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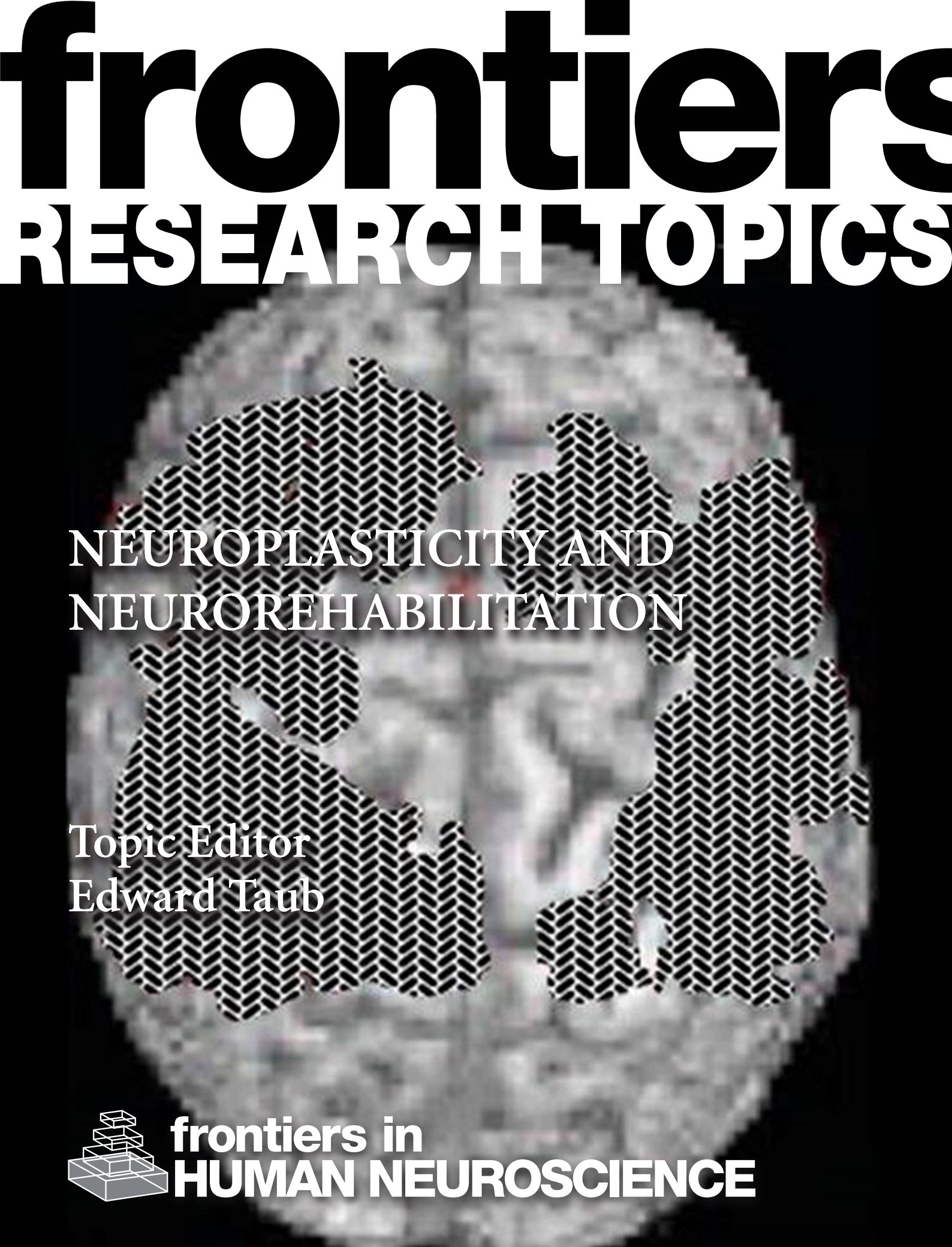

NEUROPLASTICITY AND NEUROREHABILITATION

Topic Editor
Edward Taub

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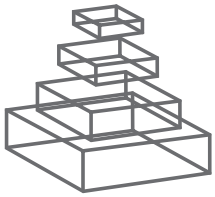

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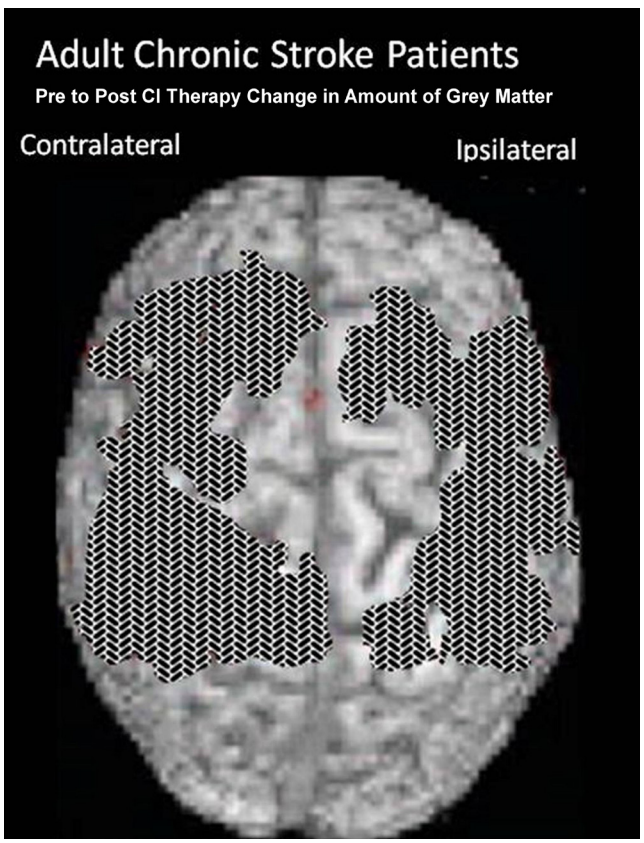
NEUROPLASTICITY AND NEUROREHABILITATION

Topic Editor:
Edward Taub, University of Alabama at Birmingham, USA



Cortical surface-rendered image of gray matter change after CI therapy in adults with chronic stroke. Gray matter increases displayed on a standard brain. Surface rendering was performed with a depth of 20mm. Cross-hatched areas indicate t statistics ranging from 2.0 to 6.7. Corrected for family-wise error.

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EDITORIAL
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Foreword for neuroplasticity and neurorehabilitation

Edward Taub*

Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA
**Correspondence: etaub@uab.edu*

Edited and reviewed by:
Hauke R. Heekeren, Freie Universität Berlin, Germany

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For much of the twentieth century, the views concerning the plasticity of the mature mammalian central nervous system (CNS) and the possibility of improvement in functional impairments in the chronic phase following substantial damage to the CNS were fixed and rarely questioned. The potential for plastic CNS change was thought to be confined to the immature organism, but in the adult the structure of the brain and spinal cord was believed to be hard-wired and unchanging no matter rehabilitation or environmental influence was applied after CNS damage. This belief was perhaps most prominently stated by Ramon y Cajal (1928), but its origin can be traced to very near the beginning of the scientific study of the nervous system, as embodied in the work of Louis Broca on the anatomic localization of motor function in the brain (Broca, 1861). Alternate views were periodically expressed (Fleurens, 1842; Fritsch and Hitzig, 1870; Munk, 1881; Lashley, 1938), but they were very much in the minority. An important experimental challenge to the standard view of the structural immutability of the CNS came in the discovery by Liu and Chambers (1958) of collateral intraspinal sprouting of dorsal root axons after spinal cord damage. Subsequent work by Goldberger (1977) and Goldberger and Murray (1974) confirmed this finding and subsequently it was demonstrated that sprouted fibers establish synaptic connections in the brain as well (Raisman, 1969; Raisman and Field, 1973; Tsukahara et al., 1975). These findings engendered considerable interest in terms of their potential relevance to recovery from spinal cord injury. However, for one-quarter century after the discovery of collateral intraspinal sprouting, there was no clear demonstration that its occurrence in the brain could have important significance for the motor or sensory function of an adult mammal. There was thus no compelling evidence-based reason for altering the prevailing view of an anatomically fixed brain.

In the field of rehabilitation, the older view concerning the limited amount of recovery of function that was possible in the chronic phase after CNS injury also had the status of axiomatic belief, which, if anything, was even more firmly held. There was a general opinion that rehabilitation treatment before the end of the spontaneous recovery period could accelerate its progress and perhaps even elevate its final level somewhat in the case of motor function, but there was no consensus for the latter belief and there was also no credible, controlled evidence that this was possible. In fact, powerful evidence to the contrary was part of the practice of every physiatrist, neurologist, and rehabilitation therapist. The process of spontaneous restitution of function was routinely observed to proceed with progressively decreasing speed until a plateau was reached, and subsequently additional recovery did not occur no matter what rehabilitation method was employed. This observation was universal and its implied principle of there being a barrier to improvement in function in the chronic phase after CNS injury was viewed as being self-evident.

The belief in the lack of potential for rehabilitative change long after CNS damage and the lack of plasticity in the mature mammalian brain, when these subjects were considered together at all, were thought to be reflections of one another. One implied and seemed to confirm the other. Hughling Jackson's hierarchical view that lower centers of the brain, capable only of providing the basis for impaired performance, substituted in function for higher damaged centers after CNS insult (Jackson, 1873, 1884) and other similar formulations powerfully influenced thought for most of the twentieth century. However, in the 1970s several investigators, Wall (Wall and Egger, 1971; Dostrovsky et al., 1976) among others, obtained findings that they interpreted as indicating that environmental influences, including training, could induce plastic change in the injured brain. These conclusions were preliminary. The experimental breakthrough came in the work of Merzenich (Merzenich et al., 1983, 1984; Jenkins et al., 1990) and Kaas et al. (1983) and their co-workers in the 1980s and early 1990s. There was a comparable development in the field of neurorehabilitation. In 1993 a paper (Taub et al., 1993), based on several decades of basic research on somatosensory deafferentation in old world monkeys (Taub, 1977, 1980), reported on a rehabilitation procedure, termed Constraint-Induced Movement therapy or CI therapy, that could produce substantial improvement in upper extremity motor function in humans many years after stroke. It was later found that CI therapy produced substantial functional (Liepert et al., 1998, 2000; Kopp et al., 1999) and structural (Gauthier et al., 2008) changes in the brain. These findings overturned the classic views on the unmodifiability of the CNS after damage and the unmodifiability of functional deficits persisting into the chronic phase after brain damage. The papers in this collection describe the subsequent findings in the field of neuroplasticity and neurorehabilitation. Many of the investigators who have made the key discoveries in these fields are the senior or co-authors of these papers. Persuasive evidence has been accumulating at a rapidly accelerating pace that the two areas are closely related. For the future, the newly developed areas of research based on these observations hold great promise for arriving at fundamental discoveries on the potential for plastic change in the damaged nervous system, how this can be produced by behavioral training, environmental influences, and other extrinsic

manipulations, and how these procedures can be employed to effect much greater recovery of function of CNS damage than had previously been thought to be possible.

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Brain plasticity-based therapeutics

Michael M. Merzenich[1]*, Thomas M. Van Vleet[1,2] and Mor Nahum[1,3]

[1] *Posit Science Corporation, San Francisco, CA, USA*
[2] *Medical Research, Department of Veteran Affairs, Martinez, CA, USA*
[3] *Department of Optometry, University of California at Berkeley, Berkeley, CA, USA*

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Etienne De Villers-Sidani, McGill University, Canada
Christopher I. Petkov, Newcastle University, UK
Gitendra Uswatte, University of Alabama at Birmingham, USA

***Correspondence:**
Michael M. Merzenich, Brain Plasticity Institute at Posit Science Corporation, 77 Geary Street, Rm. 303, San Francisco, CA 94108, USA
e-mail: mike.merzenich@positscience.com

The primary objective of this review article is to summarize how the neuroscience of brain plasticity, exploiting new findings in fundamental, integrative and cognitive neuroscience, is changing the therapeutic landscape for professional communities addressing brain-based disorders and disease. After considering the neurological bases of training-driven neuroplasticity, we shall describe how this neuroscience-guided perspective distinguishes this new approach from (a) the more-behavioral, traditional clinical strategies of professional therapy practitioners, and (b) an even more widely applied pharmaceutical treatment model for neurological and psychiatric treatment domains. With that background, we shall argue that neuroplasticity-based treatments will be an important part of future best-treatment practices in neurological and psychiatric medicine.

Keywords: brain plasticity, neuroplasticity, computerized training, aging, hemispatial neglect, schizophrenia

BACKGROUND

In the evolution of treatments of neurological and psychiatric impairments and illness, mainstream medical science has followed two broad paths. One originated with the early Twentieth Century discovery of pharmaceutical agents demonstrated to have powerful, distorting impacts on human neurology (Perrine, 1996; López-Muñoz and Alamo, 2009). Especially from about the middle of the Twentieth Century onward, drug-based medicine has been increasingly strongly supported by technically-sophisticated fundamental neuroscience, which has struggled mightily to describe and define neurological processes and diseases in specific chemical terms, on the path to their chemical manipulation for medical advantage.

The second path, emerging across the same era, began with the insights and discoveries of behavioral scientists and clinicians, who rapidly demonstrated that behavioral abilities could be beneficially modified in patients in need of behavioral adjustment or correction (Boring, 1929; Reisman, 1991). Their cognitive-behavioral therapies have been empirically elaborated in a myriad of ways, to address different levels and aspects of the panoply of symptoms expressed in neurological and psychiatric impairment and disease.

Into the present era, legions of medical professionals predominantly deploy one or the other of these two classes of therapeutic tools to address, in very different ways, the hundreds of neurological and psychiatric disorders that fall within their clinical purview. Both groups see one another as providing an incomplete treatment model. The cognitive (or physical or speech or talk) therapist attempts to correct the distorted expressions of behavior that can so obviously limit the performance abilities of the patient in treatment. Extending a long empirical tradition elaborated by Freud and extended by many others, therapists often also attempt to understand masked biographic neurobehavioral distortions that may still be contributing to current dysfunctions. In another form of treatment, cognitive therapists define the patient's behavioral weakness or limitation as a direct target for correction. If the patient has a negative mood, for example, the therapist works with the patient to improve it via various behavioral strategies; the patient's primary symptom is the direct focus of treatment. If the patient in front of them has a failing memory, to cite another example, the patient is trained to remember—or trained in ways that help them cope with their failing memory. Professionals of a more reductive and chemical persuasion find these strategies to be superficial and necessarily limited by nature. "How can the primary functional expression of a disability or illness be regarded as its cause? How can you expect behaviorally guidance or training to restore a physically and chemically wounded or functionally altered brain in ways that address the underlying causes of impairments or diseases? By what processes can all of that required, detailed chemical and connectional healing occur?"

Their primary answer for addressing those fundamental faults has been the chemical drink or cocktail designed to rebalance or correct or attenuate already-distorted brain chemistry. The behavioral clinician sees such approaches as necessarily crude and limited for addressing the complex neurobehavioral distortions that frustrate the patient in treatment—which, of course, they are. "Treat a patient with a wounded or dysfunctional brain by chemically re-distorting it? How can that provide correction in the key deficits underlying the disorder, when hundreds of chemical processes have been altered as a consequence of the wounding or the disease?" To the behavioral therapist, the exaggeration of the imagined sophistication of the neuro-pharmacological treatment

of disease is perhaps only matched by the magnitude of its actual crudity, in neurobehavioral terms.

Beginning about four decades ago, a third vision began to emerge (see Merzenich, 2013, for review). Studies in neuroscience began to elucidate, in progressively more complete form, the neurological origins of behavior. Those studies have now provided us with a first-level understanding of the rules of the processes that govern brain change, both as they account for a progression of the brain in a degrading, "aging," or distorting—or a strengthening, "rejuvenating," or corrective neurological direction. This science has also elucidated, in neurological terms, a number of important "failure modes" of the self-organizing brain that have long been given medical labels, like "depression" or "schizophrenia" or "oppositional-defiance disorder" or "Alzheimer's Disease" (see below, and Merzenich, 2013).

Importantly, after a Century of empirical studies by behaviorists trying to understand the origins of neurobehavioral limitations or distortions in humans with neurological impairment or illness, the explosively developing scientific domains of "integrative neuroscience" and "cognitive neuroscience" began to document, with increasing clarity, how and why emergent brain system alterations—expressed by plasticity itself—appear to account for functional human degradation, failure, and disaster. This science has also revealed, with increasing clarity, how limited or distorted neurological processes could be driven, via those same plasticity processes, in strengthening and correcting directions.

Because this evolving science provides a more complete understanding of the origins of—and potentially effective treatment modes for—neurological impairment and illness, we believe that it shall evolve into a foundation science for neurological and psychiatric medicine. Here, the goal is to describe the state of its current scientific development, as a platform for describing what steps can be taken to bring the rapidly developing scientific field of brain plasticity-based therapeutics into medical reality. Our brief review of core principles of neuroplasticity is followed by several practical examples of how the translation of this neurological (and behavioral) science is optimized for therapeutics.

THE SCIENCE OF NEUROPLASTICITY

Studies conducted principally over the past 40 years have allowed us to collectively establish the following principles of neuroplasticity:

THE BRAIN IS CONTINUOUSLY PLASTIC

Not so many years ago, mainstream neuroscience and neurological medicine contended that plasticity was limited to an early childhood epoch—a "critical" or "sensitive period." We now know that brain remodeling can be induced on a large scale at any age in life (see Swain and Thompson, 1993; Merzenich and de Charms, 1996; Merzenich, 2001, 2013; Weinberger, 2004; Gilbert et al., 2009). What differs as a function of age is the way in which the brain regulates plasticity. In the very young brain, almost all inputs continuously engage competitive plasticity processes. In older brains, plasticity is regulated as a function of behavioral context and outcomes.

IN THE OLDER BRAIN, A CONTEXT- AND OUTCOMES-DEPENDENT RELEASE OF NEUROMODULATORS FROM SUBCORTICAL LIMBIC SYSTEM NUCLEI ENABLE AND TRIGGER BRAIN CHANGE

In the perinatal and early-childhood "critical period," plasticity-enabling conditions are always "on." In the older child and adult brain, changes in the control of the release of "neuro-modulatory neurotransmitters"—and in the properties of the receptors in the brain that govern their actions—enable the older brain's moment-by-moment control of change; it is permitted *only* when the specific contextual conditions that enable or trigger plasticity are met, with changes arising under those special contextual-enabling conditions "saved" (driving enduring changes in connection strengths) as a function of behavioral outcome (e.g., see Merzenich, 2001, 2013 for reviews).

For example, under conditions of focused attention, any stimulus excites acetylcholine (ACh) releasing neurons in the basal nucleus of Meynert (Richardson and DeLong, 1990; Sarter et al., 2001, 2006). In the cortex, ACh inputs positively enable plasticity by (a) selectively amplifying only anticipated ("selectively attended") and (b) selectively weakening non-anticipated inputs—including those at any given cortical location that may have most effectively excited neurons before learning-induced changes were initiated (Sarter et al., 2006; Froemke et al., 2007). By this action, brain circuits enable plasticity by advantaging input strengths for those specific activities that the brain can gain in ability by changing to, and disadvantage behaviorally non-contributing inputs that they shall change from.

As a second example, noradrenaline (NA) releasing neurons in the locus coeruleus (LC) (and in nucleus accumbens and amygdala) broadly amplify neuronal activity, increasing the general level of excitability (arousal, or baseline level of attention) in subcortical and cortical structures in any closely-attended context (for example, in stimulus- or goal-seeking or other "motivated" states) (see, e.g., Aston-Jones and Cohen, 2005; Sara, 2009; Sara and Bouret, 2012). NA is also released to selectively amplify the activities evoked by unexpected (novel) input (Aston-Jones et al., 1999), conferring special powers for the representation of "surprising" inputs or activities for driving enduring representational change.

Dopamine (DA) releasing neurons in the ventral tegmental area and substantia nigra are highly specific plasticity enablers (see, e.g., Bao et al., 2001, 2003; Winder et al., 2002; Lisman et al., 2011). They are excited when the brain receives—or first predicts the occurrence of—a hedonic input (reward), or when the brain achieves or first predicts behavioral success (for which it "rewards itself") in a learning cycle (Schultz, 2007). With their release, inputs that "predict" that reward (i.e., are highly correlated with its occurrence) are selectively strengthened; competitive inputs uncorrelated with reward prediction arriving in a short post-reward epoch are selectively weakened (see Ahissar et al., 1992; Bao et al., 2003).

We now have a first-level understanding of the "rules" that control the release and the actions of these (and other) neuromodulators in learning, and of the modulator-specific ways that they nuance brain changes in experience and learning.

It should be noted that this crucial neuromodulatory machinery, controlling learning and memory abilities throughout life, is

also plastic (Nakamura and Sakaguchi, 1990; Sara and Segal, 1991; Steiner et al., 2006; Smith et al., 2011; Zhou et al., in review). The strengths, selectivity, and reliability of its actions can be significantly improved via intensive training in most individuals with neurological or psychiatric impairment or disability.

MANY ASPECTS OF THE NEUROLOGICAL REPRESENTATIONS OF INPUTS AND ACTIONS CAN BE MODIFIED BY APPROPRIATE NEUROBEHAVIORAL TRAINING

In early studies of plasticity processes, we and others conducted studies designed to reveal which aspects of the representations of inputs or actions could be improved by training, under the right contextual conditions, in the adult brain (see Merzenich, 2013, for review). It was quickly shown that we could change the selectivity of neuronal responses (i.e., receptive field sizes); the memberships of competing populations of neurons ("mini-columns;" Buxhoeveden and Casanova, 2002) that represent those selective inputs; the detailed representation of stimulus magnitudes; stimulus modulation rates; successive-signal segmentation and integration ("masking;" "sampling rate"); stimulus duration and inter-stimulus interval resolution and estimation; spectrotemporal or spatiotemporal stimulus complexity; stimulus sequencing; stimulus source location or identification; signal-to-noise conditions for stimulus representation, and response reliability—among other parameters of inputs (see, e.g., Merzenich and de Charms, 1996; Gilbert et al., 2009; de Villers-Sidani et al., 2010; Merzenich, 2013). In the domain of action, we could similarly drive improvements in response reliability; response speed; replication of timing in responding; response accuracy; response sequence reconstruction; and response fluency; among other parameters of action control. Many other scientists have extended these studies to demonstrate plasticity in other perceptual, working memory, associative memory, selective attention, sustained attention, distractor suppression, among other functional neurological abilities.

