the ipsilesional forelimb and reduce independent use of the contralesional forelimb for many functions. Motor behavior using the intact forelimb is associated with gradually evolving astrocytic and neural events in the opposite hemisphere, and many of these events seem to be use-dependent. The homotopic (nonlesioned) cortex has been regarded by some investigators as an essential contributor to functional outcome in the impaired forelimb. An alternative view is that the events in the homotopic cortex are not directly linked to recovery of sensorimotor function in the impaired forelimb but rather are linked to improved function in the nonimpaired forelimb or in ipsilateral sensory control over impaired contralesional motor programs that have become unresponsive to contralesional sensory influences. This view is supported by the details of limb use during motor tasks, by data from a novel beam walking test designed to encourage the animal to display its deficits rather than hide them with motor tricks, by behavioral analysis of cross-midline sensorimotor integration, and by experiments in which both hemispheres are damaged sequentially (in stages separated by enough time for neural events in the yet-to-be injured cortex to assume some functions). To capitalize on a potential therapeutic opportunity

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to shape synaptic efficacy for functional benefit, it may be important to include as a target the nonimpaired forelimb and interlimb coordination, perhaps early after the injury when the expression of trophic factors in astrocytes is increased. Avoiding long durations of constraint of the nonimpaired forelimb, at least until later postoperative periods, may be prudent. Intense motor training focusing exclusively on the impaired forelimb too early after some types of acute cortical injury can exaggerate the size of the injury, whereas intense early motor training of the nonimpaired forelimb has been found to enhance skilled motor function even beyond control levels. In contrast, in animal models of Parkinson's disease, early intense motor training of the impaired forelimb can have a protective effect on cellular and behavioral outcome. Moreover, forced or self-imposed nonuse of the impaired forelimb can exaggerate the extent of degeneration. If these data are relevant to Parkinson's disease in humans, the implication may be that earlier detection and intervention using motor rehabilitation techniques might be of some value in slowing the progression of Parkinson's disease. Thus, a rehabilitative strategy that is found to be successful for one type of brain insult may not be optimal for another, and the timing of the rehabilitation may be crucial.

The intact cortex after unilateral brain injury

Historical hints of possible contribution

Research animal models of brain injury and recovery of function have a long history. Well over 100 years ago Fritch and Hitzig [1] and Ferrier [2] electrically stimulated different points on the surface of the brain, confirming that movement of the contralateral limbs could be evoked from the motor cortex. Following the experimental neurology approach pioneered by DuVerney in 1697 and Flourens in the early 1800s, they then ablated this tissue, causing hemiplegia contralateral to the damage (cited in [3-6]). According to historical reviews [4,7], Nothnagel used knife cuts in 1874 to demonstrate that sensorimotor dysfunction from unilateral caudate injury was similar to that following unilateral damage to the sensorimotor zone of the cortex and noted a slow recovery of function that he suggested might be caused by compensatory events in the intact hemisphere.

Cellular activation, structural remodeling, and growth

It is now generally established that in response to unilateral damage to the sensorimotor cortex, various neural, astrocytic, molecular, and metabolic changes occur in the neocortex and underlying striatum of both hemispheres, but the degree to which the so-called intact, homotopic cortex is involved in recovery remains controversial [8-13]. Signals arising from partial denervation of interhemispheric projections may activate mechanisms of

plasticity in the intact cortex that could play a substantial role in functional outcome [13-24]. There is, however, considerable disagreement about the nature of that role, particularly with regard to recovery of function and restorative treatments.

In the homotopic cortex, plasticity-associated events reminiscent of, but probably not identical to, ontogenetic events may appear at different times after focal brain lesions or ischemic injury [17,20,25-27]. The size and type of injury may be of crucial importance, but among the earliest events can be the appearance of growth-correlated markers, typically followed later by neuronal structural changes. In the homotopic cortex, hypertrophy of astrocytes in Layer V has been observed in the first 7 to 10 days after electrolytic damage, so much so that in Golgi-stained sections they may obscure visibility of neurons in light microscopy [20]. In the uninjured brain the random staining of astrocytes and neurons does not yield such overlap in the population of stained cells. It is possible that this overlapping indicates that astrocytes proliferate and also may become unusually connected structurally and functionally to neurons.