It should be noted that these studies have also shown that all of these same (and many other) aspects of the neurological representations of inputs and actions can be driven by training, just as easily, in a degrading direction (e.g., see Merzenich and Jenkins, 1993; Zhou et al., 2011)—again by the action of normal brain plasticity processes.

THE PRIMARY PLASTIC CHANGE IS IN THE STRENGTHS OF CONNECTIONS (SYNAPSES) IN BRAIN CIRCUITS

Neuroplasticity research has extensively documented the phenomenology of—and the cellular and molecular processes underlying—the plastic remodeling of the "wiring" in brain circuits. The central governing rule was postulated by the Canadian psychologist Donald Hebb in the 1940's: "What fires together, wires together" (Hebb, 1949). This coincident-input-dependent co-strengthening of synaptic connections occurring moment by moment in time in a learning context is achieved through both a multiplicity of physical changes in synapses that amplify connection strength, and by synaptogenesis. The magnitude of such changes under near-optimum learning contexts can be remarkable: a large proportion of synapses in any directly engaged cortical zone (commonly, many millions to billions of synapses) are altered in their connection strengths as you acquire any significant skill or ability (e.g., Kleim et al., 2002). As we master any skill or ability through experience or progressive learning, these changes in brain circuitry result in the specialization of the brain as a master receiver and master controller of all of the inputs and actions supporting that mastery.

Nonetheless, the same processes that confer growth in synaptic power for inputs that contribute to neurobehavioral advance are also driven backward, for other non-behaviorally-contributing synapses, in a synaptic weakening and synapse elimination direction (see below). This "normalization" of collective synaptic input power has been extensively studied in other experimental models by depriving neurons of a major source of their inputs; in that event, synaptic strengths are rapidly adjusted to sustain neurons within a narrow electrical potential window that assures their ongoing functional viability (Horng and Sur, 2006; Cooper and Bear, 2012; Feldman, 2012).

PLASTICITY CONTROLS FUNCTIONAL RELIABILITY VIA ITS GENERATION OF NEURONAL COOPERATIVITY

Through Hebbian network plasticity, the extensively cross-wired neurons in the cerebral cortex also strengthen their connections with their nearest neighbors. When the brain is engaged behaviorally, inputs that are activated nearly simultaneous in time strengthen together, increasing their cooperativity to generate more salient (i.e., more collectively powerful, more reliable) responses. That plasticity-driven growth in local "teamwork" is a critical aspect of the improvement in the processing of information supporting learning-based advances in behavior (see Edelman, 1987; Merzenich and Jenkins, 1993; Merzenich and de Charms, 1996; Merzenich, 2013).

Learning-driven increases in neuronal response coordination are a primary determinant of the feed-forward power of any plastically strengthening cortical process. Cortical neurons at all "higher" system levels are integrators operating with short time constants. Their plasticity processes are also coincident-input dependent. The greater the coordination of neurons in the lower levels of the network that feeds them, the greater their selective powers and selectivity, and the greater the power of that input to drive plastic remodeling at higher system levels. Moreover, at the "top" of our great brain systems, coordination of activity is a primary determinant of the ability of cortical networks to sustain the reverberant activities that are selective for behavioral targets or goals (i.e., working memory) (see Wang et al., 2004; Compte, 2006). The strengths of these key plasticity-gating processes "at the top" are crucially dependent upon the strengths, i.e., collective coordination, of the inputs that feed them.

NEUROPLASTICITY DRIVES CHANGES THAT BROADLY REMODEL THE PHYSICAL BRAIN

Changing synaptic strengths and synaptogenesis involves complex physical change processes resulting from changes in genetic expression of several hundred well-described molecular processes. At the same time, there are many other physical changes induced by brain plasticity processes, collectively involving several *thousand* known molecular processes. The physical processes of neurons (the receiving "dendrites" and their synaptic "spines;"

the transmitting "axons" and the elaboration of their terminal arbors; the distributions of collateral axons that richly interconnect neurons within cortical networks; the processes and cell-to-cell contacts of closely coupled non-neuron glial cells) can be plastically altered on a large scale, resulting in changes in cortical thickness, neuropil volumes, and cortical area and subcortical nucleus volumes (see Merzenich, 2013, for review). Specific cell types can shrink or greatly expand in size, and can be greatly metabolically reduced or invigorated—all expressed through easily-documented, controlled, plastically-induced physical change. The insulating myelin can be thickened—or thinned—under plastic control (de Villers-Sidani et al., 2010; Zhou et al., 2012). Chemical factors controlling the health and vigor and operational characteristics or brain systems, or contributing to the regulation of plasticity itself—including "trophic factors," transporters, excitatory, inhibitory and neuromodulatory neurotransmitters and receptors are all altered physically, when the brain advances, or retreats, by the action of adult neuroplasticity processes (Merzenich, 2013).

BRAIN SYSTEMS ACCOUNT FOR OUR EXPLICIT BEHAVIORS

A large body of science has now shown that our expressive behaviors are a product of complex, multi-level recurrent networks (for further discussion and review, see Merzenich, 2013; Nahum et al., 2013c). In these networks, information is represented with greatest resolution in detail in place, feature, and time at lowest network ("system") levels. At successively higher levels, there is an integration of representation to progressively more complex objects, relationships and actions, as they apply in the "real world." At the "top" of brain systems, those most-completely-integrated neurological representations generate enduring neural activity that is selective for their representation. That persistent reverberant activity, providing the neurological basis of working memory, can be sustained in the human brain for tens of seconds to minutes of time (see Goldman-Rakic, 1995; Compte, 2006; Merzenich, 2013). It is important to understand that representational information is continuously fed backward from this highest (and from all other) level(s). In these recursive recurrent networks, the operational levels contributing to the representation of any aspect of input or action in brain systems are inseparable; in other words, all explicit behaviors are a product of the *system*. Therefore, when evident behaviors are distorted or impaired, as they are in the many ways that define the fundamental deficits and nuances of different specific neurological and psychiatric clinical indications, we necessarily target neurological renormalization at all system levels when designing therapeutic training programs.

IN A BRAIN SYSTEM, PLASTICITY IS CONTROLLED "FROM THE TOP"

Recent neuroscience studies have shown that through recursive re-entrant feedback (see Edelman, 1987; Grossberg, 2013; and Merzenich, 2013, for review), the representation of information "at the top" of our forebrain processing systems selectively enables plastic changes contributing to the progressive behavioral success of brain systems (see Hochstein and Ahissar, 2002). At highest system levels, behavioral targets are held, as described, via sustained target-specific activities, in working memory. That sustained persistently reverberant activity is projected backward down to "lower" system levels, where it positively enables plasticity for any fed-forward activity that can potentially contribute to a progressively improving resultant. Scientists often call the opening of this window that controls, through this top-down biasing, what the brain can change to, a "selective attention" process. In fact, "working memory" and "selective attention" can be considered to be two descriptors of the same persistent reverberant activity-based representation/feedback process (see Fuster, 2008). This process also provides the neurological basis of the brain's predictive, associative memory, sequencing construction, and syntactic powers.

The neurological processes by which feedback "from the top" biases plasticity in learning at all lower network levels are now understood, at a first level. Biasing is achieved, neurologically, by dis-inhibition processes in cortical networks controlled by convergent modulation "from the top" on the one hand (through a selective attention process), and from a cholinergic subcortical input source engaged under conditions of focused attention, the basal nucleus of Meynert (see Sarter et al., 2001, 2006; Froemke et al., 2007; Weinberger, 2007; Carcea and Froemke, 2013; also see Zhou et al., 2010), on the other hand.

Ahissar and Hochstein (Hochstein and Ahissar, 2002; Ahissar et al., 2009) have described this feedback plasticity-enabling biasing, in psychological science terms, as "the reverse hierarchy theory." According to this perspective, the brain holds a model of a behavioral event or training goal in working memory; that model, fed back to lower system levels, selectively amplifies activities (through dis-inhibition) that the brain can change *to*, as it progressively sharpens and refines, through learning, the resultant—its working memory-sustained models.

PLASTICITY ENGAGES BOTH SYNAPTIC STRENGTHENING AND SYNAPTIC WEAKENING PROCESSES

Fundamental studies of plasticity mechanisms have shown that every brief change cycle invokes a synapse-strengthening moment (e.g., strengthening all inputs whose coordinated actions moment by moment in time are correlated with a positive behavioral outcome), followed by a synapse-weakening moment (e.g., weakening all inputs occurring within a brief, following epoch of time) (Dan and Poo, 2006; Cooper and Bear, 2012). As noted earlier, this synapse weakening can be viewed as an electrically homeostatic process that contributes to the ongoing weakening of behaviorally non-meaningful intrinsic activities or inputs—that is, to a normalization of internal or background external (environmental) noise.

Viewed from another perspective, plasticity processes can be viewed as continuously competitive. Through these two-way plasticity processes, neurons in coupled "mini-columns" are continuously competing with their neighbors for the domination on neurons on their mutual boundaries (see Merzenich and Jenkins, 1993; Merzenich, 2013). By giving one coupled group the competitive advantage over their neighbors, it is easy to expand their team a 1000-fold—or, if they are a competitive loser, to reduce its "membership" many times over. By giving any one source of input a competitive advantage or disadvantage, the

territory it comes to dominate in the brain can be dramatically enlarged, or contracted; every moment of gain for the "winner" is a moment of loss for "losers." By these two-way processes, one can easily both refine (for some inputs) and degrade (other inputs)—even the most-fundamental aspects of representation of visual or auditory or somatosensory inputs in the adult brain.

AT LEAST MOST (POSSIBLY ALL) PLASTICITY-INDUCED CHANGES ARE, BY THEIR NATURE, REVERSIBLE

Plasticity engages fundamentally reversible neurological change processes. We have conducted a number of studies that have demonstrated that neuroplasticity follows Hebbian principles: the representations of inputs and actions are competitively sorted on the basis of the temporal distributions of inputs (Merzenich and Jenkins, 1993; Merzenich and de Charms, 1996). Following these principles, it is just as easy to degrade the brain's processing abilities as it is to strengthen or refine them. In the designs of therapeutic training regimes, the Hebbian "rule" must be considered, to assure that training-driven changes are always in the positive, strengthening, recovering, re-normalizing direction.

We have recently conducted a number of studies in animals that show that plasticity processes are very broadly reversible. For example, after documenting many aspects of the function, anatomy and chemistry in the brains of aged vs. young adult animals, it was shown that every measure differed markedly (de Villers-Sidani et al., 2010; de Villers-Sidani and Merzenich, 2011; Mishra et al., under review). In the aged rats' auditory cortices, time and space constants were longer and greater; response selectivity was poorer; reliability of sound feature representation was poorer; response correlation was weaker; the neuron populations representing sensory inputs were less strongly coupled, operating with far weaker cooperativity; inhibitory processes controlling "top-down" modulation were weaker; local and long range connections were more poorly myelinated; level-to-level (system) coordination (in gamma and theta frequency ranges) was less sharply localized and more weakly persistent; representational topographies were degraded; trophic factors contributing to metabolic and physical maintenance and plasticity were only weakly expressed; the normally strong adaptation to repeated identical stimuli and responses to unexpected stimuli against a continuous or repeated background were sharply reduced; the strong suppression of non-attended distractors was reduced; receptor subunits for inhibitory and excitatory processes were altered in a degrading direction; and the modulatory control processes controlling plasticity were all more weakly operating in very old vs. prime-of-life animals. After recording these manifold, significant differences between aged and young rats' brains, animals were intensively behaviorally trained in operant tasks to determine which of these operational characteristics of the brain could be "rejuvenated." Somewhat to our surprise, with training limited in these aged rats to approximately 1 h/day for about 1 month, all of these degraded operational and physical-chemical characteristics of the aged brain could be substantially if not completely restored to a "youthful" state, in aged animals (de Villers-Sidani et al., 2010; de Villers-Sidani and Merzenich, 2011; Mishra et al., under review).

Given its reversible nature, plasticity processes can just as easily be engaged in a young prime-of-life animal in ways that drive their brains in an increasingly uncorrelated pattern activity (as seen in aged animals). That has also been achieved for the auditory brain by a simpler environmental exposure strategy. By housing young, vigorous adults in an environment of non-correlated noise (believed to increase the level of internal noise in the hearing brain) for a period of several weeks, *all* of the functional and physical characteristics of the machinery of the brain noted above altered *as if* the animal had advanced over those several weeks to an "old age" status (Zhou et al., 2012; Kamal et al., 2013).

Because these reversible change processes can drive neurological changes in either an advancing or degrading direction, driving the processing and physical characteristics of the brain rapidly "forward" to simulate aging is equivalent to driving the animal backward in age: The physical and functional properties of the brain near the end of life closely correspond to those same characteristics in the brain recorded near the beginning of life (Zhou et al., 2012). That conclusion is supported by documenting the operational and physical characteristics of the machinery of the brain in very old and very young animals: they closely match one another. It is also manifested by the fact that key accelerated changes leading to "premature aging" achieved by noise exposure or by "negative" training, carried forward far enough, similarly result in the re-opening of the "critical period" (Zhou et al., 2012).

THE NEUROSCIENCE OF BRAIN PLASTICITY PROVIDES NEW INSIGHTS INTO THE ORIGINS OF THE EXPRESSIONS AND NATURES OF ACQUIRED NEUROLOGICAL AND PSYCHIATRIC IMPAIRMENT AND "DISEASE"—AND IN HOW TO DRIVE "CORRECTIVE" CHANGES IN IMPAIRED BRAINS VIA INTENSIVE TRAINING

From the study of all of these complex aspects of brain change, neuroscientists have defined the "rules" that govern them, in the terms of the brain processes that account for these aspects of brain change. We now understand necessary and optimal conditions for driving positive changes in most dimensions of brain processing, as well as the behavioral functionality that they account for. This rule-based training represents an important refinement of the more-empirically based development of "cognitive training" program designs, in several important respects. First, with this understanding, we can more directly target neurological (not merely behavioral) improvement or re-normalization. Second, this science is progressively resolving long-standing arguments about "best practices." There *is* a best way: following the brain's rules for learning-based remodeling.

Neuroplasticity research, with related studies in fundamental and integrative neuroscience conducted in animal and human models, has provided us with a new level of understanding of the neurological bases of representation of behavior. It has shown us, at a deeper level, the natures of the neurological distortions that underlie function impairment, loss, or dysfunction, specifically defined in neurological (not limited to descriptive behavioral) terms. Fundamental recovery, which addresses the central deficit underlying the disorder, must result in neurological improvement or restoration. With this rapidly growing science, we can

potentially extend our targeting of neurological dysfunction to the more elemental processes in brain systems that account for behaviorally expressed impairments.

To understand how this differs from the currently predominant approaches to neurological and psychiatric medicine, consider two simple examples: First, scientists have studied alterations in many disease states by documenting which genes are up-regulated—and which are down-regulated—in specific diseases (Gilman et al., 2012; Calciano et al., 2013; Fass et al., 2014; among several hundred citable examples). One of their goals has been to determine what specific change processes could account for the disease's emergence, on the path to the potential pharmaceutical treatment of the illness. With remarkable repetition, study after study has recorded: (1) several to many hundreds of genes that are significantly up-regulated and down-regulated in the disease or condition; and (2) a broad overlap in this pattern of change for brains studied from patients with even strikingly different clinical indications (like schizophrenia or autism or Alzheimer's disease or multiple sclerosis). For example, genetic variation in encoding brain derived neurotrophic factor (BDNF) has been implicated in neuropsychiatric disorders such as Alzheimer's disease, affective disorders, schizophrenia, and substance dependence (e.g., Zhang et al., 2006).

From a brain plasticity perspective, these gene chip results are unsurprising: any struggling brain undergoes broad-scale "plastic" revision. It is highly probable that most of the recorded changes in gene expression are a reflection of plastic remodeling; in the face of growing "noise" in neurological processes, the brain plastically adapts to retain some level of functional control (see Merzenich, 2013). Given the chemical complexity of these changes contributing to disease expression, no single drug or limited drug combination, and no simple training of an explicit behavioral ability(ies) can be expected to drive the myriad of coupled plastically-adjusting processes to correction. On the other hand, animal studies indicate that it may be possible to achieve broad-scale "reversals" in targeted brain systems via relatively simple intensive training programs repertoires.

To cite a second example, cognitive psychologists have identified the ability of brain systems to sustain reverberant activities representing specific information "held in working memory" as a primary cause of many problems, extending from ADHD through schizophrenia to mild cognitive impairment. Their predominant treatment solution has been to directly exercise this failing faculty; medically useful gains are achieved by such training (Klingberg, 2010). However, as in any form of explicit skills training, benefits do not broadly generalize to other task domains that engage working memory in real-life behaviors (Melby-Lervåg and Hulme, 2013; Rapport et al., 2013).

A brain plasticity perspective addresses this kind of problem from a deeper level of understanding. "What is the cause of the inability of the brain to generate strong, persistent activities? How and why is the system not generating the highly correlated feedforward inputs and/or neuromodulatory inputs both known to be crucial for its genesis?" From that perspective, broader training designed to increase the salience (correlated power) of representation of the details of inputs and actions at every system level and the assured or corrected function of neuromodulatory contributors to working memory processing would be deemed to also be important for achieving stronger far-transfer training impacts (see, for example, Strenziok et al., 2014).

ADVANCES IN COGNITIVE NEUROSCIENCE HAVE ALSO PROFOUNDLY CHANGED THE THERAPEUTIC LANDSCAPE

A rapid expansion and elaboration of human brain recording and imaging studies have paralleled the phenomenal growth of neuroplasticity-related neuroscience. An increasing number of those studies document aspects of training-driven plasticity itself. Still, the primary focus of this research has been the mapping of patterns of activity in brain systems that account for specific human abilities. That science provides a basis for defining alterations in abnormal brain systems accounting for almost every class of functional impairment or "illness." This science has been limited with respect to the completeness with which it records abnormality in specific neurological-process terms. Still, it provides great insights into the origins of and the bases of behavioral expressions of every important neurologically-based clinical indication—and is a crucial source of information for our designs of impairment- and disease-targeted plasticity-based therapeutic programs. It also provides an increasingly definitive basis for documenting therapeutic outcomes, where the primary goal in therapy is shifting beyond behavioral improvement to the potential re-normalization of dysfunctional brain systems. Examples of how we apply this key source of information in program designs are described below.

TRANSLATING NEUROLOGICAL (AND BEHAVIORAL) SCIENCE INTO OPTIMIZED THERAPEUTICS

Based on the neuroscience of brain plasticity, therapeutic training strategies have been created that target a growing number of clinical conditions. Here, three examples illustrate how these programs are created and validated for therapeutic use.

TARGETING NEUROLOGICAL AND BEHAVIORAL IMPAIRMENTS IN SCHIZOPHRENIA

A growing body of literature points to pervasive neurocognitive and social cognitive impairments as fundamental aspects of the expression of schizophrenia (e.g., Cirillo and Seidman, 2003; Brewer et al., 2005; Keefe et al., 2006; Eastvold et al., 2007; Becker et al., 2010; Kim et al., 2011). Deficits in perception, speed of processing, working memory, attention, executive function, social cue perception, and social and action control are recorded even before illness onset, and are associated with poor functional, societal and occupational outcome (Green et al., 2000, 2012; Edwards et al., 2001; Lencz et al., 2006; Niendam et al., 2006; Seidman et al., 2006; Simon et al., 2007; Chan et al., 2010; Kohler et al., 2010; Billeke and Aboitiz, 2013). Because the perceptual, cognitive and social cognitive deficits are generally dissociated from psychotic symptoms in schizophrenia, they are not significantly ameliorated by antipsychotic medication (e.g., Goldberg et al., 2007; Green, 2007). Specifically, second-generation dopamine-agonists antipsychotic did not show any advantage over first-generation agents in treatment of cognitive deficits in schizophrenia (Keefe et al., 2007). Similar negative effects for cognitive deficits in schizophrenia have been reported for clinical trials involving

glutamate-related or serotonergic agents (see Goff et al., 2011, for recent review).

At the same time, we hypothesize that these weaknesses in perceptual and cognitive processing underlie the catastrophic breakdown of working memory operations, which are at the heart of psychoses genesis (see Merzenich, 2013). By that interpretation, training that improves the neurological abilities that contribute to working memory and neuro-modulatory system functionality in these great brain systems should have substantial prophylactic power in at-risk individuals.

From a neurological perspective, schizophrenic brains are: poor signal resolvers, operate sluggishly, struggle to generate sustained activities supporting top-down (working memory, selective attention, associative memory, predictive) processes in prefrontal cortex (Minzenberg et al., 2009), and in frontal, posterior parietal and inferior and medial temporal areas (Heckers, 2001); have distortions in language, visual, source-reference and other operations related to psychotic symptoms (Modinos et al., 2013); have impairments in social cognition that greatly impact quality of life (see Couture et al., 2006); and have changes in fundamental neuronal processes that we associate (along with working memory degradation) with very noisy brain system processing (e.g., Hinkley et al., 2011).

Perceptual, cognitive, social, and motor control deficits along with modulatory system abnormalities are obvious, important targets for treatment in schizophrenia. From a brain plasticity perspective, fundamental neurocognitive recovery shall require brain system remodeling: high-speed, high-fidelity processing with systems engaged by progressively more complex inputs and more difficult challenges should be combined with more-explicit cognitive re-training to achieve system re-normalization. As a part of a brain plasticity-based recovery strategy, it is important to re-normalize neuro-modulatory processes controlling plasticity itself. Core deficits expressed in the illness—the usually-severe degradation of working memory processes and magnified levels of arousal and intensity—are attributable in part to abnormally high levels of expressions of dopamine and NA in these individuals (Tost et al., 2010). By that dysregulation, the contribution of these systems to plasticity itself can be significantly altered—with further distortions induced by the psychoactive drugs that target the expressions of these neuro-modulators to ameliorate this self-poisoning.

Our computerized cognitive training programs are designed to drive the brain of the schizophrenic patient broadly in the normal-ward direction, in: auditory/aural speech, visual, social cognition, executive, social, and action control domains, attention and focus, and in neuro-modulatory system control domains. In these targeted brain system, training extends from low-level perceptual processing to higher-level working memory, attention and executive and motor control processes. Subjects are trained to refine representational fidelity and operate at speed, in ways designed to reduce internal brain noise and restore more normal physico-chemical processing. Training of implicit abilities is designed to assure that all fundamental processing abilities are refined in ways that support more reliable and more sophisticated explicit operations. All exercises are progressive and adaptive, adjusting in difficulty to assure a continuously successful-but-challenging level of ongoing training. The generalization of gains to all processing that engages the targeted systems is a universal program goal.

Specific attention has been given in our exercise suite to training of social cognition, which has been recently pointed out as a particularly important target for intervention in schizophrenia. Specifically, social cognition deficits have been directly linked to poor functional outcome in schizophrenia (Couture et al., 2006) and have been found to underlie critical factors affecting daily living in schizophrenia, such as occupational status, community functioning, independent living skills, and quality of life (e.g., Couture et al., 2006; Bell et al., 2009; Fett et al., 2011). Our Social cognitive training ("SocialVille;" Nahum et al., 2013a,b) deploys socially-relevant stimuli in tasks that target affect perception, social cue perception, theory of mind and attributional style. The SocialVille exercises require progressively more complex discrimination and identification of socially-valid stimuli, while again driving progressive improvements in processing speed, working memory, and attention control. We have recently successfully completed a pilot feasibility study of SocialVille in early-phase schizophrenia patients, who completed training remotely from home using internet-connected laptops (Nahum et al., 2014).

Through collaboration with university-based scientists, different combinations of these forms of brain plasticity-based training have been applied in a large population of chronic schizophrenia and first-episode patients (e.g., Adcock et al., 2009; Fisher et al., 2009a,b; Dale et al., 2010; Popov et al., 2011; Subramaniam et al., 2012; Keefe et al., 2012; Sacks et al., 2013; see reviews by Biagianti and Vinogradov, 2013 and Fisher et al., 2013). For example, following 50 h of plasticity-based auditory training, chronic schizophrenia patients made significant gains in global cognition, processing speed, verbal working memory, and learning and memory metrics (e.g., Fisher et al., 2009a,b). In parallel, brains of trained subjects compared with controls recovered more normal M100 responses to successive signals consistent with recovery of more normal perceptual abilities resulting from training (Adcock et al., 2009; Dale et al., 2010); recovered more strongly correlated (recovered) gamma frequency responses in the lower gamma frequency (Popov et al., 2012); recovered stronger responses to rapidly successive stimuli in the gamma high-frequency domain (Dale et al., under review); more strongly synchronized alpha frequency responses for target stimuli, and more strongly de-synchronized non-target domain alpha-band responses in an attention-controlled task (Popov et al., 2012; Dale et al., under review); recovered more normal sensory gating (Popov et al., 2011); recovered more normal dorsolateral frontal responses in a working memory task (Dale et al., under review); restored more normal patterns of response in an attribution-of-source task (Subramaniam et al., 2012; see **Figure 1**); recovered more normal amygdala and ventral-lateral-frontal cortical responses in an emotion recognition task (Hooker et al., 2012, 2013); recovered more normal BDNF expression (Vinogradov et al., 2009); among other physical and functional neurological measures of plastic training-driven recovery.

While these studies are still a work in progress, taken together, they indicate that this form of computerized, neuroplasticity-based training is effective for broadly driving behavioral

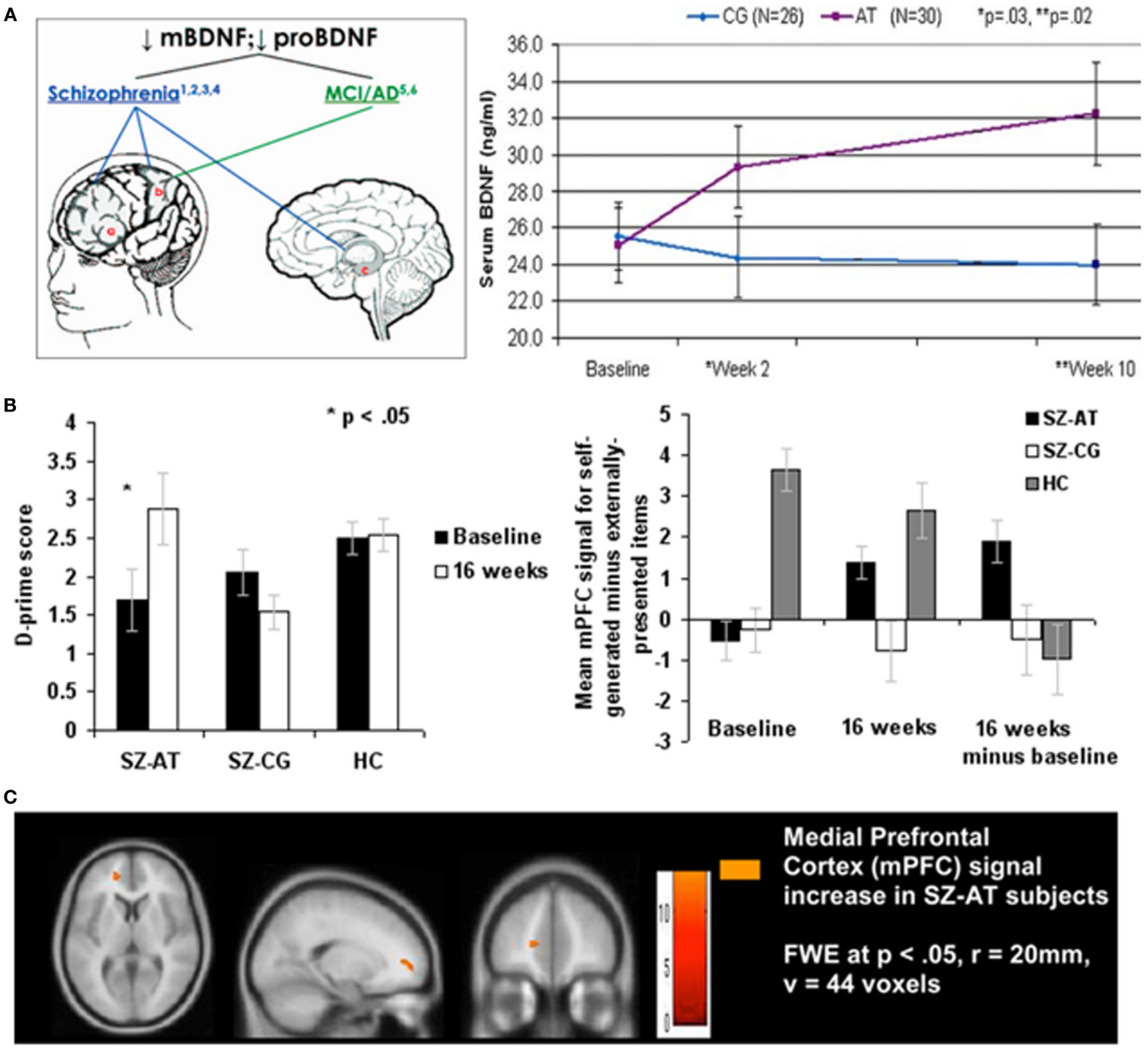

FIGURE 1 | Illustrating positive far-transfer chemical, behavioral, and brain response changes attributable to intensive brain training in patients with chronic schizophrenia (SZ). Tasks targeted the auditory-aural language/perceptual-cognitive system. Training was via computers, over a 40–50 hour-long training period. **(A)** Brain-Derived Neurotrophin Factor (BDNF) in its "pro" and mature ("m") forms is down-regulated in schizophrenia and in other chronic neurological and psychiatric illnesses (e.g., healthy aging). As a result of this brain plasticity-based training, serum levels of BDNF were elevated to normal levels; no changes were recorded in subjects who worked with equivalent intensity on progressive control video games. The up-regulated of BDNF to near-normal levels was sustained for more than a year following training program completion. Similar effects have been recorded in aging brains. Data are from Vinogradov et al. (2009). **(B)** Left: Re-normalization of abilities in a behavioral task in which subjects with SZ identify whether they or an outside agent was the source of an immediate-past action. Again, this is a transfer effect of training; no aspect of this task is represented in the completed training regime. Right: Strengthening of BOLD responses in a medial prefrontal cortical area hypothesized to be the primary cortical site for the assignment of agency in the brain. No measurable task-related activity was recorded in this area in subjects with SZ prior to training, or in computer brain-engaged SZ controls before or after training. From Subramaniam et al. (2012). **(C)** Brain images showing changes in responses recorded in this task. Abbreviations: CG, computer games control; AT, auditory training; SZ-AT, schizophrenia patients in the auditory training group; SZ-CG, schizophrenia patients in the computer games group; HC, healthy controls.

and physiological changes in a normal-ward direction in the schizophrenic brain. On this basis, we are now conducting a multi-site FDA medical device trial to further document these medical outcomes on the path to establishing medical claims for program use. Our longer-term goal is to progressively improve our training strategies to drive stronger and more complete and reliable changes in all key domains of dysfunction and loss in patients with this very complexly neurologically distorting illness.

It might be noted that given its bi-directional nature, training-driven plasticity processes are not riskless. Because we have a relatively complete understanding of the principles governing plasticity in our own medial therapeutic applications, we have not recorded negative consequences resulting from the application of any of our training tools. At the same time, an FDA approval process is important for assuring the affirmed positive values of the medical delivery of this new approach.

HEMISPATIAL NEGLECT SYNDROME

Approximately half to two thirds of patients with right hemisphere injury exhibit a complex, debilitating array of spatial and non-spatial (attention) neurological deficits (Mesulam, 1990; Heilman et al., 1993). Those deficits arise from damage or disconnection to interconnected inferior parietal or lateral frontal cortical areas, or from the subcortical basal ganglia or thalamus. Patients with neglect do not respond to stimuli on the contra-lesional side of visual space, often seemingly unaware that anything in that space exists. For example, they may lose or fail to see or find objects located in neglected space, commonly suffer from poor navigation, and disregard significant events arising in the neglected field.

In addition to this manifest visual-field-localized impairment, patients with neglect exhibit deficits in attention that are not lateralized (Husain and Rorden, 2003; Van Vleet and Degutis, 2014). Those more general deficits are general in this population, and on that basis have been argued to be fundamental to the disorder (Corbetta and Shulman, 2011). They include deficits in arousal (particularly strong in the acute phase of recovery); attention to transient events; working memory updating; spatial working memory span; and alertness and sustained attention. Importantly, these non-spatial deficits are stronger predictors of chronic spatial neglect in the post-acute recovery phase than are the visuo-spatial deficits themselves (Hjaltason et al., 1996; Husain et al., 1997; Robertson et al., 1997; Duncan et al., 1999; Peers et al., 2006). Several recent studies show that the poor regulation of intrinsic alertness is correlated with—and plausibly explains—the degree of spatial field loss ("spatial bias") in these patients (Robertson et al., 1997).

From a brain plasticity perspective, this is a particularly clear instance in which neuro-modulatory dysregulation contributing to attention control has been argued to be a strong contributor to disability. A pharmacological approach to recovery in such a case would be the administration of a stimulant drug; such drugs have been applied in this population with limited success (Fleet et al., 1987; Geminiani et al., 1998; Barrett et al., 2012). A cognitive behavioral approach would be to engage patients by heavily stimulating them to do what they can't do: respond to stimuli presented in the contralesional visual field. Again, that therapeutic approach has been applied with significant but limited success (Weinberg et al., 1977). Our neuroplasticity-based approach has applied training specifically designed to up-regulate both phasic and chronic alertness, combined with training designed to assure the recovery of more normal spatial working memory and representational salience for visual inputs arising from the affected visual field area. That approach is, again, predicated on our understanding of the normal modulatory processes in play. In the neglect patient, deficits in both tonic and phasic alertness are recorded. Tonic alertness (the background state of arousal) is highly correlated with the background level of expression of NA arising primarily from the midbrain LC (Sturm et al., 1999; Sturm and Willmes, 2001; Thiel et al., 2004). Tonic alertness is supported by a right-lateralized supra-modal network that feeds back to the LC, including the right inferior frontal, right inferior parietal and anterior cingulate regions. In contrast to the slowly changing tonic alertness, phasic alertness is the rapid modulation in alertness arising in any briefly engaging event, vital for operations such as orienting, selective attention, and the enabling of plasticity that is dependent upon these processes. This neuromodulation is largely attributed to the forebrain expression of ACh originating from the dorsal nucleus of Meynert, as well as by the phasic release of NA, again from the LC; their activation is again influenced by recurrent projections from a complex forebrain network. Phasic alertness is stronger against a platform of higher tonic alertness; both can be substantially amplified in an enduring way by relatively simple forms of training (DeGutis and Van Vleet, 2010; Van Vleet and DeGutis, 2013).

The primary strategy that we deploy to "exercise" this impaired neuromodulatory machinery is a continuous performance task in which subjects maintain a specific visual or auditory target in working memory (setting up the conditions for "top-down" neuromodulatory engagement), with those targets presented as rare events within a series of novel stimuli that are known to strongly activate limbic system sources of ACh (Richardson and DeLong, 1990; Sarter et al., 2001, 2006) and NA (Bouret and Sara, 2005). In the training task, subjects demonstrate that they are continuously attending to novel background stimuli by responding to them one by one; they demonstrate that they are holding a target stimulus in working memory by withholding their responding when it occurs (DeGutis and Van Vleet, 2010; Van Vleet and DeGutis, 2013). It might be noted that stimuli are also presented in this training in time-jittered sequences (see Wodka et al., 2009; Ryan et al., 2010) and that patients are progressively time-challenged in their responding to novel non-target stimuli (they must withhold responses for target stimuli), again because neurological studies indicate that this will drive more rapid and more enduring neurological remodeling.

With the application of this neuro-modulatory- (attention-) targeted computerized training strategy alone, most neglect patients—including individuals with brain injury arising from almost any cause—rapidly recover their ability to "see" in the affected hemifield (Van Vleet and DeGutis, 2013; see **Figure 2**). Given this initial recovery, training is now more effectively extended to more completely recover representational fidelity and spatial working memory by directly re-refining the brain systems representing spatial and spatial sequencing and working memory deficits of neglect patients as well. A brain plasticity-based program based on these principles is now the subject of a large multi-site FDA medical device trial.

AGE-RELATED IMPAIRMENT; RESILIENCE AGAINST NEURODEGENERATIVE DISEASE ONSET

By contrast to relatively sharply targeted training applied to address the problems that frustrate neglect syndrome patients,

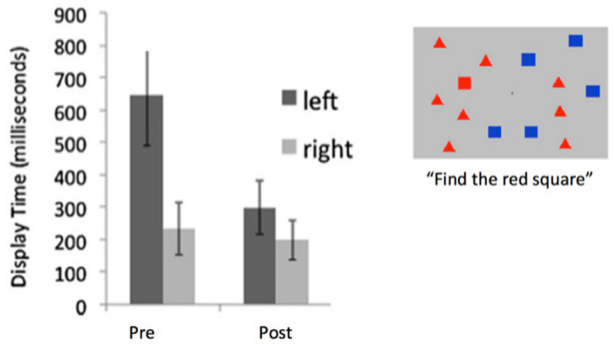

FIGURE 2 | Hemispatial neglect syndrome patients are slow to identify target stimuli arising in the neglected visual hemifield. Training designed to recover the functionality of intrincsic alertness over a period of 6 h was adequate for achieving recovery when examined within 48 h post-training. Data from 20 neglect patients with a wide panoply of brain-injury etiologies. (e.g., tumor, stroke, TBI). Display time significantly shortened following training. Note that this is a "transfer" effect, as training involving the identification of objects, scenes, or tones was presented at central fixation (i.e., not in the neglected field). Redrawn from DeGutis and Van Vleet (2010) and Van Vleet and DeGutis (2013).

neurological and behavioral changes in aging, like those in schizophrenia, are almost brain wide. The documentation of age-related deficits—and the path of the progression to an ultimately catastrophic decline to senility—have been the subject of several hundred thousand scientific reports. The average aging brain expresses major progressively-growing behavioral deficits in all of its major processing systems in perception; speed of action and fluency; phasic, sustained and divided attention; different aspects of memory; social cognition; and executive, social and action control (see Salthouse, 2000, 2012; Reitz et al., 2011; Merzenich, 2013, for review). Losses translate to about an average of one third of a standard deviation per decade in ability after ability past the age of 30 for men, and beyond the age of about 45 in women. In neurological terms, substantial degradation is recorded in all great representational systems—again, on the average—in representational accuracy; processing speed; local response and system coordination; excitatory and especially inhibitory powers (and the complex machinery that support them); tracking of rapidly successive inputs; representation of temporal details of inputs(durations, intervals, rhythmic sequences, et al.); accurate representations of sequenced inputs, scenes, scenarios; the sustained responses supporting "working memory"/"selective attention"/"associative memory" and prediction; and the more complex "mental" neurological activities supporting executive, social and motor control, ideation, and thought (see Merzenich, 2013 and Nahum et al., 2013c, for review). All of these processes, and their degradation in aging, are again contributed to by parallel atrophy of the neuro-modulatory centers regulating the release of dopamine, norepinephrine, ACh, serotonin, et alia (Barili et al., 1998; Mufson et al., 2002; Backman et al., 2010)—which crucially support the plasticity processes that account for both functional maintenance and learning-based remodeling.

These neurological changes *are* "cognitive aging": from a physical view, they are the basis of connectional dis-elaboration and disconnection, de-myelination, reduced blood flow, and neuropil and cortical shrinkage reduction attributable to dendrite, axonal arbor, glial process and synaptic simplification (e.g., see Mufson et al., 2002; Lo et al., 2011; Hahn et al., 2013; Jagust, 2013). Because of the broad picture of decline, both behaviorally and neurologically, there is a lot of re-engagement required to drive the brain broadly in a rejuvenating direction, conferring greater resilience re the onset of senile dimension and neurodegenerative diseases. At the same time, there is no simple pharmaceutical or cognitive behavioral strategy that could possibly drive the requisite, broad-scale corrections. The training program that we have designed to address these broad issues requires up to about 200 h to complete (dosing is dependent on the depth and breadth of neurological loss), with additional training on a lighter schedule required for many individuals to sustain a safe position over subsequent years. Again, all training is progressive and adaptive, and presented in game-like training formats on computers or other mobile devices. Our goal is to recover, insofar as possible, neurological representational accuracy, speed, coordination, sequencing, recording (remembering, selectively attending), noise control (distractor suppression) and executive processes in the brain. At the same time, exercises are designed to up-regulate, re-refine and re-invigorate modulatory control processes controlling learning, memory, attention states and mood.

As noted earlier, an important goal of this training is to increase resilience against the onset of neurodegenerative disease. Because we know the patterns of progression in pathology across a long epoch of time before frank "disease" onset, we increasingly understand how it relates to progressive changes in brain engagement. This understanding is also richly informed by the many factors that can accelerate the advance to Alzheimer's, Parkinson's and other neurodegenerative diseases (e.g., decreased noradrenergic activity; see Marien et al., 2004; Jardanhazi-Kurutz et al., 2010, 2011; Kong et al., 2010; McNamee et al., 2010; Koffie et al., 2011; Kalinin et al., 2012). We have extensively relied on this literature in the designs of programs to try to assure that training programs address the neurological, immunological, and vascular aspects of age-related impairment.

We have collaborated with university-based scientists who have conducted controlled studies in thousands of normally aging individuals to evaluate the effectiveness of this approach. Completed studies are still piecemeal, evaluating both the modality-specific and the general cognitive and neurological impacts of training in vision, hearing, executive control, attention, and related neuro-modulatory systems function. Studies of social cognition training are underway. All studies reveal a significant level of positive, enduring computerized training-driven improvements. To briefly summarize: (1) Training targeting the aural speech/language system have been shown to substantially improve measured listening, memory and related cognitive abilities, with significant far-transfer effects shown in quality of life/everyday life assessments (Mahncke et al., 2006; Smith et al., 2009; Zelinski et al., 2011). (Note that more than 250 additional studies demonstrating the behavioral and neurological values of this form of training have documented in studies in

children and young adults. See Merzenich et al. (1998); and www.scientificlearning.com. In studies conducted in individuals of all ages, recoveries in perceptual abilities in listening have repeatedly documented rejuvenated speed of processing, accuracy, and attention control in processing abilities). (2) Training targeting visual perception and related cognition abilities resulted, in controlled trials, in significant improvements in visual processing (e.g., Ball et al., 2007; Berry et al., 2010; Wolinsky et al., 2013; see **Figure 3**). Improvements in speed and accuracy of processing and improvements in spatial vision (saccade sampling rates; multitasking; local and global reconstructions; scene reconstruction; useful field of view) were repeatedly recorded in these studies. These aural language and visual training studies also extensively documented improvements in attention, working memory, and immediate and delayed recall, and associative memory/syntactic abilities. (3) Studies document benefits of training for executive control and temporal and spatial navigation processes in training (e.g., see Ball et al., 2007; Smith et al., 2009; Merzenich, 2013). With working memory and with the highest levels of operation in social cognition, these explicit behaviors normally directly engage frontal, posterior parietal, anterior and posterior cingulate, medial ventral and hippocampal zones that undergo disconnection as a pre-amble to AD onset. (4) Broad far-transfer effects of training are recorded—e.g., to everyday quality of life (Ball et al., 2007; Smith et al., 2009) to sustained confident independence (Edwards et al., 2009; Wolinsky et al., 2010a), to resilience impacts against the onset of depression (Wolinsky et al., 2009), to measures documenting improved brain health (Wolinsky et al., 2006, 2010b) and to sustained (Edwards et al., 2009) and safer automobile driving (Ball et al., 2010)—among other indices (Wolinsky et al., 2006, 2009, 2010a,b; Edwards et al., 2009; Ball et al., 2010). (5) Positive improvements have been shown to endure for many months to years following training completion (e.g., Wolinsky et al., 2006, 2009, 2013; Zelinski et al., 2011) (see **Figure 3**).