Other growth-related events may occur as well. An increase in fibroblast growth factor 2 (FGF-2) immunoreactive astrocytes has been found in both the ipsilesional and contralesional sensorimotor cortex beginning during the first week [15], followed by and overlapping with an increase in microtubule-associated protein-2 [28], growth-associated protein-43 (GAP-43), and synaptophysin [29,30].

Use-dependent dendritic arborization

Over several weeks, a marked increase in dendritic arborization in Layer V pyramidal neurons has been shown after large focal cortical electrolytic lesions or middle cerebral artery occlusion (MCAo) (Tables 1, 2) [14,20]. Signals from the damaged hemisphere, possibly diffusible or transportable proteins, may be important, because aspirating the tissue immediately after electrolytic lesions prevents the dendritic arborization in the homotopic cortex or possibly delays their appearance, and recovery is compromised [31]. Sectioning the corpus callosum increases arborization, but only when animals are forced to use the forelimb corresponding to that hemisphere

Table 1
Exuberant neuronal growth and pruning: Layer V basilar dendritic arborization (bifurcation counts) in the intact sensorimotor cortex after damage to the same region in the opposite hemisphere

| Sham | Lesion | | | |
|----------|----------|-----------------------|-----------------------|----------|
| | Day 3 | Day 18 | Day 30 | Day 120 |
| 18.3±0.2 | 21.1±0.8 | 27.6±2.9 ^a | 24.5±0.3 ^a | 22.4±1.2 |

^a Significantly different from sham.

Table 2

Stroke leads to increase in dendritic branches in the intact cortex

| | Standard environment | Complex environment |
|------|-------------------------|-------------------------|
| Sham | 38.2 ± 2.2 | 45.8 ± 2.1 ^a |
| MCAo | 51.7 ± 1.8 ^a | 54.9 ± 3.6 ^a |

Basilar dendritic branches per neuron in the intact cortex at day 18 after MCAo. Raising animals in a more complex environment further enhances the degree of branching.

^a Significantly different from sham.

Abbreviation: MCAo, middle cerebral artery occlusion.

From Biernaskie J, Corbett D. Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemia. *J Neurosci* 2001; 21(14):5272-80.

by immobilizing the other forelimb [15]. Thus, partial denervation of projections from the opposite hemisphere may create a fertile milieu for neuronal growth that is enhanced by behavioral demand. The extent to which neural and glial events may be use-dependent, injury-dependent, and time-dependent has relevance for rehabilitative therapy and is discussed later.

When dendritic arborization reaches its peak extent, after a couple of weeks or so in the electrolytic lesion model, the dendrites are spine-sparse despite their exuberant branching relative to dendrites from undamaged rats (Table 3) [32]. Over the ensuing weeks the density of dendritic spines increases as the arbors enter a slow phase of "pruning" that may be associated with use-dependent synapse selection [13,20,21,26,33,34]. The pruning process, which varies considerably in extent and in number of weeks or months in which it reaches its maximum, is not caused by a return to more symmetrical use of the forelimbs. Thus, the animal continues to use its non-impaired forelimb preferentially for most complex movements during the period of pruning [13]. In addition, immobilization of the impaired forelimb beginning at the time of peak dendritic arborization does not interfere with the pruning [21,26,33].

As the dendritic arbors are pruned, synaptogenesis also occurs, although it is not known whether the two events are directly linked [19,35,36]. Electron microscopy reveals a large increase in multiple axodendritic synapses and discontinuous expanded postsynaptic receptors in the

Table 3

Spine density in the intact cortex after sensorimotor cortex lesion: Layer V dendritic arbors are spine-sparse at peak growth despite being more branchy, but by day 33 spine density increases. Data are dendritic spines (per 10- μ m segment) in the intact cortex at day 18 or day 33 after unilateral damage to sensorimotor cortex

| | Day 18 | Day 33 |
|--------|-------------------------|-------------------------|
| Lesion | 13.1 ± 1.2 ^a | 29.2 ± 1.2 ^b |
| Sham | 22.3 ± 1.2 | 23.1 ± 1.2 |

^a Significantly different from sham.

^b Significantly different from sham and from lesion day 18.

Table 4

Synaptogenesis: Multisynaptic boutons in intact (opposite) cortex after unilateral injury to sensorimotor cortex. Data are synapses (per neuron) in which an axon terminal makes connections with more than one dendrite or at multiple locations on a single dendrite (Jones et al, 1999)

| Sham | Postlesion, day 18 | Postlesion, day 30 |
|-----------|--------------------|-------------------------|
| 750 ± 180 | 410 ± 109 | 1840 ± 219 ^a |

^a Significantly different from sham and from postlesion day 18.