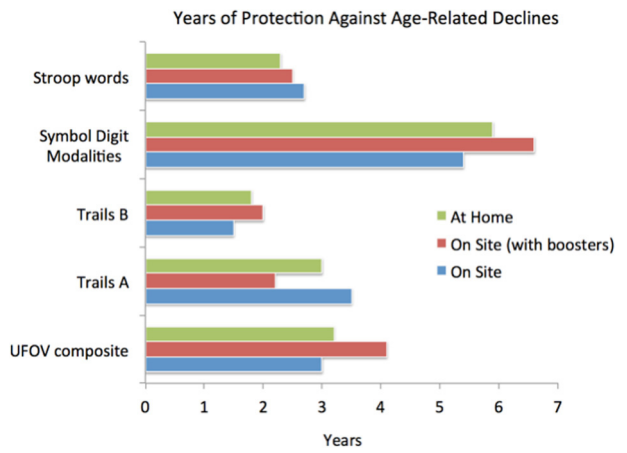

FIGURE 3 | Illustrating the magnitudes of gains for a limited computer delivered epoch of training (about 10 h) in a large ($n = 670$) cohort of healthy aged participants. Training was conducted "at home" or in a clinical center at the University of Iowa. One population in the clinical center completed a 4-h "booster" training session 6 months after initial training program completion. All patients were behaviorally assessed before, immediately after, and 1 year after training program completion. Here, gains are expressed as an estimate of the number of years before assessment scores would be predicted to fall below pre-training scores; these highly significant gains had an average endurance of 3–4 years. Note that the "UFOV composite" reflects the approximately 1 *SD* gain in brain speed and visual control within an expanded visual field achieved directly from the training. All other measures represent near and far-transfer effects (i.e., benefits shown in untrained cognitive domains). Adapted from Wolinsky et al. (2013).

Does this form of training delay AD onset? Does it block, and can it reverse neuropathology progressions? Answering that question is the current goal of a large controlled internet-delivered trial currently underway. A growing body of evidence provides increasingly compelling evidence that this may, indeed, be the case. By training thousands of individuals at risk for AD onset, this question should be answerable, with finality, in the immediate future.

IMPEDIMENTS FOR DELIVERING THESE NEW TREATMENT STRATEGIES TO PATIENTS IN NEED

The evolution of this new medical strategy for treating neurological and psychiatric illness is a "textbook example" of a disruptive technology. Its medicine is delivered via computers and other smart devices, at very low cost, without any requirement for the immediate presence of a medical professional. The scientific principles that support its use are poorly understood by most of the professionals who would normally prescribe and deliver this medicine. Most medical schools still focus on chemical and anatomical aspects of neuroscience on the path to creating pharmaceutically-focused medicine. Most graduate training in the psychology help professions still focus on "cognitive therapy" approaches to rehabilitation, with limited formal training in fundamental or integrative neuroscience on a level that informs this brain plasticity-based translational approach. Technological approaches are not the norm for a majority of practitioners in both of these large professional communities (see McMinn et al., 1999); even the minimum requirements in automated patient monitoring of compliance and progress potentially requiring professional participation and response delivered via the internet is beyond routine clinical practice for many specialists.

The delivery of these programs has also been confounded by a difficulty that the clinical community and public has in distinguishing between more classical cognitive therapeutic approaches delivered by computer from programs developed with application of a brain plasticity-based approach. Professional scientists have repeatedly described the limitations of the former—for example for achieving generalization beyond the directly trained tasks—describing their findings as demonstrating the limited values of any computer-delivered therapy. A result is great confusion for the professional community and public about the validity of all computer-delivered therapeutics in the brain health field.

Finally, the delivery of this form of medicine is impacted by the demands for compliance required of the patient. As we have noted earlier, driving the brain of an individual with schizophrenia or an individual at high risk for Alzheimer's Disease onset in a broadly re-strengthening direction can require many hours of intensive training potentially only achieved over a period of

months. To substantially delay and to potentially achieve reliable prophylaxis against the onset of neurodegenerative disease, or to prevent schizophrenic onset, some almost-daily exercise may have to be undertaken for the rest of the patient's life. From a health perspective, the gains from this form of medicine often completely justify that effort; about 50–100 h to improve the level of cognitive ability for a patient with schizophrenia, for example (as was applied, for example, in Fisher et al., 2009a,b), is, after all, only about 1/100th the span of 1 year in their life. Clinical trials requiring this level of participation are now underway. Still, a public that is educated in ways that result in the broader patient acceptance of these forms of treatment as medicine is a key to their more successful, wider application.

Toward that end, the evaluation of program effectiveness through an FDA medical device process or its equivalent is a crucial part of our implementation strategy, and FDA-level trials are now underway for treatments designed to improve or recover the neurological status of patients with schizophrenia, traumatic brain injury, and stroke.

AN ALTERNATIVE VISION OF PREDOMINANT FUTURE TREATMENT MODES IN NEUROLOGICAL AND PSYCHIATRIC MEDICINE

As our understanding of our fundamental human neurology grows, the more we can expect it to be brought to bear as the basis of neurotherapeutic medicine. Up to this point, brain medicine has primarily followed a chemical therapeutics approach. When deficits are attributable to processes operating across complexly self-organizing brain systems whose functionality is impacted by several dozen major variables and is implemented through hundreds or thousands of gene-regulated chemicals, drug treatments necessarily have limited impacts that can rarely if ever be regarded as curative. Any "real cure" invariably requires complex brain rewiring that only the brain itself can achieve. This is the primary reason why there has been no major fundamentally new drug approved for use for more than 20 years. It is also the primary reason why addressing issues of aged infirmity that occur at an end stage of functional deterioration shall be a failure. In the end, only the brain, through its intrinsic plastic processes, can sustain or repair itself, at the level required to sustain high functionality. Those same good reasons explain that while pharmaceutical treatments can often rescue individuals with major neurologically and/or psychiatrically illness, the distortions manifested in their illnesses including the neurological alterations that are the basis of their disease routinely remain unaddressed (e.g., Opler et al., 2014).

In part because of the unsatisfactory clinical outcomes from chemical medicine, equally flourishing clinical practices try to address neurological impairment and psychiatric illness by documenting behavioral abnormalities as a premise for engaging the patient to directly address them. Again, the therapist commonly looks for a linchpin in behavior that can ameliorate the broader clinical symptoms. Because this strategy is usually removed from the underlying etiological causes of system distortions or failures in the brain, it again often fails to face up to the mechanistic realities that account for the clinical problems borne by the patient.

Brain plasticity-based therapeutics, still in its infancy, represents an attempt to address those real neurological distortions, on a level at which something closer to a fundamental neurological correction can potentially be achieved. As we understand how brain systems organize themselves, in detail, through their native plasticity processes, we understand with increasing clarity what plasticity itself has contributed to disease symptoms. Even more importantly, we understand how to harness these powerful intrinsic brain change processes to drive positive neurological corrections, on the path to something closer to a "cure." Initial attempts to drive neurological correction on the requisite broad scale have usually generated still-incomplete brain remodeling; this translational science is still at a primitive stage, and very much a work in progress. At the same time, the brains of individuals with clinical conditions as complex as those that apply to the patient who is schizophrenic, is frail and struggling and at risk for collapse in aging, or has suffered from formerly-inexplicable visual field "blindness" following brain injury or stroke are clearly driven in a significantly improving—indeed, at least in most neurological respects re-normalizing—direction, by intensive computer-based training designed on these scientific bases. This form of medicine is inexpensive to deliver, as all it requires for operation is internet-connected tablet/computer/smart-phone (see Fernandez, 2011), and it is rapidly scalable for immediate application across the world.

We believe that this represents the advent of a new era in brain health medicine. In the future, we can expect to see a healthy re-integration of chemical, cognitive-behavioral, and brain plasticity-based therapeutic strategies. At the core, brain plasticity-based therapeutics can be expected to drive fundamental re-normalizing corrections for distorted brain systems. Those treatments shall be often supplemented in their actions by drugs or gene therapies that help patients overcome specific biological weaknesses assignable to genetic faults, or that augment the power of plasticity processes to accelerate positive neurological recovery. They shall also be supplemented by cognitive behavior approaches that more appropriately and more powerfully localize therapies to treat plasticity-induced distortions that spring from each patient's unique biographical history. With these novel therapeutics, treatment of the distorted chemical brain merges with treatment of the distorted behavior, on the grounds of brain plasticity based therapeutics.

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Conflict of Interest Statement: The authors all work for a for-profit company on the development of the therapeutic training programs. Those programs, and the science that supports their designs and uses, are described in this review.

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The functional significance of cortical reorganization and the parallel development of CI therapy

Edward Taub[1]*, Gitendra Uswatte[2] and Victor W. Mark[3]

[1] *Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA*
[2] *Departments of Psychology and Physical Therapy, University of Alabama at Birmingham, Birmingham, AL, USA*
[3] *Departments of Physical Medicine and Rehabilitation, Neurology, and Psychology, University of Alabama at Birmingham, Birmingham, AL, USA*

Edited by:
Bogdan Draganski, University of Lausanne, Switzerland

Reviewed by:
Antoni Rodriguez-Fornells, University of Barcelona, Spain
William C. Gaetz, The Children's Hospital of Philadelphia, USA

****Correspondence:***
Edward Taub, Center for Psychiatric Medicine 714, University of Alabama at Birmingham, 1720 2nd Avenue South, Birmingham, AL 35294-0018, USA
e-mail: etaub@uab.edu

For the nineteenth and the better part of the twentieth centuries two correlative beliefs were strongly held by almost all neuroscientists and practitioners in the field of neurorehabilitation. The first was that after maturity the adult CNS was hardwired and fixed, and second that in the chronic phase after CNS injury no substantial recovery of function could take place no matter what intervention was employed. However, in the last part of the twentieth century evidence began to accumulate that neither belief was correct. First, in the 1960s and 1970s, in research with primates given a surgical abolition of somatic sensation from a single forelimb, which rendered the extremity useless, it was found that behavioral techniques could convert the limb into an extremity that could be used extensively. Beginning in the late 1980s, the techniques employed with deafferented monkeys were translated into a rehabilitation treatment, termed Constraint Induced Movement therapy or CI therapy, for substantially improving the motor deficit in humans of the upper and lower extremities in the chronic phase after stroke. CI therapy has been applied successfully to other types of damage to the CNS such as traumatic brain injury, cerebral palsy, multiple sclerosis, and spinal cord injury, and it has also been used to improve function in focal hand dystonia and for aphasia after stroke. As this work was proceeding, it was being shown during the 1980s and 1990s that sustained modulation of afferent input could alter the structure of the CNS and that this topographic reorganization could have relevance to the function of the individual. The alteration in these once fundamental beliefs has given rise to important recent developments in neuroscience and neurorehabilitation and holds promise for further increasing our understanding of CNS function and extending the boundaries of what is possible in neurorehabilitation.

Keywords: CI therapy, cortical reorganization, neurorehabilitation, neuroplasticity, stroke, traumatic brain injury, cerebral palsy, multiple sclerosis

INTRODUCTION

Research on Constraint-Induced Movement therapy or CI therapy has demonstrated that the deficit in motor function following damage to the central nervous system (CNS) produced, for example, by stroke can be substantially improved in the chronic phase many years after the injury. Numerous experiments, to be described below, have shown that CI therapy is accompanied by large changes in the function and structure of the brain and that these changes are correlated with the magnitude of the improvement in motor function that the treatment produces. That chronic stroke patients could functionally benefit from rehabilitation, that the mature human brain evinces considerable potential for rewiring and remodeling, and finally that these clinical and neurobiological phenomena are inter-correlated, all contradict firmly held traditional views, as described in the Foreword to this collection of papers.

NEUROPLASTICITY

ANIMAL STUDIES

As noted in the Foreword the first clear example of the capacity of the CNS to change structurally after damage was the seminal discovery of intraspinal axonal sprouting after hemipyramidotomy in monkeys in the mid-1950s by Liu and Chambers (1958). Axonal sprouting was subsequently found to occur in the brain as well as the spinal cord, but it was never clearly shown to be causally associated with changes of importance to the function of an organism. It was generally recognized that axonal sprouting might well have functional relevance, but since a direct demonstration was lacking, general interest in the phenomenon began to wane over time. However, there was a resurgence of interest in the potential of the CNS for neuroplastic change in the last two decades of the twentieth century stimulated by the work of a number of investigators including Kaas, but especially Merzenich. They used single unit recording to demonstrate that the removal of afferent input from a body part in new world monkeys dramatically affects its representation in the brain.

The best known early study involved the removal of input from a primate digit by amputation. The somatosensory cortical representation of the hand area was identified using microelectrode mapping from two to eight months after surgical amputation of either digit 3 only or both digits 2 and 3. In both types of surgery, the cortical representation field of the remaining intact

digits expanded to occupy most of the original cortical territory of the now amputated fingers (Merzenich et al., 1984). This study showed that inputs can cross borders of the representation zones of separate digits to "invade" nearby cortical representations, in the dramatic terminology of the Merzenich group. The study also suggested to its authors that the extent of reorganization did not extend beyond 2 mm from the original representational boundary of the digit.

In a striking extension of this work, the Merzenich laboratory demonstrated that not only were cortical representation zones altered by a decrease in afferent input, but the converse was also true. Substantially increased "behaviorally relevant" input from a body part that had to be closely attended to resulted in an increase in the size of its cortical representation, a phenomenon known as use-dependent or skill-related reorganization. In their study (Jenkins et al., 1990), microelectrode maps were obtained of the somatosensory hand representation in cortical area 3b before, immediately after, and three weeks after monkeys underwent somatosensory discrimination training. Some of the monkeys were conditioned to keep the fingertips of one or more of the longest digits of this hand in contact with a grooved rotating disk to receive a reward. The grooved surface of these disks required that monkeys carefully regulate the amount of pressure applied to the disk with their fingertips to maintain contact long enough to be rewarded with a food pellet. The grooved surface also resulted in changes in tactile stimulation when the disk rotated. This "behaviorally relevant" stimulation of the fingers, to which close attention had to be paid in order for hungry monkeys to respond correctly and obtain food reward expanded the cortical representation zones and shifted the representation borders of the digits by a maximum of 2 mm. Other monkeys in the study were conditioned to keep their fingertips in contact with a stationary smooth disk to receive a reward. There was no need to carefully regulate pressure applied with the fingertip to remain in contact so that the task did not require as much attention as the task for the first group of monkeys. The second group of animals did not show cortical reorganization.

The work of Pons et al. (1991) challenged the idea of a 2-mm limit in the amount of reorganization that could occur. Fingers, palm, upper limb, and neck of monkeys were deafferented in the laboratory of one of us (ET) by serial dorsal rhizotomy so that the brain was deprived of inputs from these areas. Tactile evoked responses recorded from the cortex twelve years after the deafferentation indicated that the adjacent area representing the face had invaded the deafferentation zone; that is, the cortical area that had once received input from the deafferented parts of the body now received input from the face whose afferent supply from the periphery remained intact. The expansion of the border of the representation zone of the face greatly exceeded 2 mm. It was observed to take place over the entire representation of the arm (10–14 mm), and was designated "massive" cortical reorganization (Pons et al., 1991). This work aroused the interest of investigators in part because it suggested that plastic brain reorganization could take place over an area large enough to represent an entire arm or leg and thus might be relevant to the rehabilitation of function after brain injury. A portion of the data is shown in **Figure 1**.

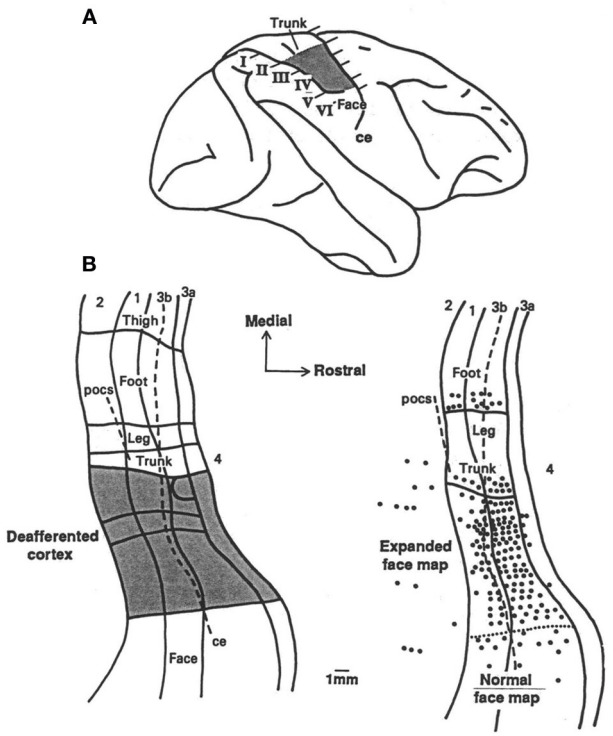

FIGURE 1 | (A) Lateral brain view showing the portion of the postcentral cortex that was deprived of its normal inputs by the deafferentation procedure ("deafferented zone" marked by shading) and the locations of the six parasagittal sections (I through VI) illustrated on the left. **(B)** Two flattened maps of SI, the first showing the deafferented zone (marked by shading), and the second the recording site density in the animal illustrated (CM3). The second map shows that tactile stimulation of the face evoked response that "invaded" the entire (deafferented) arm area. Adapted from Pons et al. (1991).

Even the tenet that new neurons are not produced in the adult mammalian brain (e.g., Rakic, 1985) has come under challenge and been overthrown (reviewed in several articles in this collection of papers). In the early 1990s, Gould et al. published data that confirmed earlier work (Altman and Das, 1965), which was largely ignored, that demonstrated the formation of new neurons, that is, neurogenesis, in the adult rat hippocampus (Gould et al., 1992; Cameron et al., 1993). Two other groups of researchers, at close to the same time, showed that neurogenesis takes place in the olfactory bulb in the adult mammalian brain (Corotto et al., 1994; Lois and Alvarez-Buylla, 1994). As with other forms of plasticity (see above), neurogenesis appears to be experience-dependent: both enriched environments and exercise increase neurogenesis in the hippocampus, and presumably elsewhere (reviewed in van Pragg et al., 2000).

By the middle of the 1990s, the view that the adult brain had substantial capacity for plasticity had gained a strong foothold. CNS reorganization had been shown to occur in adult non-human animals following a variety of interventions, including peripheral nerve section, dorsal root section, digit amputation, sensory input increase, and extensive behavioral training. The concept that the borders between the receptive fields of

individual digits were fixed gave way to the idea of borders that were dynamically determined by the amount of sensory input to each receptive field. The importance of the behavioral relevance of changes in sensory input for stimulating plastic changes was also established. Furthermore, investigators showed that the production of new neurons, perhaps the most radical form of plasticity, takes place even in the adult brain.

HUMAN STUDIES: THE RELEVANCE OF CORTICAL REORGANIZATION TO THE FUNCTIONING OF THE INDIVIDUAL

While these animal studies provided persuasive evidence that the boundaries of cortical representation zones were not fixed and could be altered dramatically by marked changes in afferent input in the mature non-human mammalian brain, this type of cortical reorganization had not yet been demonstrated in humans. Moreover, in all of the animal research it remained possible that the observed changes in cortical representation zones were an epiphenomenon. It was clearly recognized by investigators at that time that there was as yet no compelling evidence indicating that the reorganized cortical representation zones were not just sitting in somatosensory cortex at the top of the brain with no functional significance for the organism.

The occurrence of plastic cortical reorganization in humans was demonstrated in 1994 in upper extremity amputees by two groups using magnetic source imaging, one in San Diego (Yang et al., 1994) and another in Germany (Elbert et al., 1994). The second group, consisting of Elbert, Flor, Taub, and others, continued with a series of studies focused on determining the possible relevance of cortical reorganization to the sensory experience and behavior of humans.

There were major advantages to using human subjects to study the possible functional significance of cortical reorganization. First, humans could speak and report on their sensory experience immediately, while with animals it would take considerable time, often weeks to months, to establish a meaningful system of communication on the basis of conditioned response paradigms to achieve the same result. Moreover, with animals an investigator would have to have a clear idea of what he was looking for to establish a useful method for obtaining specific information through a training program, while with humans important and unexpected information could be obtained from chance remarks that a subject might adventitiously make, especially with respect to unexpected alterations in sensory experiences, as might occur in reporting phantom limb phenomena. A second major methodological advantage of working with human subjects was that they often have long histories of particular types of sensory experiences or performance of specific kinds of behavior which can be easily identified, such as years of carrying out the intensive, repetitive movements involved in practicing a musical instrument. Similarly, because of the existence of health care systems and other social institutions, substantial numbers of humans with particular types of chronic pathology, such as long-term amputees, are potentially available for immediate study. These factors made possible a number of what are, in effect, naturally occurring experiments relevant to the study of the functional significance of cortical reorganization which could be accomplished without requiring extensive or arduous procedures before the experimental measurements could be made.

In an extension of the first study by our group, it was found that the loss of sensory input after upper extremity amputation and the consequent severance of peripheral nerves resulted in massive cortical reorganization that had a very strong direct relationship to the severity of phantom limb pain (PLP) experienced by the amputees in the chronic phase (Flor et al., 1995). The correlation between amount of cortical reorganization and severity of PLP was $r = 0.93$, which explained almost 85% of the variance in cortical reorganization (**Figure 2**).

Persons with congenital limb aplasia often do not experience PLP or other phantom limb phenomena. In five individuals with congenital limb aplasia and no PLP, neuromagnetic source imaging revealed minimal reorganization of primary somatosensory cortex (SI). Five other individuals who had traumatic amputations after early childhood but who had no PLP also showed minimal reorganization in SI. However, a group of subjects whose amputation occurred in adulthood and did have PLP showed the usual, previously reported cortical reorganization in SI (Flor et al., 1998). In addition, as in the case of the Jenkins et al. monkey study, we found that an *increase* in sensory input, or an increased reliance on sensory input, led to the converse phenomenon, use-dependent cortical reorganization. For example, magnetic source imaging revealed that string instrument players have an increased (or otherwise changed) cortical representation of the left hand, which performs the complex task of fingering the strings, but not the right hand, which has the less dexterity-demanding task of bowing the strings (Elbert et al., 1995) (see **Figure 3**). It was further found that blind individuals who employed three fingers on a hand simultaneously to read Braille showed substantial enlargement of the hand area compared to sighted non-Braille-reading persons (Sterr et al., 1998). Additionally, the medial to lateral order of the representations of the three "reading" fingers on the convexity of the cortex was altered or "smeared" in the

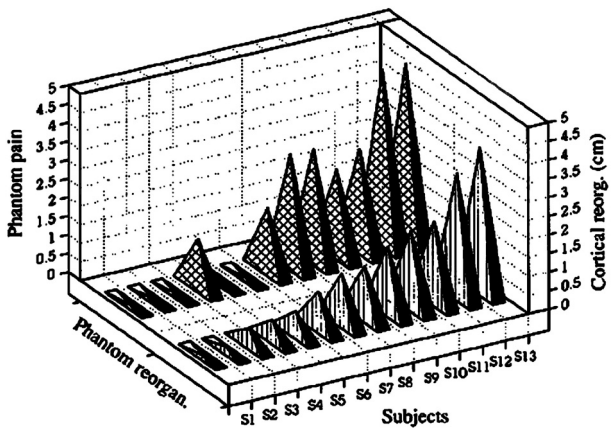

FIGURE 2 | Amount of cortical reorganization (in cm) for each subject plotted against intensity of phantom-limb pain as measured with the Multi-dimensional Pain Inventory. Reprinted from Flor et al. (1995).