From Jones TA, Chu CJ, Grande LA, et al. Motor skills training enhances lesion-induced structural plasticity in the motor cortex of rats. *J Neurosci* 1999;19(22):10153-63.

intact sensorimotor cortex (Table 4) [19,35,36]. These synapses are similar to those formed after long-term potentiation (LTP) in the hippocampus, an established activity-related model of learning and memory [37] in which the *N*-methyl-D-aspartate (NMDA) receptor is believed to be at least partially involved. The process of pruning can be prevented by administration of an NMDA antagonist (MK-801) or by self-administration of ethanol, beginning at the peak of dendritic arborization and continuing during the pruning period [25,34,38]. Blocking the pruning process is associated with adverse functional effects on behavior. *N*-methyl-D-aspartate receptors are widely believed to be linked to learning. Pruning may reflect use-dependent synapse selection associated with behavioral activity and learning in the nonimpaired limbs. Animals with unilateral lesions learn to use the limbs corresponding to the intact hemisphere to compensate for loss of normal function in the impaired forelimb.

Fragile recovery

N-methyl-D-aspartate-dependent hyper-excitability and decreased expression of γ -aminobutyric acid (GABAergic) receptors also occur in both the ipsilesional and contralesional cortex within the first weeks after injury [39,40] and may contribute to neural activation and ongoing functional recovery. Indeed, NMDA antagonists (eg, MK-801, ethanol) or GABAergic agonists (eg, diazepam) administered during later time periods following recovery can transiently reverse functional gains achieved in the contralateral forelimb [25,38,41-43]. In the case of NMDA antagonist drugs, the reinstatement of impairment can last up to 1 week beyond the half-life of the drug. This marked vulnerability to NMDA antagonists suggests that recovery of function is extremely fragile and depends on normal glutamatergic activity, at least during the period of dendritic growth, pruning, and synaptogenesis.

The integrity of the NMDA synapse may also be critical chronically for injury sustained during early development. Thus, rats that sustain hypoxic-ischemic injury unilaterally during the neonatal period (postnatal day 7) develop a contralesional forelimb-placing deficit from which they recover

fully by adulthood, but the unilateral placing deficit is reinstated on exposure to an NMDA antagonist [96].

What function might the homotopic cortex provide?

Although the function of the intact forelimb specifically is only now being investigated under conditions in which pruning and synaptogenesis have been blocked, it is known that several weeks after cortical injury the intact forelimb can guide complex exploratory behavior, can help prevent the impaired forelimb from errors in motor tasks [21], and is capable of skilled reaching ability at a level that significantly exceeds reaching ability of uninjured animals [8]. The gradual appearance of synaptogenesis in the homotopic cortex may reflect this capacity.

Additionally, the authors have recently found that the timing of synaptogenesis is closely correlated with the appearance of a qualitatively unique stage of recovery from vibrissae-evoked placing deficits. After unilateral focal ischemic injury to the forelimb sensorimotor cortex by the endogenous vasoconstricting agent, endothelin-1, or by MCAo restricted to the neocortex, qualitatively unique stages of recovery of limb placing occur, as demonstrated in a battery of cross-midline and non-crossed placing tasks. In these tasks the animal is held so that its forelimbs are suspended without support, and the left or right vibrissae are brought into contact with a stable surface such as a table edge. The normal animal rapidly places the forelimb closest to the table. Beginning late in the second week after the injury, but not early after the injury, the ipsilesional set of vibrissae (intact side of the body) gains the capacity to elicit placing from the contralateral (impaired) forelimb, at a time when the contralateral vibrissae cannot reliably elicit placing from the contralateral forelimb (M.T. Woodlee, D.A. Adkins, T.A. Jones, T. Schallert, unpublished observations). This observation indicates that, parallel with synaptogenesis in the homotopic cortex, when the vibrissae on the contralateral side of the midline seem to be denied access to motor programs for contralateral limb use, the capacity of the intact hemisphere to process sensory input from its corresponding vibrissae and to activate contralateral motor programs is increased. The ability of the animal to use sensory input from the ipsilateral side of the body to control motor programs involving both forelimbs would probably impart an enormous advantage in coordinated motor behavior at a time when the animal cannot efficiently use sensory input to the injured hemisphere to control the impaired forelimb. The link between use-dependent neural events and this novel cross-midline placing reaction currently is circumstantial. It remains to be investigated directly whether the growth and pruning of dendrites [20], synaptogenesis [19,35,36], or the rewired projections to brain stem and spinal nuclei associated with the intact cortex after focal brain injury [16] might mediate this key stage of functional recovery.

Complex dendrites from complex environments

When injured animals are housed socially in environments that are enriched (ie, less impoverished than standard housing conditions although far less complex than the natural environment of a rat), or when they are given acrobatic training, the level of trophic factor expression in astrocytes, dendritic arborization, and synaptogenesis is greatly enhanced [1,11,12,14,15,44,45], as shown in Table 2 for animals with MCAo [14]. This enhancement of expression is important not only because animals with impairment may suffer from self-imposed motor impoverishment or fatigue (especially for behaviors that involve both forelimbs and hindlimbs), but also because different laboratories handle animals in ways that might differentially influence the extent of neural growth. Indeed, the Schallert lab ensures that even animals in standard housing conditions are gently handled, are very tame and familiar with the experimenters and testing conditions, and are exposed frequently to environments that encourage social and motor activity.

Possible help from pharmacology and biotechnology

In addition to complex environments, there may also be chemical ways to boost injury-related structural events in the intact hemisphere by drugs delivered even after the infarct size reaches its maximal extent. Noradrenaline and neurotrophic factors have been implicated in neuroplasticity and are among the many agents that have been investigated for possible therapeutic strategies. For example, administration of amphetamine and other agents such as FGF-2 or osteogenic protein (OP-1) that have been found to improve recovery seem to target the homotopic cortex [1,11,13,29,46] and may require physical therapy to be most effective [47]. Whether these agents provide clinically meaningful improvement in the function of the impaired forelimb has not been established. Indeed, the level of improvement so far described in preclinical studies has been small in terms of the effect on tasks that specifically evaluate the functional integrity of the impaired limbs without the assistance of the nonimpaired limbs.

Recently, Chen et al [16] reported that following MCAo, infusion of the endogenous agent, inosine, into the ventricles on the intact side of the brain induced marked neuronal growth (more than from injury alone) from the Layer V cortex opposite the infarct. Inosine levels are known to increase in the brain in response to trauma, so the authors infer that exogenous infusion of this or related agents might be enhancing normal injury-related signals for rewiring. Track-tracing methods indicated that cortical connections from the intact hemisphere to the denervated red nucleus and cervical spinal cord were unexpectedly increased. Although these connections were shown to increase slightly in untreated MCAo rats (an injury-dependent and perhaps also a behavior-dependent effect), inosine apparently greatly exaggerated the connectivity and promoted improvement of function, particularly in a reaching test [16]. About 18% of the inosine-treated animals made

attempts to use their impaired forelimb spontaneously to reach through the bars of a cage to obtain food in the Whishaw reaching test whereas no untreated rat attempted to reach [48]. Even the inosine-treated animals were successful on only 7% of their attempts, however. When the nonimpaired forelimb was restricted during the test (a bracelet prevented the animal from putting this limb through the bars of the cage to obtain food), a comparable number of untreated animals attempted to reach with the impaired limb but gradually ceased responding, whereas over the ensuing weeks half of the inosine-treated animals continued to attempt to reach and were accurate on about 18% of their attempts. (Some of these animals, however, had a head start from their previous willingness to attempt reaching when their nonimpaired forelimb was not prevented from reaching.) Future studies should ascertain whether any of the untreated animals could have achieved a comparable level of reaching success with similar levels of practice and whether inosine had any effect on general activity, alertness, or appetite. It could be argued that the level of accuracy achieved in the inosine group was not preclinically meaningful, but a number of other tests were consistent with a partial enhancement of function (eg, postural asymmetry when suspended, tongue extension, placing, and inhibitory control of the forelimb during swimming) [49-52], indicating that inosine should be explored further.

Inosine is a purine nucleoside that is synthesized from adenosine and readily enters cells to activate signaling pathways that may stimulate neuronal growth. Because environmental complexity, social behavior, and motor training also increase injury-related structural events, it would be interesting to investigate whether endogenous inosine levels are increased by manipulations that increase behavioral demand.