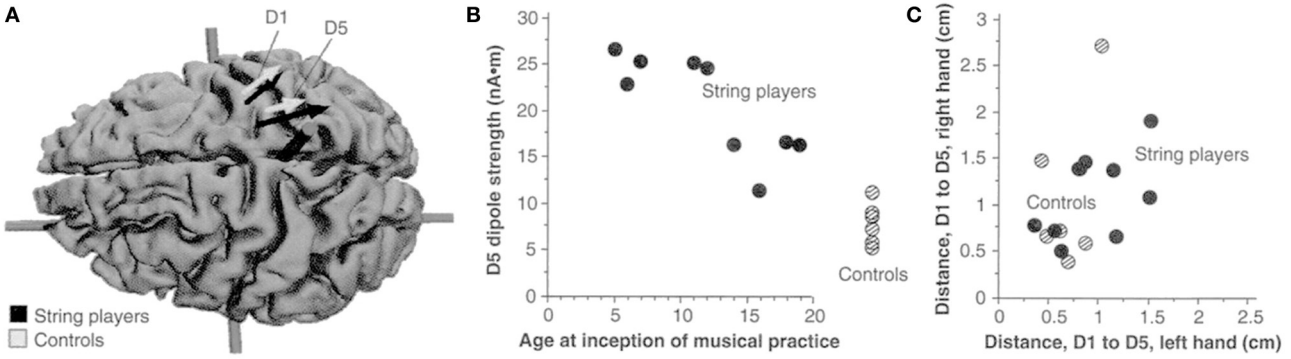

FIGURE 3 | (A) Equivalent current dipoles elicited by stimulation of the thumb (D1) and fifth finger (D5) of the left hand are superimposed onto an MRI (magnetic resonance imaging) reconstruction of the cerebral cortex of a control, who was selected to provide anatomical landmarks for the interpretation of the MEG-based localization. The arrows represent the location and orientation of the ECD vector for each of the two digits' averaged across musicians (black) and controls (shaded). The length of the arrows represents the mean magnitude of the dipole moment for the two digits in each group. The average locations of D5 and DI are shifted medially for the string players compared to controls; the shift is larger for D5 than for DI. The dipole moment is also larger for the musicians' D5, as indicated by the greater magnitude of the upper arrow. **(B)** The magnitude of the dipole moment as a function of the age of inception of musical practice; string players are indicated by filled circles, control subjects by hatched circles. Note the larger dipole moment for individuals beginning musical practice before the age of 12. **(C)** Scatterplot of the Euclidean distances (in centimeters) between the cortical representations of DI and D5. This distance for the musicians' left hands was greater than that in controls, but this difference is not statistically significant. Reprinted from Elbert et al. (1995).

three-finger Braille readers. This alteration in cortical topography correlated with impaired ability to detect which finger was being touched during tactile threshold determinations; there was no difficulty in determining that one of the fingers had been touched but errors were made in designating which. Control subjects in this experiment were blind individuals who read Braille with one finger. They exhibited an increased representational zone of that finger. However, there was no significant smearing of the representational zones of the digits, nor was there a significant decrease in ability to detect which of the fingers of the hand was being touched during tactile threshold testing. There was thus a strong correlation between the topography of the perceptual disorder and the topography of the altered cortical digital representation zones.

We have also identified a similar phenomenon in terms of motor control of the digits. In focal hand dystonia the individual has difficulty with independently moving two or more digits. It develops most frequently in musicians who often practice their instrument for many hours most days of the week over many years, making repetitive digital movements. For example, an affected pianist might be unable to flex the forth digit to strike a key without at the same time flexing the fifth digit. This problem would, of course, be disabling for musical performance. Magnetic source imaging revealed that there is a smaller distance (fusion) between the representations of the digits in somatosensory cortex for the hands of dystonic musicians than for the hands of non-musician control subjects (Bara-Jimenez et al., 1998; Elbert et al., 1998). This followed up earlier work with animals in the Merzenich laboratory by Byl et al. (1996, 1997) and Wang et al. (1995). In later work, a core area of the auditory cortex was found to be enlarged by a factor of 1.8 in the blind compared with sighted individuals (Elbert et al., 2002). This territorial expansion is consistent with the demonstrated increased ability of the blind to accurately localize acoustic sources in peripheral auditory fields (Muchnik et al., 1991; Lessard et al., 1998; Röder et al., 1999). Blind individuals do not receive more auditory stimulation than sighted individuals. However, to interact effectively with their environment, they have to rely on non-visual, primarily auditory input to a greater extent. There has been considerable other work on cross-modal plasticity in congenitally blind humans. Both auditory (Kujala et al., 1992, 1995a,b, 1997; Alho et al., 1993; Weeks et al., 2000) and tactile (Rösler et al., 1993; Uhl et al., 1993; Kujala et al., 1995a; Röder et al., 1996, 1997; Cohen et al., 1997) stimuli come to be processed in visual cortex. In another study, tinnitus sounds in tonal tinnitus were shown to be related to cortical reorganization in a tonotopic map region in auditory cortex at the dominant frequency of the tinnitus sounds (Mühlnickel et al., 1998). Tinnitus might thus represent a type of auditory phantom phenomenon.

The original stimulus for the work by our group just described above was an experiment by Ramachandran that generated considerable attention. After amputation most patients report spontaneous phantom sensations that seem to emanate from the now-absent body part. It was well known that stimulation of the amputation stump could elicit sensations that were referred to the phantom limb as well as being perceived on the amputation stump (Mitchell, 1871; James, 1887; Cronholm, 1951). In addition, early investigators (Henderson and Smyth, 1948; Cronholm, 1951) and later Ramachandran et al. (1992a,b) reported on upper extremity amputees in whom phantom sensation could also be elicited by tactile stimulation of the face ipsilateral to the amputation. Since the map of the hand on the somatosensory homunculus in the primary somatosensory cortex is flanked by the ipsilateral face laterally and the arm/trunk medially, Ramachandran and coworkers (1992a,b) maintained

that this mislocalization of tactile facial stimulation was a direct perceptual correlate of the type of invasion of sensory inputs from these sites into the hand area described in the animal experiments on cortical plastic reorganization by Merzenich et al. (1984) and Pons et al. (1991). Moreover, Ramachandran and coworkers (1992a,b) argued that the phantom sensations arise because in primary somatosensory cortex (SI) somesthetic input from the face area laterally and the upper arm area medially take over the synaptic spaces vacated by degenerating afferent connections in the representational zone of the now missing segment of limb. Ramachandran described subjects who had a precise topographic isomorphism between locations receiving tactile stimulation on the face and the perceived locations of referred sensations on the phantom limb. There was a strict one-to-one relation between a stimulation point on the face and a specific point on the phantom limb. In one subject, this topographic isomorphism was accompanied by some sensory modality specificity; water allowed to wash across the face was perceived as fluid streaming along the phantom limb. The phenomenon was termed facial remapping and was viewed as the basis not only of the mislocalization of tactile stimulation of face and amputation stump to the phantom limb, but of the spontaneous phantom limb experience (Ramachandran and Hirstein, 1998; Ramachandran and Rogers-Ramachandran, 2000). However, as noted above, while Flor et al. (1995) reported a very strong correlation between the magnitude of cortical reorganization in SI and the severity of PLP, the extent of the plastic cortical change in SI was not found to be related to topographically isomorphic remapping or to any other phantom phenomenon including the presence, frequency, length, and intensity of telescoping of the phantom limb (i.e., the progressive shortening of the phantom limb over time). Tactile stimulation of the face gave rise to referred or mislocalized sensation to the phantom limb in 4 of 13 subjects but not in the remaining 9 subjects in the Flor et al. experiment. Moreover, in 3 of the 4 cases it was unrelated to the cortical reorganization in SI, and had precise topographic isomorphism in just one case. Ramachandran reported initially that facial remapping was present in 30–40% of his subjects. However, Knecht et al. (1996, 1998) in a systematic study found no evidence of topographic isomorphism between facial stimulation and phantom experience in any of the eight subjects studied. In follow-up studies, Ramachandran reported that orderly topographic remapping was far less common than observed in his original study; the disparity may have been due to selective referral of cases since his interest in such cases was well-known in the clinical community in his region. Knecht et al. (1996, 1998) found that mislocalization of facial stimulation to the phantom limb in our subjects occurred, but it was not topographically ordered on the phantom nor was it reproducible over time. In addition, topographically imprecise mislocalization of sensory stimulation could be elicited not only from stimulation of the face and ventral chest wall ipsilateral to the amputation stump whose representations border the amputation zone in SI, but almost equally often from the contralateral surface of the face and chest wall, which do not. Moreover, topographically precise facial remapping is apparently a relatively rare phenomenon occurring in less than 7% of the amputee population and is, therefore, of unclear general significance.

THE NEURAL BASIS OF PHANTOM LIMB: THE DISJUNCTION BETWEEN PHANTOM LIMB PAIN AND OTHER PHANTOM LIMB PHENOMENA

As noted, the type of cortical reorganization associated with PLP is not associated with other phantom limb phenomena. The reason for this puzzling disparity may lie in the results of an experiment performed in the laboratory of Edward Jones in collaboration with one of us (ET) with macaque monkeys that many years earlier had undergone serial section of the sensory roots of all the spinal nerves innervating one of their upper extremities. With immunocytochemical techniques, it was shown that the procedure had different, and in effect opposite, effects on the lemniscal component of the somatosensory system, which transmits tactile and body-position information from the spinal cord and brain stem to processing centers in the brain, and the spinothalamic component of the somatosensory system, which is the major pathway for the transmission of pain information to the brain. In the monkey lemniscal, non-nociceptive system, a loss of cells in the brain stem and thalamic nuclei was observed. In contrast, the activity of thalamic neurons in the central pain pathway increased. A down-regulation of inhibitory γ-aminobutyric acid (GABA) type A receptors in nuclei associated with the central pain system in the thalamus was also detected. With this loss of GABA inhibition, one might expect an increase in pain-related CNS activity.

The amputation of an extremity transects both motor and sensory nerves—transection of the latter resulting in deafferentation. Furthermore, most researchers believe that the phenomenon of central pain, of which the CNS component of PLP would be an example, results from an imbalance of nociceptive and non-nociceptive somatosensory inputs (Casey, 1991). If the neurological consequences of upper extremity amputation in humans are similar to those that accompany somatosensory deafferentation in macaques, then an imbalance in nociceptive and non-nociceptive inputs caused by the increase in activity in spinothalamic pathways may induce or modulate cortical somatosensory reorganization and lead to perturbations that are perceived as PLP. Another possibility is that cortical reorganization driven by the reduction in afferent input after amputation produces or contributes to the imbalance in nociceptive and non-nonciceptive inputs between the spinothalamic and lemniscal circuits. These possible scenarios may explain why the extent of cortical reorganization is related to PLP.

A role for cortical reorganization in PLP is not inconsistent with the extensive evidence that peripheral mechanisms, particularly those involving the amputation stump, also play a role. Indeed, as noted, members of our research group have confirmed the earlier observation of Sherman et al. (1984) of a positive correlation ($r = 0.53$ in our group's study) between PLP and pain experienced in an amputation stump (Lotze et al., 1999). In conjunction with our results showing cortical reorganization in SI after amputation, this correlation suggests that both central and peripheral processes may interact and contribute to phantom limb pain.

Another experiment from our group suggests the nature of the neural basis of non-painful phantom phenomena (Flor et al., 2000). During tactile stimulation of intact portions of the body in upper extremity amputees, on the occasions when mislocalization

of sensation to the phantom limb occurred, it was accompanied by: (1) elevated activity in posterior parietal cortex, which is known to be devoted to elaborating and maintaining a representation of the body and its parts (Stein, 1989; Kew et al., 1994; Bonda et al., 1995), (2) elevated activity in SI, as well as to (3) decreased activity in secondary somatosensory cortex (SII) that might be associated with a disinhibition of the activity in posterior parietal cortex and SI. Thus, non-painful phantom experiences seem to be based on a widely distributed neural network in multiple cortical regions. PLP, however, appears to have a more localized cortical basis in SI.

NATURE OF THE ASSOCIATION OF CORTICAL REORGANIZATION WITH SENSORY EXPERIENCE AND BEHAVIOR

The origin of phantom limb phenomena appears to be considerably more complex than envisioned in the Ramachandran hypothesis. However, the hypothesis was both novel and ingenious and it certainly raised the question of the possible relation between cortical reorganization on the one hand, and sensory experience and behavior on the other. Identifying the exact nature of this relationship is important, but underlying this question are the joint issues of the causality and independence of the two phenomena. Modulation of the flow of somatosensory input can produce changes in the representation of the body in primary somatosensory cortex. It is also followed by such changes in perception as the experience of PLP after amputation. Is there a causal relationship between cortical reorganization and PLP so that the two have an invariant relation, or can they be uncoupled from one another? This question is of more than academic interest. As will be described below, cortical reorganization is related to the recovery of function produced by Constraint-Induced (CI) therapy after stroke and other types of damage to the CNS. A question of considerable pragmatic import for CI therapy is whether the observed cortical reorganization is simply an epiphenomenon that occurs as a result of the increased extremity use produced by the therapy, but has no independent status or functional significance; or are the two inextricably related functionally in such a way that altering either one invariably changes the other. If the latter, one could attempt to increase the cortical reorganization associated with CI therapy by some means other than or in addition to the procedures now constituting the treatment, perhaps by pharmacological means, and thereby increase the therapeutic effect. From a practical point of view, the question of the possibility of reciprocal influence is more important than the thornier issue of causality which requires determination of invariant antecedence in time of one process by the other and evaluation of such other factors as possible multiple causation.

In the area of phantom limb phenomena, there are a number of experiments that are relevant to the question of independence and the possibility of mutual influence. Miltner et al. (1999), Taub et al. (1999), Weiss et al. (1999) demonstrated in chronic upper extremity amputees that a Sauerbruch prosthetic limb, which is operated by muscular activity of the amputation stump, eliminated PLP in five of nine subjects and reduced PLP in two other subjects, all of whom reported experiencing PLP prior to wearing the Sauerbruch prosthesis. In contrast, a group of patients who wore a cosmetic prosthesis that did not increase use of the residual limb showed no mean change in amount of PLP. Lotze et al. (1999) also found that each of four upper extremity amputees who made extensive use of a myoelectric prosthesis and had PLP before prosthesis use reported an absence of PLP after long-time use. Seven of eight subjects who either had no prosthesis, a cosmetic prosthesis, or wore myoelectric prostheses for reduced amounts of time reported a continuation of PLP. In addition, fMRI measurements revealed a correlation between cortical reorganization and amount of PLP. It is of additional interest that in another experiment two-point discrimination training on the amputation stump decreased PLP (Flor et al., 2001). The improved sensory discrimination in this study could have been due to the alterations in the cortical map that were demonstrated to occur. The observation that prolonged use of a functional prosthesis dramatically reduces PLP is of therapeutic importance, since otherwise PLP is a relatively treatment-resistant disorder. However, though the two functional prosthesis studies and the stump discrimination training experiment are suggestive, they are not conclusive in demonstrating that cortical reorganization is not an epiphenomenon with respect to PLP. It is possible that manipulation of the amputation stump affected afferent input from neuromas or other structures in the residual limb. For example, Lotze et al. (1999) showed that PLP had a moderate correlation with stump pain ($r = -0.53, p < 0.05$). Further evidence of the relation of PLP to stump pain is summarized by Sherman (Sherman et al., 1984; Sherman, 1997), though the relationship is moderate rather than strong. It is also possible that manipulation of the stump distracted attention away from perception of pain and it is this rather than the alteration of somatosensory maps in the brain that reduced PLP. The latter consideration is less likely to explain the results from another experiment from our group by Birbaumer et al. (1997). Neuroelectric source imaging was used to assess changes in cortical reorganization in SI after anesthesia of an amputation stump produced by brachial plexus blockade in six PLP patients and four pain-free amputees. Three of six phantom limb subjects in the first group experienced a virtual elimination of current PLP attributable to anesthesia that was mirrored by a very rapid elimination of cortical reorganization in somatosensory cortex. Cortical reorganization remained unchanged in three PLP amputees whose pain was not reduced by brachial plexus blockade and in the phantom pain-free amputation controls though the actual peripheral manipulation was the same in all subjects. Thus, these results show that the cortical reorganization was not simply an epiphenomenon, but instead had a functional relationship to PLP.

While these findings establish a functional link between cortical reorganization and PLP in upper extremity amputees, they do not indicate the direction of that relationship or whether it might be bidirectional. The amputation stump anesthesia greatly reduced afferent input from that portion of the body. This could have eliminated the preexisting cortical reorganization, which in turn eliminated the PLP, or oppositely, the stump anesthesia could have eliminated the PLP, which had the effect of abolishing the cortical reorganization. Alternatively, the peripheral input from the stump may have been independently

maintaining both cortical reorganization and PLP. Another possibility is that PLP and cortical reorganization after limb amputation are different manifestations of the same process; they are the same phenomenon that expresses itself with different characteristics in the different domains of CNS activity and subjective experience.

The case for a reciprocal relation between sensory experience and cortical reorganization could be made more strongly by demonstrating that it is possible to affect PLP by altering the somatosensory map through some means other than physically manipulating the amputation stump or reducing afferent input from it. An experiment by Katz and Melzack (1991) and some studies from our group are relevant in this regard (Knecht et al., 1996, 1998; Weiss et al., 2004). Katz and Melzack (1991) showed that transcutaneous electrical nerve stimulation (TENS) of the ipsilateral ear significantly reduced PLP. Since there was no manipulation of the amputation stump, the most plausible way in which this effect could have been achieved would be by affecting the invasion of the amputation zone of the somatosensory map from the neighboring intact face area. Thus, change in phantom pain perception would have been mediated by altering the somatosensory map and could not be explained by manipulating peripheral structures directly associated with the amputation stump. In the area of non-painful phantom sensation, Ramachandran's report of individual cases of topographic facial remapping, while not effectively explaining the phantom limb phenomenon does indicate that phantom sensation can be produced by stimulation of a region of the body not contiguous with the residual limb. The most plausible explanation for this phenomenon is that the reorganization of cortical somatosensory maps following the amputation established new neural connections that could be responsible for the mislocalized sensations. Knecht et al. (1996, 1998) reported routinely producing perceived sensation in the phantom by stimulating the face and ventral chest wall, whose cortical representations border the amputation zone, but rarely locations elsewhere on the body. Care was taken not to stimulate the amputation stump. In perhaps the most telling case, Weiss et al. (2004), using intact human subjects, abolished sensation from the radial and medial three-quarters of the hand by pharmacological blockade of the radial and median nerves. Magnetic source imaging indicated that the cortical representations of the little finger and the skin beneath the lower lip, which are adjacent to opposite sides of the deafferented cortical region, had moved closer together, presumably because of their expansion across the deafferentation zone. Paired-pulse transcranial magnetic stimulation revealed motor cortex disinhibition for two muscles supplied by the unaffected ulnar nerve. In addition, two notable perceptual changes were observed: increased two-point discrimination ability near the lips and mislocalization of touch of the intact ulnar portion of the fourth finger to the neighboring third finger whose nerve supply was blocked and whose cortical representation was invaded by the intact portion of the fourth finger representation. Of particular interest for the present purposes was the immediate improvement in two-point discrimination on the face by abolishing a major portion of sensation of the hand, two locations distant from one another on the body. This is a counterintuitive result that can best be understood as being mediated by changes in the somatosensory maps where they are adjacent to one another.

SUMMARY: THE FUNCTIONAL SIGNIFICANCE OF CORTICAL REORGANIZATION

The line of investigation into the functional significance of cortical reorganization following alterations in afferent input just described can be summarized as follows.

- String players with many years of practice on their instruments have an enlarged or otherwise changed cortical representation of the fingers of the hand that has the complex task of fingering the strings, while the opposite hand, which has the less dexterity-demanding task of bowing the strings, does not.
- Blind individuals who read Braille with three fingers exhibit a disordered or smeared representation of those three digits. This is correlated with an inability to reliably identify which finger is being touched during tactile threshold determinations.
- A similar phenomenon occurs in the motor control of the digits. Musicians with focal hand dystonia who have difficulty making independent movements of individual fingers also show a decreased (fused) representation of the digits in SI.
- Blind individuals exhibit a large expansion of a core area in auditory cortex. This is consistent with the increased ability of the blind to localize sounds and their increased reliance on sound to orient in space.
- The sounds experienced in tonal tinnitus have been found to be related to cortical reorganization. There was a marked shift of the cortical representation of the tinnitus frequency into an area adjacent to the expected tonotopic location.
- The magnitude of cortical reorganization in primary somatosensory cortex (SI) was found to be very strongly correlated with severity of phantom limb pain (PLP).
- Congenital amputees and amputees with traumatic amputations when adult who experienced no PLP showed minimal cortical reorganization in SI. Traumatic amputees who reported PLP exhibited massive cortical reorganization in SI correlated in magnitude with the PLP.
- A phantom limb phenomenon which is non-painful—mislocalization of sensation to the phantom limb during tactile stimulation of intact portions of the body—was found to be accompanied by elevated activity in posterior parietal cortex, which is known to mediate the perceived location of body parts in space, and in SI, as well as decreased activity in secondary somatosensory cortex (SII).
- Prolonged use of a functional prosthesis operated by muscular or myoelectric activity in an upper extremity amputation stump is very strongly correlated with a decrease in PLP, including its frequent elimination. Myoelectric prostheses are also correlated with a reduced amount of cortical reorganization in SI.
- Sensory discrimination training on the amputation stump results in a decrease in PLP.

Of particular importance with respect to the nature of the relation of cortical reorganization and sensory experience are the following findings.

- Stimulation of the face and ventral chest wall, which are physically distant from the residual limb of upper extremity amputees, but are the source of invasion of the amputation zone in SI, can give rise to sensations mislocalized to the phantom limb. This occurs very rarely in intact individuals.
- Anesthesia of an amputation stump by brachial plexus blockade was carried out in six PLP patients. Three of the six subjects with PLP experienced a virtual elimination of the PLP during the blockade while the three others did not. The three patients whose PLP disappeared showed an elimination of cortical reorganization in SI during the brachial plexus blockade while cortical reorganization remained unchanged in the three patients whose PLP was not reduced.
- Subjects with intact arms were given a peripheral nerve blockade that abolished sensation from the first three digits of a hand and from the radial portion of the fourth digit. Tactile stimulation of the intact ulnar portion of the fourth digit was frequently mislocalized to the anesthetized third digit. Of even greater interest was the fact that two-point discrimination on the face improved. Both sensory phenomena were correlated with movement of the cortical representations of the face and the intact fifth digit so that they were in closer approximation across the deafferented cortical region.