The possibility is often suggested that the intact cortex can be fueled by drugs to take over functions lost from the damaged hemisphere. Investigators widely disagree on what should constitute instances of vicarious brain repair, reorganization, compensation using response substitution, or easing of diaschisis. A delayed lesion of the intact sensorimotor cortex and related area has not yet been demonstrated to reverse the level of recovery in the impaired forelimb per se, as it should if one assumes that this region simply takes over function lost by the injury. The most striking outcome is that the previously nonimpaired forelimb becomes the more impaired of the two forelimbs [49,53-56].

As noted previously, in reaching tasks after single unilateral lesions the intact limb and postural adjustments combine to improve outcome, so it is essential to examine movement kinematically before concluding that improvement of function is associated with improvement in the use of the impaired forelimb. The involvement of the homotopic cortex might well reflect, at least in part, enhanced function of the nonimpaired forelimb for reaching or other motor tasks or contralesional sensorimotor integration that permits sensory input corresponding to the intact hemisphere to control motor function bilaterally.

Any drug that enhances an animal's behavioral activity might indirectly have an influence on structural changes in the intact hemisphere. A drug that would even partially restore a single component of function in the impaired limbs might begin to increase overall motor capacity or energy available and thus lead indirectly to incremental shaping of behavior toward greater motor complexity. The behavioral change, in turn, might promote structural events that would further facilitate experience-dependent mechanisms supporting recovery. Amphetamine may be one such drug, because it is known to increase motor activity and memory consolidation. It is not clear whether inosine affects motor activity. Chen et al [16] do report subtle behavioral improvements in response to the drug, so the behavioral changes might affect the structural events more than the other way around. Alternatively, the behavioral and structural events may interact and mutually enhance each other over time [13,21].

A drug-related improved capacity to use alternative strategies might be considered a worthy goal. In the clinic, however, alternative strategies can promote learned nonuse of a partially impaired limb or cognitive processes [57,58]. In clinical trials it would be important to not rely on global outcome measures, to monitor carefully the specific behaviors being changed by the treatment, and to target these changes with concurrent physical therapy.

Use-dependent dendritic arborization and the role of injury

There is considerable support for the view that reactive plasticity sometimes depends in part, or entirely, on the interaction of injury- and behavior-related signals. Many reports have already been mentioned indicating that, in addition to brain-repair mechanisms, animals readily learn to adopt alternative motor strategies, or "tricks", after injury or drugs to compensate for dysfunction [20,21,23,48,59-69]. These compensatory strategies may both drive and be derived from events in the homotopic cortex. After brain injury, dendritic arborization and synaptogenesis may be the end product of new motor learning that becomes necessary because of the injury.

Because injury to the sensorimotor cortex leaves the contralateral limb partially impaired, rats show chronic preferential use of the unaffected forelimb and hindlimb to initiate weight shift and for complex movements. It has been suggested that the nonimpaired forelimb, which largely corresponds anatomically to the intact hemisphere, is increasingly used to prevent foot slips on narrow beams, rotating poles, and grids and for exploration along vertical surfaces. When the rat is walking forward along the length of a pole that is narrow and rotating, the tail deviates laterally toward the impaired side of the body, apparently to compensate for enhanced reliance on the intact limbs for weight-bearing steps [70,71]. Transplantation interventions combined with exposure to a complex environment had no effect on speed or errors of rats negotiating this rotating pole but did improve function because the tail no longer deviated (ie, there was less need to use

the tail, presumably because there was improved function in the bad limbs). During reaching, the nonimpaired limbs become involved in compensatory postural adjustments, as revealed by high-speed kinematics analysis of reaching [48]. These motor-learning strategies in the nonimpaired limbs may contribute to synaptogenesis and task-related synapse selection in the non-lesioned hemisphere, which could solidify the motor programs. Treatments that have clinical potential should improve reaching success as well as normalizing the way an animal reaches.

Taken together, these studies would predict that structural events observed in the intact cortex are closely linked to experience. To address this idea more directly, Jones and others [21,26,33,72-74] examined motor behavior after brain damage in relation to anatomic changes in the intact cortex and then directly manipulated motor behavior at different postinjury time points. Dendritic arborization was largely prevented by partially immobilizing the good forelimb so that it could not be used for motor function during the first few weeks after injury (Table 5) [21,26,74-76].

Unlike injured rats, unoperated control rats forced to use one forelimb by the immobilization of the other limb showed no detectable increase in dendritic arborization, at least not in Layer V pyramidal neurons (Table 5). Brain injury may, in certain instances, prime surviving tissue in the homotopic cortex, thereby enabling the animal to rely more efficiently on the nonimpaired limbs, using them as a behavioral "crutch" to negotiate vertical exploration and to engage in beam or grid walking, feeding, and social interaction more efficiently [20,21,26,36,73]. This view may help in understanding why injured tissue and motor learning are needed for the structural changes to take place (Table 5), why enriched environments and motor training procedures increase the level of arborization and synaptogenesis (see Table 2), and why functional

Table 5
Neuronal growth after injury is use-dependent and injury-dependent: Layer V basilar dendritic arborization (bifurcations) of pyramidal neurons at day 18 after unilateral damage to the opposite hemisphere in ipsi-casted (intact forelimb immobilized for first 15 days), uncasted (2-holed vest), and contra-casted (impaired forelimb immobilized) rats

| Sham | Lesion | | |
|-------------------------|------------|-------------------------|---------------|
| No cast | No cast | Ipsi-casted | Contra-casted |
| 21.1 ± 0.8 ^a | 25.3 ± 0.7 | 21.5 ± 0.7 ^a | 24.1 ± 0.6 |

^a Significantly different from lesion—no cast.

Without injury, neuronal growth is not detectable even with excessive forced use. Layer V basilar dendritic arborization in noninjured rats forced to use one forelimb exclusively for 18 days by casting the other forelimb, and in uncasted rats

| Control (no cast) | Control (one casted forelimb), Hemisphere corresponding to | |
|-------------------|--|------------------|
| | Overused forelimb | Nonused forelimb |
| 20.2 ± 0.4 | 19.8 ± 0.4 | 19.0 ± 0.5 |

outcome is improved in complex motor tasks, including standard beam-walking tests. The critical site of action for amphetamine and other noradrenergic agonists, which facilitate recovery of function when administered during beam-walking tasks but not when delivered in the home cage [47], seems to be the intact hemisphere and not the injured hemisphere [46].

Revealing learned motor compensation: the ledged beam

The method of behavioral testing and its interpretation becomes a key issue because motor strategies often considerably mask a chronic impairment. Using current behavioral methods, it is difficult to observe these compensatory strategies chronically even with slow motion videometric analysis and kinematics [12,48,65]. Even after sustaining extensive damage to motor regions of the forebrain, rats may compensate so adeptly that it becomes a major enterprise to detect the deficit. Assessing hind limb function, which is a key region of infarct after MCAo, is particularly difficult.

The authors have adopted a unique approach to this problem. They developed a method of revealing hidden limb impairments in animals long after unilateral ischemic injury. A novel beam-walking task was used that seems to quantify the extent of compensation. The overall goal was to begin to disentangle brain-repair mechanisms from learned motor compensation, essentially by detecting "cheating" in rats when they are tested [10].

The primary feature of this new beam-walking task is that there are ledges along both sides of the beam. The upper surfaces of the ledges are 2 cm lower than the upper surface of the beam and provide an alternative support surface that can be used by an animal for stepping with the impaired hindlimb. As the animal traverses the beam, compensatory adjustments in posture or weight bearing in the nonimpaired limbs become unnecessary. The animal instead uses this step-down ledge to keep from slipping as it walks, effectively revealing the otherwise hard-to-detect impairment in the limbs contralateral to the brain injury. An additional feature is that the beam is wide at the starting point and tapers gradually to a narrow end near the goal, challenging the animals with varying levels of difficulty.

The authors have consistently found that, without the ledge, animals with unilateral infarcts caused by MCAo can gradually learn to keep the impaired limbs from slipping as they traverse the tapered beam. At first they might use an odd, sidestepping pattern; later they might use a less-awkward pattern of walking that involves tail deviation and subtle limping or hopping movements efficient enough to allow the animals to show little or no deficit. These motor patterns are difficult to detect and are nearly impossible to quantify, but based on their endpoint performance, the animals seem to have "recovered." In a similar time frame, impaired animals exposed to a tapered beam with the support ledge display an easily measured deficit in their impaired limbs up to 450 days after injury (the limit of the authors'

investigations). Whenever the ledge is removed, the animals quickly learn that the ledge is missing and begin to adapt by walking down the beam without slipping. Each rat adopts its own idiosyncratic motor strategy. This ability to walk along the beam does not mean, however, that the animals are recovered. When the ledge is returned to the beam, the animals learn that it is there and, unlike normal animals that rarely use the ledge for stepping, the injured animals readily begin to re-use the ledge by stepping down with the contralesional hindlimb (and, at the narrow end, the contralesional forelimb). Thus, the learned tricks for compensating for a motor deficit are unmasked by the ledge. That is, the animals no longer are required to use their tricks because the ledge is available as a crutch, and its use can easily be quantified (eg, in terms of the percentage of contralateral versus ipsilateral ledge-dependent steps relative to the total number of steps taken by the animal as it traverses the beam).