None of these experiments individually demonstrate conclusively that the alteration of cortical reorganization zones resulting from changes in afferent input has a functional relation to sensory experience or that the two phenomena can mutually influence one another. Individual experiments might be amenable to alternate interpretations. However, the combination of the many experiments involving different experimental manipulations, different domains of experience, and different methods of measurement constitutes a strong body of evidence that the two phenomena, cortical reorganization and sensory experience, can interact with one another in both directions with important consequences for the individual. Each experiment can plausibly be explained by this reciprocal functional relationship. More importantly, it is the weight of the evidence that is important rather than any individual study for giving credence to the close connection between plastic cortical change and change in perception.

TWO DIFFERENT KINDS OF CORTICAL REORGANIZATION: INPUT-DECREASE AND INPUT-INCREASE

In the pages above, many examples have been presented indicating the way in which modulating the flow of afferent input is followed by changes in the cortical representation of the body in somatosensory cortex and in other areas of the cortex for other sensory modalities. Loss of sensory input results in an invasion of the deafferentation or amputation cortical zone by innervation from adjacent still-intact portions of the body or by other intact segments of the sensorium. Loss of input may also result in a contraction of cortical representation zones, as occurs after stroke, in both SI and primary motor cortex (MI) representing the affected arm (Liepert et al., 2000) (see below), presumably resulting from the learned nonuse of that extremity. A prolonged increase in behaviorally relevant sensory input leads to an opposite result, an expansion of the cortical representation of the stimulated part of the body. Input-decrease or injury-related cortical reorganization, which is often the result of damage to the CNS (e.g., stroke, blindness) or peripheral structures (e.g., extremity amputation, presumed injury to the cochlea in tinnitus), is often related to consequences that are adverse to the organism, such as PLP, inability to correctly localize the site of tactile stimulation, and nonuse of an affected upper extremity after stroke. However, the effects of input-decrease cortical reorganization can also be positive, such as the increase in two-point discrimination on the face after pharmacological blockade of radial and median nerves in the arm. Input-increase or use-dependent cortical reorganization usually has results that are positive for the individual, such as skill acquisition. The results of neither input-decrease nor input-increase cortical reorganization are inherently adverse or positive for the individual, but in terms of the direction of territorial cortical change, they are phenomenologically opposite. However, the mechanisms involved in the two types of reorganization may be similar. The two processes mentioned most frequently in past discussions of this issue are sprouting from neighboring neural elements and unmasking of previously silent synaptic connections. To these can be added deafferentation hyperexcitability in the case of loss of afferent input, and neurogenesis in the case of input-increase cortical reorganization (Taub et al., 1995; Knecht et al., 1996). The extent to which these, and possibly other as yet unknown, mechanisms contribute to the emergence of input-decrease and input-increase types of cortical reorganization in the adult nervous system and whether these processes are different shortly after intervention and at a later time, are important issues that await resolution by future research.

An interesting example of both types of cortical reorganization taking place concurrently in the same adult nervous system as a result of a single intervention has been described by Elbert et al.(1997). Following upper extremity amputation, magnetic source imaging revealed that tactile stimulation of the lip evoked responses not only in the area of SI corresponding to the face, but also within the cortical area that would normally correspond to the now absent hand. This invasion of the cortical amputation zone in one hemisphere was accompanied by a significant increase in the other hemisphere in the size of the representation of the digits of the intact hand, presumably as a result of an increased importance of sensory stimulation consequent to a greater dependence on that hand because of the loss of the contralateral extremity.

POSSIBLE CONFUSION BETWEEN DIFFERENT TYPES OF NEURAL PLASTICITY

In a sense any change occurring in the CNS either as a result of environmental influences, metabolic activity, or the passage of time can be characterized as neural plasticity. These changes can include a large number of processes. A very partial list of them, some of which overlap, would include: learning, increase in afferent input, decrease in afferent input, synaptogenesis, neurogenesis, long term potentiation, other alterations in synaptic strength, Hebbian rewiring, axonal sprouting, increase in the density of dendritic arborization, pruning, various forms of cellular

atrophy, and so on. The multiplicity of these processes is a potential source of serious confusion when discussing possible mechanisms of a particular type of experimental outcome. The work that gave rise to the current greatly expanded interest in neuroplasticity came from the Merzenich laboratory. These experiments were discussed originally as cortical reorganization and this usage was followed by the early group of investigators in the field. This term was operationally descriptive of what was being studied. The studies described above all have to do primarily with this phenomenon. Later investigators began referring to this phenomenon in a generic sense as neuroplasticity. Cortical reorganization is certainly an example of neuroplasticity, but it has an as yet not completely known relation to some of the just-cited processes and probably no relation to some of the others. For the sake of clarity and to aid rigorous analysis, it would probably be best to return to the term originally used to describe the processes studied in the experiments described above, cortical reorganization, rather than employing the generally used but potentially confusing generic term "neuroplasticity."

CI THERAPY

PREVAILING BELIEFS PRIOR TO CI THERAPY RESEARCH

Approximately two decades before evidence was beginning to accumulate indicating that, contrary to long-standing belief, the mature mammalian brain is capable of extensive reorganization, another essentially correlative and even more strongly held belief in neurorehabilitation came into serious question. As noted in the Foreword, it was common clinical observation that following stroke or other types of substantial brain damage in humans there was typically a period of slow recovery of function that was usually greatest early after injury and then progressively slowed. At one year after injury patients were routinely observed to have reached a plateau in their motor recovery and only rarely did they exhibit any but the most modest improvement for the rest of their lives (Twitchell, 1951; Bard and Hirschberg, 1965; Parker et al., 1986). Whatever motor function patients had at that point was thought to be close to the maximum that could be achieved no matter what therapeutic intervention was employed. This view was so firmly embedded in clinical belief that it was rarely mentioned in the literature. It was just a given in the field based on general clinical experience.

ANIMAL RESEARCH: SOMATOSENSORY DEAFFERENTATION IN MONKEYS

This belief was brought into question by research with animals, though relevance to the human case was not fully appreciated at first. The matter was clearest in the case of somatosensory deafferentation research with monkeys.

When monkeys are surgically deprived of somatic sensation from a single forelimb by the serial section of dorsal roots, the monkeys do not use that limb again; there is no spontaneous recovery of purposive movement. This was a classic observation in neuroscience (Mott and Sherrington, 1895) that has received multiple replications (Sherrington, 1931; Lassek, 1953; Twitchell, 1954; Knapp et al., 1963). However, in the case of single forelimb deafferentation, it was found that this result could be reversed by the application of two behavioral techniques. If the deafferented limb is trained, especially by the behavioral technique termed shaping, or if the intact limb is given prolonged restraint for approximately one week, the monkeys use the affected extremity purposefully when unrestrained for a variety of purposes (summarized in Taub, 1977, 1980; Taub et al., 1977). The movements are not normal; they are clumsy since somatic sensation is not present to guide fine-grained coordination or to correct errors. However, the movements are very extensive; these include thumb-forefinger prehension (Knapp et al., 1963; Taub and Berman, 1968) and reasonably accurate eye-hand coordination in pointing at a visual target (Taub et al., 1975a). Thus, while the movements are not normal, they are effective. Use of the two behavioral techniques resulted in the conversion of a useless deafferented arm into an extremity that could be used extensively. This clearly constitutes a substantial rehabilitation of movement, though that term was not generally used in connection with animals. Except in the earliest experiments (Knapp et al., 1959, 1963; Taub and Berman, 1963; Taub et al., 1965), each of the animals were in the chronic phase when training began, having had surgery more than six months earlier. Thus, when the same two techniques were later applied to humans after stroke, the results with monkeys suggested that there was no reason not to try working with patients who were also in the chronic phase post-stroke, notwithstanding the traditional wisdom in the rehabilitation field that this would not be productive. If the techniques were to work at all in humans after CNS damage, there did not appear to be any reason why they would not work in the chronic phase after CNS damage as they had in monkeys.

During the course of the past century, several investigators had found that a behavioral technique can be used in animals (Ogden and Franz, 1917; Lashley, 1924; Tower, 1940; Chambers et al., 1972) or humans (Franz et al., 1915; Bach-y-Rita and Bach-y-Rita, 1990; Bach-y-Rita, 1992) to improve motor performance substantially after neurological damage (Ogden and Franz, 1917; Lashley, 1924; Tower, 1940; Chambers et al., 1972). However, in the case of the animal research none of these observations was embedded in a formal theoretical context that allowed the formulation of predictions, nor was the generality of the mechanisms clearly recognized. Consequently, these findings remained a set of disconnected observations. In the case of the human research, the reports were not accompanied by descriptions of explicit protocols that a potentially interested clinician could employ and no clear outcome measures were used. These factors probably resulted in a general lack of attention and discouraged attempts at replication.

LEARNED NONUSE

Constraint-Induced Movement therapy or CI therapy was the first clearly described and replicable neurorehabilitation technique shown to be capable of producing a substantial improvement in motor function in the chronic phase after stroke and other types of CNS injury. It was derived from the basic behavioral neuroscience research with deafferented monkeys just described. The application of the same techniques used to convert a useless deafferented forelimb to an extremity capable of extensive purposeful movement was based on a conceptual framework that emerged from the primate experiments.

The learned nonuse mechanism was proposed as a means of resolving a central enigma posed by the Mott and Sherrington experiment of 1895 (Mott and Sherrington, 1895). Why did monkeys not use a single deafferented limb? Sherrington's reasonable answer had been that extremity deafferentation interrupted the afferent arm of spinal reflexes, and it was this that abolished use of the extremity even though motor innervation remained intact. Hence the idea emerged that spinal reflexes were the basic building blocks from which behavior was elaborated; it was the fundamental tenet of Sherringtonian reflexology. This was a pervasive view in neurology for the first 70 years of the twentieth century. However, the two simple behavioral techniques noted above enabled very extensive purposive use of a deafferented limb from which all myotatic reflex activity had been abolished. This demonstration and later control experiments showed that the Sherringtonian reflexological explanation of the primate unilateral deafferentation experiments could not be correct. What then could account for the absence of purposive movement after unilateral forelimb deafferentation? The need to address that salient question led to the formulation of the concept of learned nonuse. Several converging lines of evidence suggested that nonuse of a single deafferented forelimb is a learning phenomenon involving a conditioned suppression of movement. The restraint and training techniques appear to be effective because they overcome learned nonuse. The formulation and the evidence for it are described in detail elsewhere (Taub, 1977, 1980; Taub et al., 2006b).

EARLY ATTEMPTS TO APPLY THE PRIMATE DEAFFERENTATION MODEL TO HUMANS AFTER STROKE

The initial studies of the application of therapeutic techniques tested in deafferented monkeys to humans were carried out by Ince (1969); Halberstam et al. (1971), and (Ostendorf and Wolf, 1981; Wolf et al., 1989). The first two studies involved the transfer of the simple conditioned response technique used with the deafferented monkeys that Ince had observed in Taub's laboratory (Taub and Berman, 1963; Taub et al., 1965) directly to the rehabilitation of movement of the paretic upper extremity of patients with chronic stroke. The results were positive, but the range of movements trained were limited and consequently, except in a single case, the scope of motor improvement was also limited. This is consistent with what had been observed in the primate deafferentation studies. When only a single conditioned response was trained, motor improvement was limited to that conditioned response. It was only after these two initial attempts at human application were completed that the much greater scope of motor improvement and generalization of training effect produced by the training technique of shaping (Skinner, 1938, 1968) was observed in the primate deafferentation work (Taub et al., 1975a,b).

In 1980, an article was published presenting the learned nonuse formulation and suggesting that on this conceptual basis the same two techniques used to overcome long-standing nonuse of the more-affected limb in chronically deafferentated monkeys could be transferred to humans and might be of value for improving chronic motor deficits after stroke (Taub, 1980). Wolf and co-workers used just one of the two techniques, restraint of the less-affected extremity, to induce remediation of less-affected arm function (Ostendorf and Wolf, 1981; Wolf et al., 1989). Though the effect size was small ($d' = 0.2$), it was reliable. There was no report of whether the improvements transferred to the life situation. However, the results appeared promising, especially since training had not been used and there was some question of compliance by some patients with the instruction to wear the restraint device, a sling, for most of waking hours during the intervention period. This type of intervention involving only use of a restraint device is termed *Forced Use* therapy; it is not CI therapy since it consists of only one of the four primary components of CI therapy (see below).

DEMONSTRATION OF EFFICACY OF CI THERAPY AT THE UNIVERSITY OF ALABAMA AT BIRMINGHAM (UAB)

Taub et al. (1993, 2006a) employed both the more-affected arm training and contralateral arm restraint portions of the protocol used with deafferented monkeys, and also used a set of behavioral techniques termed the *transfer package* (Morris et al., 2006; Taub et al., 2006a,b, 2013b). Training of the more-affected arm in the laboratory was carried out using shaping, as was done in the later deafferented monkey studies. This approach, i.e., CI therapy, was applied to the rehabilitation of persons with a chronic upper extremity hemiparesis in two studies (Taub et al., 1993, 2006a) that employed attention-placebo control groups and emphasized transfer of therapeutic gains in the laboratory to the life situation. As noted, patients with chronic stroke were selected as subjects for this study because in the primate deafferentation research, substantial motor rehabilitation was possible well into the chronic phase. In addition, according to the research literature at the time, there was no evidence that any treatment could produce further recovery of function one year after stroke. Therefore, any marked improvement in the motor function of individuals with chronic stroke after an intervention that lasted just two weeks would be of particular therapeutic significance. After a long-standing plateau, the probability would be very low that an abrupt, large improvement in motor ability could be due to spontaneous recovery.

The subjects in the first two experiments in this laboratory were patients with chronic stroke who had experienced CVAs from one to twenty years earlier (mean = 4 years) (Taub et al., 1993, 2006a). Patients were randomly assigned either to an experimental group or a placebo comparison group. The treatment patients received all aspects of the CIMT protocol described below. The control patients were given a placebo procedure. All experimental and control patients had passed a minimum motor criterion before intake into the study (Wolf and Binder-Macleod, 1983); they could be characterized as having a mild/moderate level of deficit. The treatment groups demonstrated a significant increase in motor ability as measured by a laboratory motor test (Wolf Motor Function Test or WMFT) (Wolf et al., 2001; Morris et al., 2001) over the treatment period ($p < 0.01$); more importantly they showed a very large increase in spontaneous arm use in the life situation over the two-week period as measured by the Motor Activity Log (MAL) (Taub et al., 1993), an instrument with strong clinimetric properties (Uswatte et al., 2005, 2006b). In the larger of the two studies (Taub et al., 2006a), the amount of

spontaneous real-world more-affected arm use went from 9% of the amount of use of that extremity compared to before stroke at pre-treatment to 52% at post-treatment, an approximate 5 times increase. The subjects had approximately 80% retention of improved arm use when tested two years after treatment. Thus, the improvement was long-term. The control patients exhibited no change or a decline in arm use over the same period in both experiments.

These results have been confirmed in a large, multisite randomized clinical trial (RCT) in patients 3-9 months after stroke, i.e., the EXCITE trial (Wolf et al., 2006, 2008), and several hundred other smaller studies (Langhorne et al., 2009; Stevenson et al., 2012). Studies that have used attenuated (Butefisch et al., 1995; Foster et al., 1996; Peter and Leidner, 1997; Van der Lee et al., 1999; Dromerick et al., 2000; Charles et al., 2001; Gritsenko et al., 2001; Johnson et al., 2001; Kedlaya et al., 2001; Liepert et al., 2001; Platz et al., 2001) or partial versions (Ince, 1969; Halberstam et al., 1971; Ostendorf and Wolf, 1981; Wolf et al., 1989) of the full protocol have reported positive results but had smaller gains than the two RCTs from our laboratory. The usual missing component was the transfer package (TP, described below). Where our methods were replicated in laboratories set up with the help of and monitored by one of us (ET), the results were very similar (Kunkel et al., 1999; Miltner et al., 1999; Sterr et al., 2002). CI therapy for stroke patients with mild to moderate deficits has now entered clinical practice, with insurance reimbursement provided by a few but not all companies, and is becoming a regular part of the curriculum in physical and occupational therapy academic programs (Morris and Taub, 2010). Consistent with the increasing clinical acceptance of CI therapy, there has been a steady and substantial growth in peer-reviewed publications on CI therapy trials, reviews, and basic neuroscience research over the past 20 years (**Figure 4**). As of this writing, 523 peer-reviewed papers have been published.

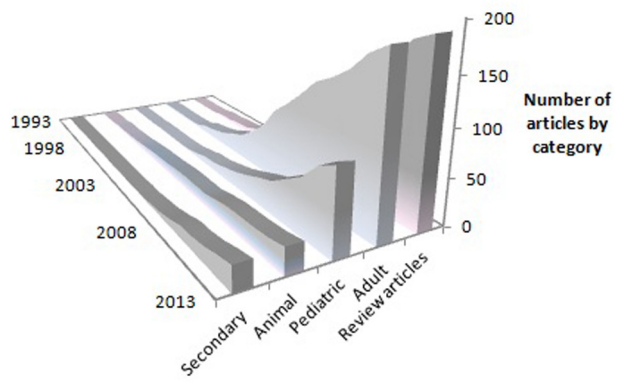

FIGURE 4 | Cumulative growth in peer-reviewed journal articles on CI therapy according to year of publication. Primary-data reports are represented by the "Adult," "Pediatric" and "Animal" categories. "Secondary" indicates articles that present subsequent analyses of previously published primary data. "Review articles" represent overviews or discussions of CI therapy, or proposals for new trials prior to data collection.

CHRONICITY AND AGE AT TREATMENT

The longest delay between CNS insult and treatment in this laboratory/clinic was 50 years; the stroke occurred when the individual was five years old and he was 55 when treated. The magnitude of his improvement was as great as the laboratory average where mean chronicity is 4 years (Taub et al., 2006a). Similar results have been obtained with many patients who received treatment from 20 to 50 years after stroke.

The potential for improvement persists unchanged throughout the lifespan. Several patients have been in their 90s and many have been in their 80s; their treatment effects did not differ in magnitude from individuals in their 20s or teens.

COMPONENTS OF CI THERAPY

The upper-extremity CI therapy protocol, as currently practiced in the UAB laboratory, consists of four basic components (Taub, 2004; Taub et al., 2006a,b): (1) intensive training of the more-affected arm for multiple days; (2) training with a behavioral technique termed shaping; (3) the transfer package (TP), a set of behavioral techniques designed to facilitate transfer of therapeutic gains from the treatment setting to daily life; and (4) discouraging behaviors that compensate for the nonuse or reduced use of the affected function.

To discourage use of the less-affected arm to compensate for the reduced effectiveness of the more-affected arm after stroke, a padded mitt is worn on the less-affected hand to prevent its use for a target of 90% of waking hours for the entire treatment period. (The target number of hours is less for more-impaired patients who must use an assistive device to walk safely). The amount of time the device is worn is recorded by a timer inserted in the device. A resting hand splint and sling ensemble was used in early experiments. It has been found that restraint of the less-affected arm is the least important component of CI therapy, and can be dispensed with entirely if the training conditions are arranged appropriately (Taub et al., 1999; Sterr and Freivogel, 2003; Ploughman and Corbett, 2004; Uswatte et al., 2006a). For CI Aphasia therapy the use of gestures and nonverbal sounds is strongly discouraged, and for Lower Extremity CI therapy, physical restraint of a body part is also not used.

Shaping is a training method in which a motor or behavioral objective is approached in small steps by "successive approximations" (i.e., a task is gradually made more difficult with respect to a participant's motor capabilities). Its principles were explicitly formulated by Skinner (Skinner, 1938, 1968) and they have been applied to the rehabilitation of movement in this laboratory (Taub et al., 1993, 1994). For rehabilitation, shaping involves providing immediate and very frequent feedback concerning improvements in the quality of movement and frequent encouragement. Further details of the shaping process employed can be found elsewhere (Taub et al., 1994; Taub and Uswatte, 2006; Morris and Taub, 2010).

The TP consists of a set of techniques in common use in the behavior analysis field for the treatment of a variety of conditions, but they have not been used systematically in rehabilitation. The TP techniques used here are: behavioral contracts, daily home diary, daily administration of the Motor Activity Log to track amount and quality of use of the more-affected arm in 30

important activities of daily living (ADL), problem solving to overcome perceived barriers to more-affected arm use in ADL performance, written assignment during treatment of practice at home both of tasks carried out in the laboratory and use of the more-affected arm in specified ADL, post-treatment home skill practice assignments, and weekly telephone calls for the first month after laboratory treatment in which the MAL is given and problem solving carried out. These techniques have been described elsewhere (Taub et al., 2006b, 2013b). It might be emphasized here that virtually all of the CI therapy articles in the literature report treatment effects that are positive, but they are typically considerably smaller than those reported from this laboratory. However, it is rare for these other studies to use all of the components of CI therapy as enumerated above. A recent study from this laboratory (Taub et al., 2013b) replicates the smaller treatment effects reported in many of the articles in the CI therapy literature, but shows that smaller treatment effects occur when either shaping or the elements of the transfer package (especially the latter) are omitted from the treatment protocol.

SEVERITY OF DEFICIT

Most of the patients treated at UAB could be characterized as having deficits that were mild/moderate, defined primarily as having the ability to extend 20° at the wrist and 10° at each of the metacarpophalangeal joints of the fingers (i.e., Grade 2 according to a classification system used in this laboratory based on active range of motion) (Taub et al., 2013a). Experiments have also been carried out with patients with moderate and moderately severe deficits (Grades 3 and 4) (Taub et al., 1999). Their treatment change for spontaneous use of the arm in the life situation was somewhat less than for higher functioning patients, e.g., increases of approximately 400 and 350% for patients with moderate and moderately severe deficits, respectively, compared to approximately 500% for patients with mild/moderate deficits, but the treatment changes were nevertheless very large. Most recently, work has been carried out with patients with useless, plegic hands that were initially fisted (Taub et al., 2013a; Uswatte et al., manuscript submitted for publication). The standard CI therapy protocol was supplemented with some conventional physical rehabilitation procedures, including some from neurodevelopmental treatment (NDT), and functional electrical stimulation (FES). The adjuvant procedures were used to maintain the fingers in a sufficiently extended and aligned position so that CI therapy training procedures could be carried out. At the end of treatment, the patients exhibited a 186% improvement in the real-world use of the more-affected arm. It had been converted into a useful "helper" in the life situation (e.g., keeping a piece of paper in place while writing with the less-affected hand, holding a toothpaste tube while unscrewing the cap, bearing body weight for bed mobility). We estimate that CI therapy is applicable to at least 50% of the chronic stroke population with motor deficit, perhaps more.