The injured cortex after unilateral brain injury

Use-dependent exaggeration of injury

Forced physical therapy of the impaired forelimb (by constraining the nonimpaired forelimb) beginning immediately after cortical (but not striatal) injury has been found to be detrimental to both surviving neural tissue and subsequent functional recovery [11,33,77]. The nonimpaired forelimb was immobilized in a plaster of Paris cast, and remained immobilized for 1 to 2 weeks, forcing the animal to rely exclusively on the impaired forelimb. It was expected that this intense, early, targeted therapy would improve function in the impaired forelimb, so that the rats would not need to rely on the nonimpaired forelimb, and would enhance arborization of dendrites in the peri-injury area. This manipulation, however, damaged otherwise survivable tissue (Table 6) [33]. When forced overuse was delayed until the second week after injury, however, the injury did not become worse [77,78].

Coulbourne (F. Coulbourne, unpublished data, 2002) found that visual cortex injury is not exaggerated by forced forelimb use (visual-stimulation therapy was not tested), whereas cortical lesions in the forelimb area double in size. These and other data suggest that use-dependent exaggeration of

Table 6
Use-dependent exaggeration of sensorimotor injury: Forced use of the impaired forelimb (by constraining the nonimpaired forelimb) during the first 7 days (early cast) but not during the second 7 days (late cast) caused delayed loss of tissue

| Sham | Lesion (no cast) | Lesion (early cast) | Lesion (late cast) |
|-----------|------------------------|------------------------|------------------------|
| 256 ± 1.2 | 228 ± 2.0 ^a | 210 ± 1.9 ^b | 227 ± 1.8 ^a |

^a Significantly less than sham.

^b Significantly less than lesion (no cast).

Data are from Stereologic analysis of volume of remaining tissue (in mm³) ± SE.

injury is not caused by stress-related steroid hormones, temperature, or other systemic influences, at least not without a complex interaction with behavior-related events acting near the site of injury.

Mild reach training, which prevents loss of the motor map after ischemic injury to the paw area in rats and monkeys [22], causes a transient loss of the motor map and impairment in reaching success (J.A. Kleim, unpublished data, 2002). It seems possible that the early reach training, which was not excessive, reversibly taxes the peri-infarct tissue, although ultimately there is improved function and expansion of the motor map controlling the forelimb. Although behavioral and neural plasticity has been reported for constraint-induced training of the impaired limb experimentally and clinically [22,57,58,79,80], and forced resting of the impaired limb can retard recovery and neural plasticity [1,13], intense rehabilitation strategies targeting the impaired hemisphere should be used cautiously for a certain unknown period immediately after the injury to obtain maximal effectiveness from constraint therapy. In the clinic, small gains in function associated with intense early therapy would by no means guarantee that tissue was not damaged. Indeed, potential functional gains might be offset by tissue damage or might become vulnerable to stressors chronically. Also, the tests selected to evaluate the effects of therapy might not be sensitive to some of the extended damage because the areas of extended damage may have unique functions not targeted by the tests used.

Jones and colleagues began acrobatic training in cortically injured rats 2 days after the lesion [19]. The training was carried out in a gradual manner, beginning with light training on the easier acrobatic tasks in the battery of tasks eventually given. The training did not exaggerate injury; it increased the number of synapses formed per Layer V neuron in the homotopic cortex and enhanced functional recovery. Apparently, gradual increases in motor training can be effective even when administered soon after the injury.

Use-dependent effects in a parkinsonian model

A feature of Parkinson's disease shared by animals treated with 6-hydroxydopamine (6-OHDA) or other neurotoxins that mimic the loss of dopamine cells in the substantia nigra is a reduction in motor activity in the affected limbs. Even in the early stages of the disease and with only partial loss of dopamine cells in the animal models' physical activity declines as a function of level of degeneration [81-83]. The authors have found that physical activity continuously imposed during the first week after exposure to neurotoxic levels of 6-OHDA improves motor ability and protects the dopamine cells in animal models [1,11,84]. The nonimpaired forelimb was constrained during this period to force forelimb use during the period of degeneration. The cells that are protected by physical therapy remain vulnerable to later decreases in motor activity, however [85]. In the clinic, physical activity for parkinsonian patients can be beneficial [77,86-91,95].

The authors also found that forced restraint of the impaired forelimb (forced rest) in animals with mild subclinical doses of 6-OHDA for the first 7 days after neurotoxin exposure exaggerates neurochemical damage and chronic dysfunction [85].

The implication is that dopamine cells might be salvageable if degeneration is detected early, but the neuroprotection is fragile. That is, motor function must continue after exposure to neurotoxins despite the apparent normal integrity of the nigrostriatal system. In the animal models, it is likely that after early physical therapy the animals sustain motor function at a sufficiently high level to retain integrity of the nigrostriatal cells because of the neuroprotection conferred by continued use. To the extent that the animal data are relevant, it might be important for people in the premorbid stages of parkinsonism to avoid slipping into a sedentary lifestyle after the completion of rehabilitative therapy to avoid the risk of reinvigorating the degenerative process. Parkinson's disease typically begins during later years of life when the impetus to move might be reduced even in normal people. It is not surprising, therefore, that in animal models the aging process can reinstate recovered deficits sustained during young adulthood unless motor enrichment procedures are used [65].

The role of use-dependent increases in the expression of neurotrophic factors in parkinsonian models is being explored for their possible contribution to the beneficial effects of forced use of a forelimb on neurochemical and behavioral outcome. In sham-operated animals, forced forelimb use increases FGF-2 bilaterally and increases glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) in the hemisphere corresponding to the overused forelimb [92,93]. In addition, physical exercise and motor training can increase neurogenesis, angiogenesis, and other plasticity-related events [15,94].

Summary

Over a century ago the intact cortex was proposed to contribute to recovery from unilateral brain injury, but its possible role in functional outcome has become more appreciated in recent years as a result of anatomic, metabolic and behavioral studies. Although use of the contralesional limb is naturally impaired after sensorimotor cortex injury, neural and astrocytic events in the intact hemisphere may give rise to, and may be influenced by, an enhanced ability to compensate for lost motor function. The debate is still open as to whether the neural changes are generally compensatory in nature, with activity in the homotopic cortex leading to greater capability in the nonimpaired limb, or whether they are actually a matter of reorganization in the homotopic cortex leading to connections to denervated targets in the opposite hemisphere, thus allowing the homotopic cortex to control motor programs there. Although both phenomena may occur to some degree, there is mounting evidence in support of the former view. Careful

behavioral techniques have been developed that can expose compensatory tricks, and the time course of these behaviors correlates well with anatomic data. Moreover, if the intact cortex sustains a second lesion after recovery from the first, forelimb sensorimotor function specific to the first-impaired side of the body is not worsened.

Partial denervation of callosal fibers coming from the injured hemisphere, plus preferential use of the good forelimb caused by a cortical injury, may increase trophic factors in the intact hemisphere. These and related events seem to provide a growth-favorable environment there that permits motor learning in the intact forelimb at a level of skill exceeding that which a normal animal can attain in the same period of time. There are anecdotal cases in human neurologic patients that are consistent with these findings. For example, a colleague of the authors who sustained a unilateral infarction that rendered his dominant right hand severely impaired noticed that soon after the stroke he was able to use his left hand for writing and computers as well as he had ever used his right hand.

Cross-midline placing tests also indicate that the structural events observed in the intact cortex may potentiate projections to the damaged hemisphere. These changes may help restore the capacity of tactile information projecting to the intact hemisphere to control limb placing in the impaired forelimb.

Neural events in the injured hemisphere can be affected by behavior differently than the neural events in the intact hemisphere. Different therapeutic strategies might well be used on opposing limbs at different times after unilateral sensorimotor cortex injury to optimize recovery (and, indeed, to avoid exaggerating the insult). Finally, the details of reorganization in both hemispheres differ greatly depending on the type of brain injury sustained (eg, in stroke versus Parkinson's disease), suggesting that an approach that considers the role of both hemispheres is likely to be beneficial in research on a broad variety of brain pathologies.

Acknowledgments

The authors thank Ted Lin, Jitsen Chang, Deanna Adkins, and Theresa Jones for their contributions to this work.

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