LEARNED NONUSE FORMULATION AS THE ORIGIN OF THE MULTIPLE APPLICATIONS OF CI THERAPY

The concept of learned nonuse (LNU) was developed in the context of primate deafferentation experiments. As noted, it was proposed as an alternate to the reflexological explanation of the results of unilateral forelimb deafferentation, which those experiments showed could not be correct (Taub, 1977, 1980; Taub et al., 2006b). However, LNU was not formulated as being specific to the case of somatosensory deafferentation. The central tenet was based on the regional loss of neuronal excitability observed to follow any substantial damage to the CNS. Thus, if the LNU formulation was correct, as the experimental tests of two counterintuitive predications seemed to indicate (Taub, 1977, 1980; Taub et al., 2006b), then it ought to apply to other types of CNS damage. This line of reasoning led initially to the attempt to improve motor deficit after stroke in humans by the same two techniques that had been employed with unilaterally deafferented monkeys: intensive training of the more-affected arm and restraint of the less-affected arm (with shaping and the transfer package techniques added). Once the LNU formulation and the techniques used to overcome LNU after deafferentation in monkeys were shown to be applicable to humans after stroke (Taub et al., 1993), the extension of these techniques to motor deficits resulting from other types of damage to the CNS in humans was straightforward. The LNU formulation predicted, even required, that they be efficacious. Thus, after the initial work with patients with chronic stroke, the CI therapy protocol was applied to improve the motor deficit after a number of other types of damage of the CNS. Each of those attempts has been successful to date and they include: traumatic brain injury (Shaw et al., 2003), multiple sclerosis (Mark et al., 2008), cerebral palsy and pediatric motor disorders of neurological origin across the full range of age from one year old through the teenage years (Taub et al., 2004, 2007, 2011).

In a substantial number of stroke patients, speech becomes very effortful and often embarrassing because of halting and slow verbal production and incomplete understanding. The person compensates by greatly reducing attempts to speak, by remaining entirely silent and using gestures and other nonverbal means of communication, or by allowing caregivers to take over speaking for them (Croteau and Le Dorze, 2006). The demonstration that motor deficits are modifiable in chronic stroke raised the possibility that verbal impairment could also be rehabilitated by an appropriate modification of the CI therapy protocol. Indeed the LNU formulation predicted that this was a strong possibility. In an incomplete translation of the CI therapy protocol used for improving motor deficits, aphasic patients with chronic stroke who had previously received extensive conventional speech therapy and had apparently maximally recovered in their language ability were induced to talk and improve their verbal skills using a single exercise involving shaping for three hours each weekday over a two-week period. There was no physical restraint. The intervention was formulated by Pulvermüller and Taub and was termed Constraint-Induced Aphasia therapy (CIAT I), and the results were positive (Pulvermüller et al., 2001). This study has since been replicated (e.g., Bhogal et al., 2003; Meinzer et al., 2004, 2007; Maher et al., 2006; Kirmess and Maher, 2010). However, this intervention was only an incomplete translation of CI Movement therapy. The initial aphasia treatment protocol was modified to more closely resemble the CIMT protocol (Johnson

et al., 2014). To date, 6 patients have been treated with the new protocol (CIAT II). Their results have considerably exceeded those obtained with CIAT I and are comparable to the results obtained with CIMT.

A final point to be made is that the use of the CI therapy protocol to improve the motor deficit after stroke stems primarily from the LNU formulation, as does each of its subsequent applications to other pathological conditions. The fact that these predicted applications have been successful constitutes an additional source of evidence in support of the LNU formulation.

An adaptation of CI therapy has been used to treat lower limb impairments, first after stroke, then after spinal cord injury, fractured hip (summarized in Taub et al., 1999) and multiple sclerosis (Mark et al., 2013). Approximately 90% of patients with chronic CVA ambulate but may do so with a degraded pattern of coordination. These disordered patterns may be partly due to the persistence of degraded patterns of movement learned in the early postinjury period and "locked in" by permitting ambulation and thereby being rewarded, before spontaneous recovery of function would have enabled an improved mode of ambulation. This phenomenon may be viewed as *learned misuse* rather than *learned nonuse*. For the leg, the less impaired extremity is not restrained because under these conditions the resulting ambulation would involve simply substituting one degraded pattern of coordination (i.e., gait with one leg prevented from having full movement) for another. Patients are given intensive shaping to promote an improved pattern of walking and other uses of the legs for many hours on each weekday over a period of three weeks, and they also receive a transfer package of techniques equivalent to that used for the upper extremity, to facilitate translation of improvements achieved in the treatment setting to everyday activities in the life situation. The results from 48 patients to date have been virtually as good as for the arm. Initially, we thought that it might be more difficult to overcome learned misuse than learned nonuse, if it was possible at all. In the case of learned misuse, bad habits of coordination need to be overcome before more appropriate patterns of coordination can be substituted. In the case of learned nonuse of the upper extremity after stroke, there is simply an absence or greatly reduced amount of extremity use in the life situation; surmounting improper coordination as an initial step is not a primary problem. We were surprised that our expectation of a substantially reduced lower-extremity treatment outcome proved to be incorrect.

Another application of the CI therapy protocol made on a non-theoretical basis and unrelated to LNU was to focal hand dystonia in musicians (Candia et al., 1999, 2002). This intervention was based on the fact that CI therapy not only overcomes LNU so that a patient can more fully make use of the impaired motor function that he still retains in the activities of daily living, as revealed by the MAL, but it also improves the impaired motor function. It was the latter aspect of CI therapy that was thought to make it appropriate for use to correct the incoordination of the digits that occurs in focal hand dystonia. This expectation was confirmed. In another non-theoretical extension of CI therapy, the increased use element of the intervention was used to reduce PLP after amputation (Weiss et al., 1999).

For further details on the various forms of CI therapy including details of their protocols, results, and the nature of the modifications from the basic CI Movement therapy paradigm that enabled its broad application, the reader is referred to recent review articles (Taub and Uswatte, 2009; Uswatte and Taub, 2013) and to the data papers cited.

AFFERENT-INCREASE CORTICAL REORGANIZATION ASSOCIATED WITH CI THERAPY

In a seminal series of studies described above, Merzenich et al. showed that increased use of a limb and the resulting increase in afferent inflow leads to an expansion of the cortical representation zone of that body part in new-world monkeys (Jenkins et al., 1990; Recanzone et al., 1992a,b,c). It was also noted above that Elbert, Taub, Flor, and coworkers (Elbert et al., 1994, 1997; Braun et al., 2000) reported that the same phenomenon occurs in humans. Importantly, it has also been shown in the experiments described above that the altered cortical topography in response to increased use of an affected body part has functional significance for the function of the individual. As the initial experiments of CI therapy were being carried out, it became apparent that this intervention involved a substantial increase in the use of the body part being treated. Therefore, it seemed plausible that cortical reorganization would occur as a result of CI therapy and might in turn be at least partly responsible for its therapeutic effect. This type of consideration had already been entertained some years before and was the basis of an NIH grant awarded to one of us (ET) and M. Goldberger in 1980 to study the effect of somatosensory deafferentation in monkeys on collateral axonal sprouting in the spinal cord and brain. It was thought that this might provide an explanation for some of the behavioral phenomena that had been observed in these animals. This research was unexpectedly interrupted after the monkeys had been surgically prepared but before results could be obtained. It was these animals who 12 years later were subjects in the experiments by Pons and coworkers (1991) and from the laboratory of E. Jones (Rausell et al., 1992; Woods et al., 2000) reported on above.

Consistent with this line of analysis it was found that CI therapy-type interventions involving training of extremity use after a CNS injury results not only in improved extremity function, but in reorganization of brain activity. Nudo et al. demonstrated this first in new world monkeys (Nudo et al., 1996). They showed that the area surrounding a motor cortex infarct that would not normally be involved in control of the hand came to participate in that function at the same time that performance on an experimental task involving manual dexterity improved. This animal study was followed by an elegant series of confirmatory experiments. Parallel collaborative studies with humans were carried out by one of us. For example, in adults whose upper extremity function had been enhanced by CI therapy after stroke, Liepert et al. (1998, 2000) used focal transcranial magnetic stimulation to show that the cortical representation of an important muscle of the hand (abductor pollicis brevis) was greatly enlarged. CI therapy had led to an increase in the excitability and recruitment of a large number of neurons in the innervation of movements of the more-affected limb adjacent to those originally involved in control of that extremity prior to treatment. At about the same

time, Kopp et al. (1999) using EEG source imaging, obtained similar findings and also found that the motor cortex ipsilateral to the more-affected arm, which normally controls movements of the less-affected arm, had been recruited to generate movements of the more-affected arm. The finding that CI therapy is associated with substantial changes in brain activity was confirmed in other early studies in which one of us (ET) collaborated involving the Bereitschafts (readiness) Potential (Bauder et al., 1999) and positron emission tomography (Wittenberg et al., 2003). To date, there have been more than 20 studies, many involving functional magnetic resonance imaging, that have obtained similar results (summarized until 2006 by Mark et al., 2006).

These studies employed functional brain imaging and brain mapping techniques to demonstrate that CI therapy could alter the *function* of specific brain regions. The question remained whether CI therapy could measurably alter brain *structure* in humans. Starting at the beginning of the first decade of this century it was shown that experienced taxi drivers have significantly expanded hippocampi (Maguire et al., 2000), jugglers acquire significantly increased temporal lobe density (Draganski et al., 2004), and thalamic density significantly declines after limb amputation (Draganski et al., 2006). Moreover, in an animal model of stroke, CI therapy combined with exercise reduced tissue loss associated with the brain damage (DeBow et al., 2003). Accordingly, structural imaging studies became a logical next step toward understanding the nature of the CNS changes that follow administration of CI therapy and whether any changes that occur are correlated with clinical improvements.

Longitudinal voxel-based morphometry (pre- vs. post-treatment) was performed on subjects to evaluate the contribution of the TP to CI therapy clinical outcome (Gauthier et al., 2008). It was found that structural brain changes paralleled changes in amount of use of the impaired extremity for activities of daily living. Groups receiving the TP showed profuse increases in gray matter tissue in sensorimotor cortices both contralateral and ipsilateral to the more-affected arm, as well as in bilateral hippocampi. The aforementioned sensorimotor clusters were bilaterally symmetrical and encompassed the hand/arm regions of primary sensory and motor cortices as well as the supplementary motor area and portions of Brodmann's area 6 (**Figure 5**, left side). It was of importance that increases in gray matter were significantly correlated with increases in use of the more-affected arm in daily life, as measured by the MAL, for the sensorimotor clusters on both sides of the brain and the hippocampus region of interest (r's > 0.45). Groups that did not receive the TP showed relatively small improvements in real-world arm use and failed to demonstrate gray matter increases. Thus, the change in the brain's morphology was directly related to administration of the TP which in turn substantially increased the amount of real-world use of the more-affected arm. The fact that the anatomical change is directly related to the TP lends increased credibility to the importance of the TP. The type of motor improvement associated with the increase in gray matter resulting from CI therapy in adults after stroke (**Figure 5**, left panel) is shown in **Figure 6**.

In another study (Sterling et al., 2013), children with hemiparetic cerebral palsy also showed increases in gray matter in the bilateral sensorimotor cortices (**Figure 5**, right side). These changes showed a strong correlation with improvements in spontaneous real-world arm use as recorded on the pediatric version of the MAL. More restricted gray matter increases occurred in children than in adults. This finding is consistent with previous research which has shown that, compared to children, adults show significantly more widespread cortical activation when a manual

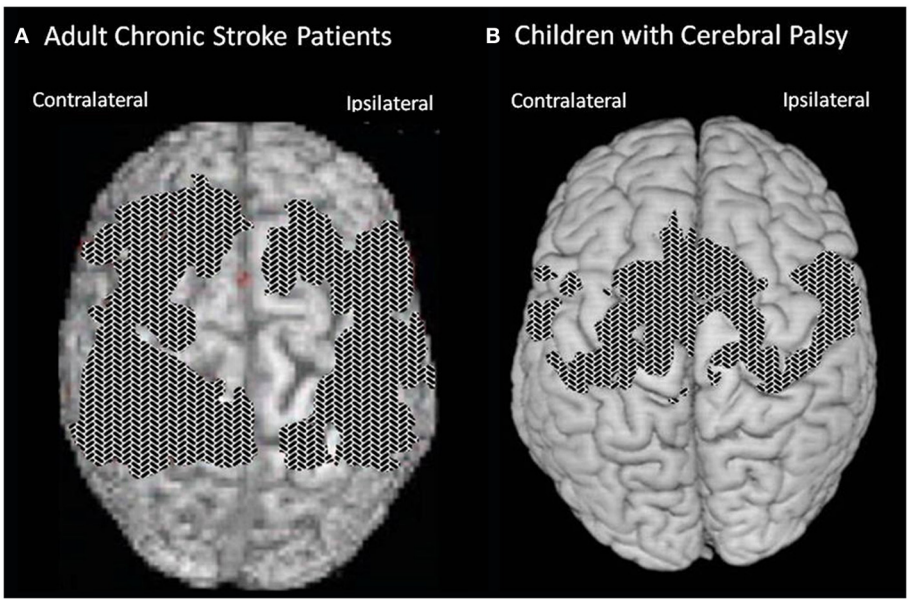

FIGURE 5 | Cortical surface-rendered image of gray matter change after CI therapy in (A) adults with chronic stroke and (B) children with hemiparetic cerebral palsy. Gray matter increases displayed on a standard brain. Surface rendering was performed with a depth of 20 mm. Cross-hatched areas indicate t statistics ranging from 2.0 to 6.7. Corrected for family-wise error.

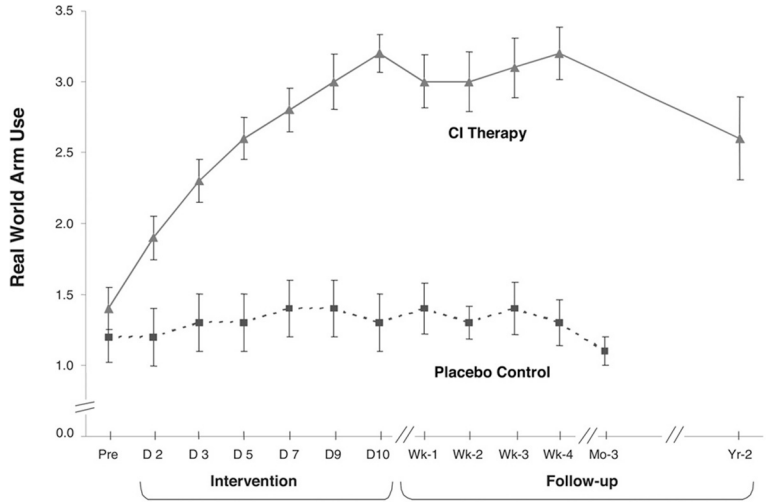

FIGURE 6 | Mean MAL arm use scores from CI therapy ($n = 21$) and placebo control ($n = 20$) patients with chronic stroke. CI therapy subjects showed a very large improvement in arm use outside the laboratory from pretreatment to post-treatment (1.8 ± 0.6; $P < 0.0001$; $d' = 3.0$). Before treatment the data indicate that these patients were using the more affected arm 14% as much as before stroke, while after 2 weeks of treatment it was 52%, an almost 4 times increase. Controls showed little change. In follow-up, CI therapy subjects retained all of their immediate treatment gains 4 weeks after therapy and showed only a 23% decrease after 2 years from post-treatment levels of real-world arm use. Reprinted from Taub et al. (2006a).

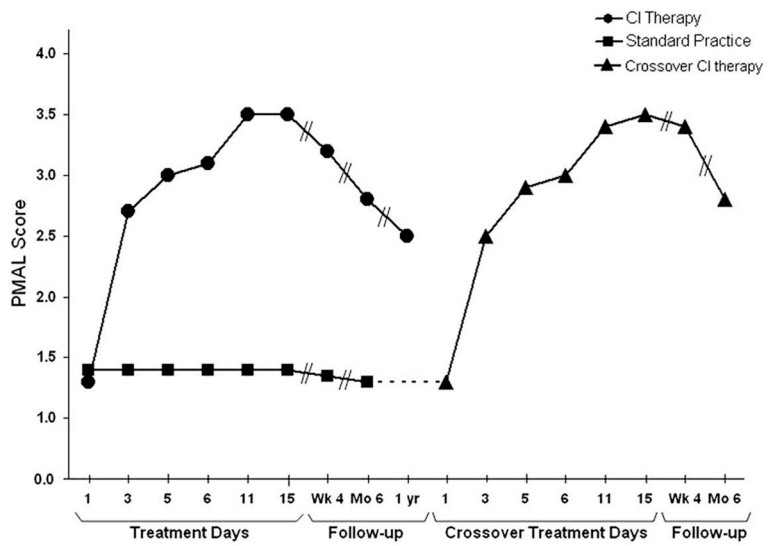

FIGURE 7 | Spontaneous use of the more impaired arm (Pediatric Motor Activity Log score) of young (2-6 years) children with hemiparetic CP receiving Constraint-Induced Movement therapy or standard occupational therapy. Data for the Constraint-Induced Movement therapy group are shown before and during treatment and 1, 6, and 12 months after treatment. Data for the control subjects are shown at corresponding times for 6 months after treatment, at which time they were crossed over to Constraint-Induced Movement therapy. After crossover data are shown for treatment and 1 and 6 months afterward. The data are similar to those for adults shown in **Figure 6**. For both the children given CI therapy first and those given the intervention after crossover, the amount of spontaneous use of the more affected arm in the activities of daily living in the real-world environment increased from approximately 15% compared to use of the less affected arm to approximately 65%. Reprinted from Taub et al. (2011).

task is performed including not only bilateral sensorimotor cortices, which also occurs in children, but more anterior motor areas as well (Mall et al., 2005). The motor improvement that was associated with the increase in gray matter resulting from CI therapy in young children with CP (**Figure 5**, right panel) is shown in **Figure 7**.

It is not possible to make a causal attribution regarding the observed cortical structural changes after CI therapy and improvement in motor function. The gray matter increase could be either a cause or an effect of increased motor ability and behavioral change, or it could simply be an independent accompaniment. However, the correlation between increases in

gray matter volume and magnitude of motor improvement does raise the possibility of a causal relationship. Moreover, the earlier research on the functional significance of cortical reorganization by Elbert et al. described above strongly suggests that cortical reorganization can have a causal role in the function of an individual and this could apply to CI therapy as well. Future research with either animals or humans in whom CI therapy is administered and cortical structural change is either enhanced or suppressed by some means other than changes in use, for example by administration of a pharmacological agent, may resolve this issue conclusively.

In both the adult and pediatric CI therapy studies increases were observed in the volume of the posterior portion of the hippocampus, which may have included the adjacent subventricular zone. The hippocampus is known to be involved in learning and memory and these two processes are associated with the improved limb use that occurs with CI therapy; the hippocampus is also responsive to amount of physical exercise. Evidence also indicates that stem cells are located at this site in the adult mammalian brain (Eriksson et al., 1998; Yamashima et al., 2004) and simulated stroke in animals can increase the quantity of these cells (Yamashima et al., 2004). One might speculate that the increases in gray matter observed in the hippocampal region and possibly in the sensory and motor areas of the brain are mediated in part by increased production of neuronal stem cells that might participate in the migratory repair of an infarcted area (Kolb et al., 2007). Alternatively, or in addition, gray matter increases may result from rehabilitation–induced increases in dendritic arborization, synaptic density (Briones et al., 2006), and possibly gliosis or angiogenesis. Determining which of these processes or combination of processes is responsible for the observed increase in gray matter following CI therapy awaits future research.

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Harnessing the power of neuroplasticity for intervention

Bryan Kolb* and Arif Muhammad

Canadian Centre for Behavioural Neuroscience, University of Lethbridge, Lethbridge, AB, Canada

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Antoni Rodriguez-Fornells, University of Barcelona, Spain
Edward Taub, University of Alabama at Birmingham, USA

****Correspondence:***
Bryan Kolb, Canadian Centre for Behavioural Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada
e-mail: kolb@uleth.ca

A fundamental property of the brain is its capacity to change with a wide variety of experiences, including injury. Although there are spontaneous reparative changes following injury, these changes are rarely sufficient to support significant functional recovery. Research on the basic principles of brain plasticity is leading to new approaches to treating the injured brain. We review factors that affect synaptic organization in the normal brain, evidence of spontaneous neuroplasticity after injury, and the evidence that factors including postinjury experience, pharmacotherapy, and cell-based therapies, can form the basis of rehabilitation strategies after brain injuries early in life and in adulthood.

Keywords: brain plasticity, neurorehabilitation, recovery of function

Over the past 20 years, our understanding of how the brain is changed by experience, usually referred to as *neural plasticity*, has exploded. Once believed to be fairly constant in its organization and function, it has become clear that the brain is inherently capable of changing after injury to enable at least some behavioral restitution. What is less clear, however, is what factors might potentiate (or attenuate) the endogenous response to injury and what rules might guide the reparative changes.

Although the idea of brain plasticity is well recognized in neuroscience and rehabilitation, it is not easily defined and can refer to many changes ranging from behavior to molecular events such as gene expression (e.g., Shaw and McEachern, 2001). Nonetheless, it is possible to identify some general principles that can guide us in harnessing the power of neural plasticity to design new rehabilitation strategies.

CHALLENGES IN STUDYING NEURAL PLASTICITY AND BEHAVIOR

CORRELATION IS NOT CAUSATION

By its nature, behavioral neuroscience is correlational. Consider an example. If a laboratory animal is trained in some type of motor task such as reaching for food with fine forelimb movements, we can look for accompanying changes in the structure of cells in the putative motor circuit (e.g., Withers and Greenough, 1989). Now if we administer a drug such as nicotine while animals learn the task we may find enhanced learning and a correspondingly larger change in the neural circuit (e.g., Gonzalez et al., 2006). But what caused what? The drug may have acted directly on the motor circuit making it easier to change, or the drug could have had some less direct effect such as increasing activity, which in turn enhanced the behavior and synaptic changes. Furthermore, just because neurons change with the learning does not mean that those changes have anything to do with the memory of the task *per se*, but could simply reflect the motor activity inherent in performing the task. As a result, behavioral studies seeking to link behavioral and synaptic changes are often criticized for "simply showing correlates." The problem is even larger when we are studying the effects of treatments on motor recovery after brain injury. Stroke may produce motor deficits and these deficits may be reduced with a treatment such as nicotine and this may be correlated with synaptic changes. There is little doubt that the drug changed behavior but we do not know what caused the synaptic changes.

One solution is to try to break the correlations and see if the behavioral change persists. For example, if we can prevent the structural changes but the behavior still improves, then they are not related. But even if we cannot break the correlation, we still do not have proof of causation. The challenge is to prove how the synaptic changes arose, such as by showing that a change in gene expression is caused by a treatment and that the epigenetic change leads to the behavioral changes. For the majority of studies, this is impractical. Proving causation in behavioral neuroscience is an extremely difficult process but for the purposes of designing treatments to facilitate improvement from brain injury, it probably does not matter. As a beginning it is sufficient to demonstrate that behavior is improved and that the improvement is associated with changes in the brain.

WHAT IS RECOVERY?

It is common to refer to functional improvement after brain injury as "recovery." But this term is ambiguous. It might imply a complete return of function, a marked improvement in function, or just some improvement. It is rare indeed for true return of the pre-injury state to occur. We like to refer to this as the "three-legged cat problem." If a hindlimb of a cat is seriously injured, it is not uncommon for a veterinarian to amputate the leg. Postoperatively the cat has great difficulty in moving around but over a period of months eventually adapts and can be surprisingly agile. Has the cat recovered? No. True recovery would require the regrowth of the missing limb. Rather, the cat has compensated for the lost limb. It would be simple to design a task to demonstrate things that the cat would have difficulty doing. Nonetheless, for the most part the cat adapts well. A similar phenomenon occurs after brain injury. Functional improvement

may occur but there is still dead or dysfunctional tissue. The person has learned to cope with the disability, either physically or cognitively. As we search for mechanisms underlying functional improvement we need to wary of thinking that there is true recovery because this may mislead our investigations (see review by Alaverdashvili and Whishaw, 2013).

WHAT IS THE BEST LEVEL OF ANALYSIS?

Research on the neural basis of rehabilitation can be conducted at many levels of analysis beginning with behavior, and moving increasingly more reductionistic to neural imaging and electrophysiological recording from the scalp, cerebral mapping, invasive physiological recordings, neuronal morphology, genetics and epigenetics, and finally to proteins and other molecules. The choice of level will depend upon the question being asked. For example, if the goal is to develop pharmacological therapies, the appropriate level will likely be more molecular in order to examine synaptic mechanisms or structure. On the other hand, to understand how compensatory neural circuits are organized it makes sense to examine neural circuitry using neural imaging or to identify changes to sensory or motor maps. The key point here is that there is no "correct" or "best" level. Studies need to be at all levels if we are to understand how to design the best therapies.

WHAT ARE APPROPRIATE ANIMAL MODELS?

Although many studies can be undertaken in humans without prior preclinical studies in laboratory animals, our understanding of the mechanisms underlying the neural basis of rehabilitation must usually begin in the laboratory using animal models. The choice of the best animal model is problematic. One significant issue is that larger brains, such as humans, have a relatively larger amount of white matter compared to gray matter than smaller-brained mammals that are normally used in laboratory studies (Zhang and Sejnowski, 2000). One consequence of this is that humans are more likely to have white matter injury than lab animals with similar gray matter injury. Approximately 25% of human strokes involve subcortical white matter injury and although there are several animal models that attempt to mimic human white matter injury, to date none are ideal (Hainsworth and Marjus, 2008; Sozmen et al., 2012). The failure to adequately mimic human white matter injury is problematic in trying to model the neural basis of rehabilitation. Specifically, a real question is whether treatments that are effective in stimulating enhanced compensation in laboratory animals with gray matter injury generalize to people with white matter injury.

A second problem is related to how results are generalized from lab animals to human patients. Animal studies generally have well-defined injuries with far less variance than in human conditions. In addition, animal studies rarely include very large injuries because smaller injuries generally show a much better response to therapies than larger injuries. Human clinical trials typically choose patients with larger injuries, presumably because they are perceived to have greater need of help, even though the proof of principle in a translation to humans might be easier to demonstrate in people with less severe symptoms.

A third problem relates to models outside primary sensory or motor regions. It is common to make unilateral injuries when studying treatments for motor cortex injuries but bilateral injuries when studying cognitive functions such as those performed by the prefrontal cortex, posterior parietal cortex, or medial temporal regions. Bilateral injuries are needed in such studies in rats because unilateral lesions produce only very small deficits. In contrast, humans normally have only unilateral injury to prefrontal, posterior parietal or temporal regions but they can have quite substantial behavioral symptoms. Given that studies of rats with unilateral motor cortex injury have shown that the intact contralateral hemisphere plays a significant role in functional improvement (e.g., Jones and Schallert, 1992; Gonzalez and Kolb, 2003), we might predict a similar mechanism with unilateral prefrontal injuries. Indeed, this may be the reason that the unilateral lesions in rodents show such minimal functional effects. One possible solution here is to focus the animal studies on the neural compensation rather than the behavior, although this seems counterintuitive.

BRAIN INJURY MAY HAVE EFFECTS ON SOMATIC ORGANS

One assumption of most studies of rehabilitation and neural compensation is that changes in the nervous system are closely related to functional outcomes. There are a few clinical reports, however, that suggest some relationship between stroke and somatic organ function, and especially kidney function (e.g., Losito et al., 2012). In most such studies the implications are either that cardiovascular problems and renal problems either share predisposing conditions (e.g., high blood pressure) or that renal problems increase the risk of stroke. Another possibility, however, is that brain injury induces functional changes both in brain and somatic organs. A recent study looked at molecular epigenetic changes in kidney, heart, and liver of rats who had suffered ischemic stroke (Kovalchuk et al., 2012). They found changes in all three organs but the effects were largest in the kidney where they found changes in methylation, acetylation, gene expression, and microRNA expression that were still present 4 months poststroke. These changes may significantly alter kidney function, which in turn may influence the course of functional improvement. This study serves as a warning that rehabilitation programs may need to be wary of the effects of stroke on distal tissues and organs.

GENERAL PRINCIPLES OF PLASTICITY IN NORMAL BRAIN

Before we address treatments that enhance functional outcomes and neural plasticity after brain injury we will briefly review key principles of plasticity in the normal brain.

NEURAL PLASTICITY IS FOUND IN ALL NERVOUS SYSTEMS AND THE PRINCIPLES ARE CONSERVED

All animals, including very simple ones like *C. elegans*, can show various forms of learning, which is correlated with neuronal plasticity (e.g., Ardiel and Rankin, 2010). This plasticity includes both pre- and postsynaptic changes and are remarkably similar to those observed in animals with much more complex nervous systems. It is believed, for example, that there are NMDA-like changes in learning in both invertebrates and mammals (e.g., Roberts and Glanzman, 2003). There are likely differences in the details, such as the nature of gene expression changes and second messengers but the general principles appear to be conserved across diverse phyla. This is important because it allows researchers to use a wide range of models to search for the neural mechanisms of plasticity.

A WIDE VARIETY OF EXPERIENCES ALTER THE BRAIN THROUGHOUT THE LIFESPAN

Virtually any experience can change the brain, especially if there is an associated behavioral change (**Table 1**). We learn and remember, create new thoughts, and behavior changes throughout our lifetime. Changing behavior requires changes in the neural circuits that underlie it. For example, there are many studies showing that learning neuropsychological tasks is associated with synaptic changes in regions believed to be requisite for the learning (e.g., Greenough and Chang, 1989; Kolb et al., 2008). Similarly, repeated exposure to psychoactive drugs can result in long-lasting changes in behavior, which is correlated with large alterations in morphology of neurons in many brain structures including medial and orbital prefrontal cortex, nucleus accumbens, caudate-putamen, and hippocampus (see **Figure 1**) (e.g., Robinson and Kolb, 2004). Indeed, it is the capacity of drugs such as amphetamine and nicotine that have led to their use as treatments for brain injury (see below). Indeed, the role of experiences in general in changing the brain is especially important as we search for treatments for brain injuries.

Table 1 | Factors affecting the synaptic organization of the normal brain.

Factor	Example reference
1. Sensory and motor experience	Greenough and Chang, 1989
2. Task learning	Comeau et al., 2010
3. Gonadal hormones	Mychasiuk et al., 2012
4. Psychoactive drugs	Robinson and Kolb, 2004
5. Neurotrophic factors (e.g., NGF, FGF-2)	Monfils et al., 2008
6. Natural rewards (e.g., sex; social interaction)	Fiorino and Kolb, 2003
7. Prenatal experiences	
7. Social play	Bell et al., 2010
8. Aging	Kramer et al., 2004
8. Stress	McEwen, 2005
9. Anti-inflammatories (e.g., COX-2 inhibitors)	Silasi and Kolb, 2007
10. Diet (e.g., choline)	Meck and Williams, 2003
11. Electrical stimulation:	
Kindling	Teskey et al., 2006
LTP	Monfils et al., 2004
LTD	Monfils and Teskey, 2004
Surface cortical stim	Adkins et al., 2008

PLASTIC CHANGES ARE AREA DEPENDENT

It is generally assumed that powerful experiences such as complex housing would produce similar neuronal changes. In fact, it is not uncommon for investigators to choose one structure (often the hippocampus) as a surrogate for how experiences are altering the brain. It has been therefore surprising to us that this is wrong. For example, when one compares the effects of amphetamine on the medial prefrontal and orbital prefrontal cortex, parietal cortex, nucleus accumbens, CA1 of the hippocampus, and the dentate gyrus the effects are wildly different (Crombag et al., 2005). Thus, whereas neurons in the medial prefrontal cortex, nucleus accumbens, and CA1 show increased spine density, neurons in the orbital frontal cortex show a decrease in spine density and the other structures no change at all (see **Figure 2**). Similarly, when Mychasiuk et al. (2012) examined the effects of prenatal stress on gene expression in hippocampus and medial prefrontal cortex they found over 100 genes changed in each region but there was virtually no overlap in which genes changed. Using one structure or the other (or blood) as a surrogate marker for epigenetic change throughout the brain is clearly misleading.

The area-dependent nature of plastic changes in the brain has obvious implications for developing therapies for brain injury. The therapies need to be informed by where the changes are hoped to occur.

THE DEGREE OF PLASTIC CHANGE IS RELATED TO BOTH THE RELEVANCE OF AN EXPERIENCE AND THE INTENSITY OR FREQUENCY OF THE EVENTS

Experiences that highly relevant to an animal are likely to produce much more rapid neuronal changes than less relevant experiences. Fowl become imprinted on a moving object appearing shortly after their hatching, which in the natural world would normally be a parent. There are immediate changes in the chick's hyperstriatum after visual imprinting including decreased spine density, increased NMDA receptor density, and increased immediate early gene expression (e.g., Horn, 2004). In contrast, experiences that are perceived as irrelevant may not lead to neural changes, even with extensive experience. An example can be seen in the training of pigeons to peck an illuminated key to obtain food vs. training to peck a key to avoid an aversive stimulus. Pigeons come prepared to associate pecking with food but not with aversion avoidance. In the latter case they appear to be unable to learn to avoid shock by pecking. Although we are unaware of any studies searching for plastic changes in the avoidance-type paradigm, it is a safe bet that there would be few if any to be found.

One factor related to relevance is motivation. One reason that laboratory animals are placed on mild food deprivation schedules when they learn tasks is because hungry animals are more highly motivated to solve the tasks to obtain food reward than sated ones. Both listening to and learning music can be a strong motivator for people and provides benefits for stroke recovery (e.g., Sarkamo and Soto, 2012; Grau-Sanchez et al., 2013).

Not only is relevance important, but so is intensity. For example, drug studies show that low doses of psychomotor stimulants produce more restricted synaptic changes than high doses (e.g., Diaz Heijtz et al., 2003). A similar effect can be seen with the duration of complex housing on cortical pyramidal cells (e.g., Greenough and Chang, 1989).

Both these phenomena are directly relevant to designing rehabilitation programs. For example, Kollen et al. (2006) reviewed treatment outcomes after stroke and concluded that the optimal rehabilitation programs encorporate high intensity therapy with a strong emphasis on functional training for relevant tasks. These studies had no measure of brain changes but the behavioral benefits of the therapy provide a strong suggestion that the treatment did alter cerebral organization.

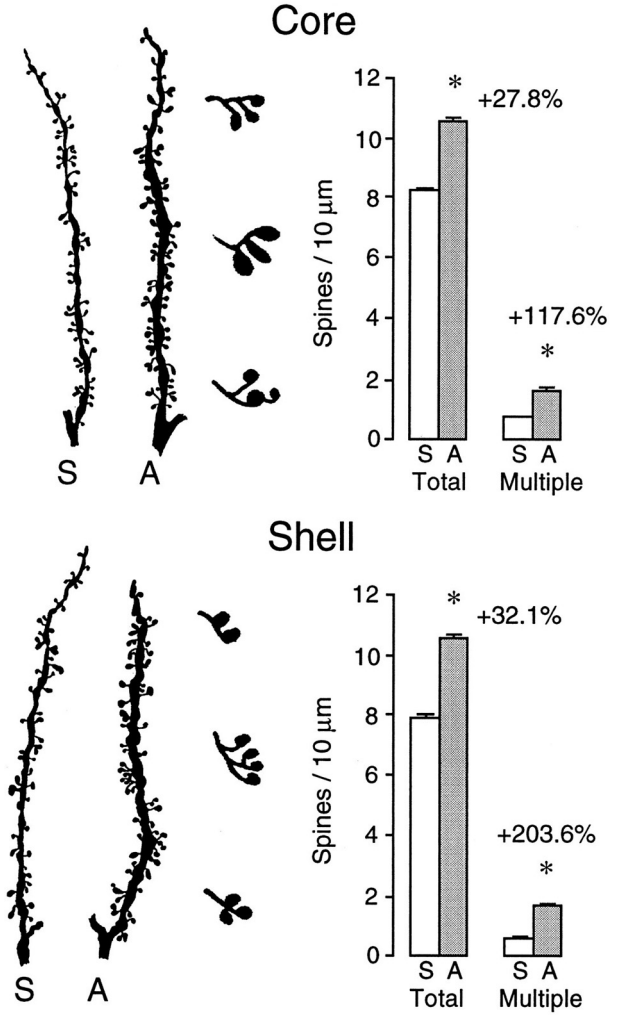

FIGURE 1 | Camera lucida drawings of representative segments from medium spiny neurons in the core (top) and shell (bottom) subregions of the nucleus of saline- or amphetamine-treated rats. The drawings to the right of the segments illustrate multiple heading spines, which show a very large drug-induced increase (After Robinson and Kolb, 1997). *$p < 0.01$.

PLASTIC CHANGES ARE TIME-DEPENDENT

Plastic changes can change over time. We noted above that one of the most powerful experiences to change neural networks is exposure to psychomotor stimulants such as amphetamine and nicotine. Cocaine has similar actions: it increases spine density in both medial frontal cortex and nucleus accumbens as effectively as amphetamine (Robinson and Kolb, 1999). After a month of abstinence the effect persists, just as it does for amphetamine and nicotine. However, in contrast to the latter drugs, which show persisting effects after 3 months of abstinence, the effects of cocaine are largely dissipated (Kolb et al., 2003a,b,c), a result consistent with the behavioral and neurophysiological effects of cocaine (Henry and White, 1995). Not only does the effect of cocaine diminish over time, it has recently been shown that it is possible to reverse the effects of cocaine on synaptic function in nucleus accumbens by administering a cystine prodrug, N-acetylcysteine (Moussawi et al., 2011). This result is obviously important for its implications in treating cocaine dependence but more generally in that it shows that neural plastic changes can be reversed.

Time-dependent changes can be seen in many other paradigms as well. Comeau et al. (2010) placed rats in complex environments for varying lengths of time. They then compared the dendritic changes in medial prefrontal and parietal cortex. Previous studies had shown that after 90 days of such housing there were large dendritic changes in parietal cortex but virtually none in prefrontal cortex (Kolb et al., 2003a,b,c). The Comeau et al study found although there was no dendritic change visible after 14 days, there were significant changes after 4 days. The opposite was true in parietal cortex—there were clear changes at 14 days but none at 4 days. This result was unexpected because although it was anticipated that plastic changes might have area-dependent schedules of change, it was not anticipated that one change would replace another. This result has implications for the design of rehabilitation programs. Different brain regions may respond to treatments following a different timetable.

EXPERIENCE-DEPENDENT CHANGES INTERACT

Although laboratory studies are often designed to present animals with a single large experience (such as a drug), in real life experiences are not singular events. We have experiences beginning *in utero* and continuing throughout life. The effects of these experiences accumulate, a process referred to as metaplasticity (e.g., Abraham, 2009). Basically, metaplasticity reflects a change in the biochemical, physiological, or morphological state of neurons or synapses that alters their subsequent ability to change state. Little is known about these interactions but if we are to understand why brain-injured patients respond differently to treatments, we need to recognize that people have different histories, including drug histories.

Most laboratory studies of metaplasticity have used some type of priming experience such as a drug or hormone followed minutes or days later high frequency electrical brain stimulation to induce long-term potentiation. Another way to study metaplasticity in the laboratory is to provide animals with one significant experience (such as a drug) and then later train animals on behavioral tasks or place them in complex environments. Both experiences are known to produce large changes in cerebral organization but what happens when they are combined? The short story is that previously exposing animals to amphetamine, cocaine, or nicotine (and even prenatal nicotine) dramatically attenuates or blocks the later effect of complex housing or behavioral training (e.g., Kolb et al., 2003a,b,c; Hamilton and Kolb, 2005; Mychasiuk et al., 2014). Similarly, when Muhammad et al. (2011) gave infant rats tactile stimulation with a fine brush 15 min per day from birth until weaning they found an attenuated response to amphetamine in adulthood. They chose tactile stimulation because like drugs and complex housing, tactile stimulation produces large changes in dendritic organization in multiple cerebral regions (e.g., Richards et al., 2012). The found a marked attenuation of the effects of amphetamine given in adulthood. Thus, it appears that drugs can reduce the effects of later experiences but early experiences can also reduce the effects of drugs. This may become important when we try to translate the effects of drugs

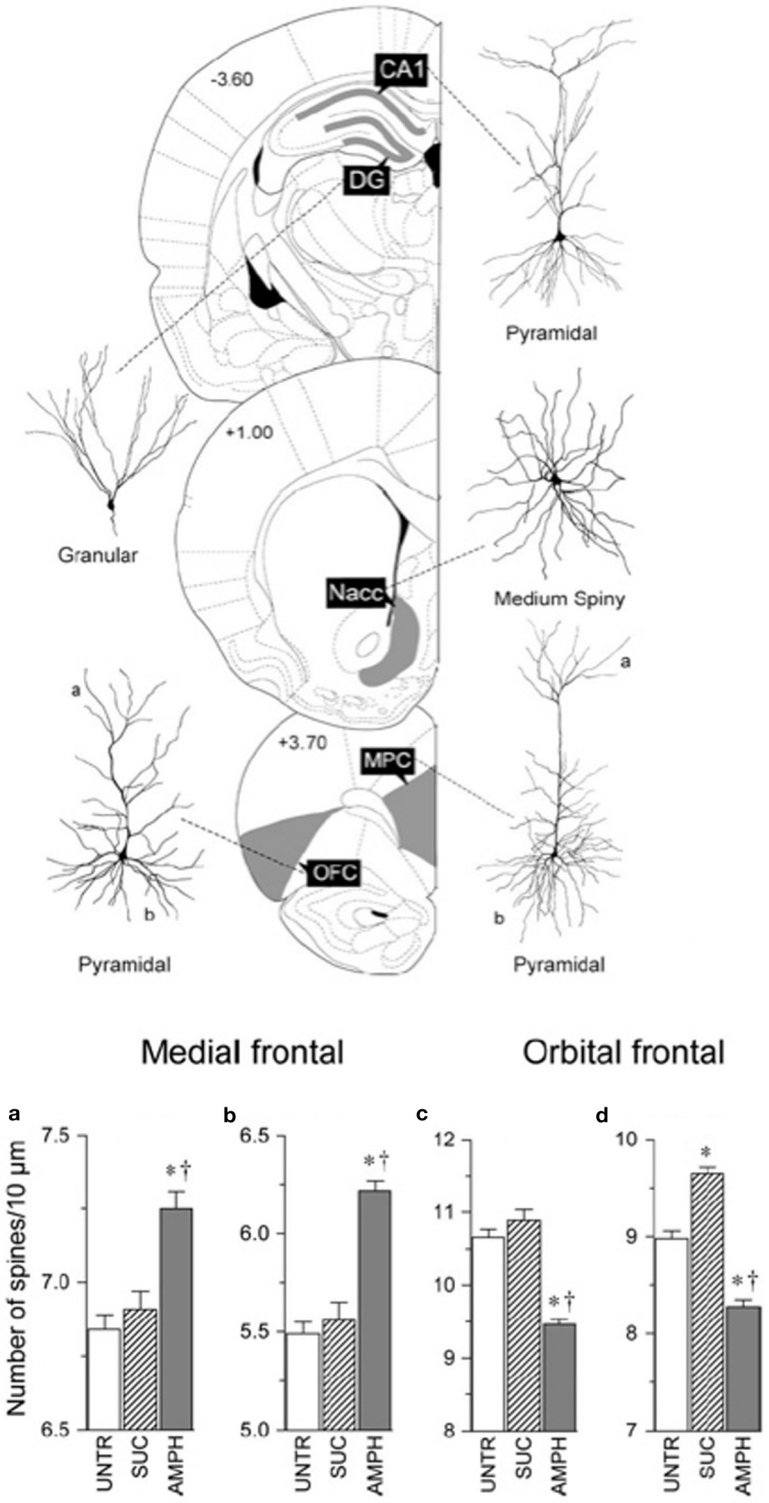

FIGURE 2 | Top: Illustration of location of, and cell morphology, for the analysis of the effects of amphetamine on neuronal structure. **Bottom**: Summary of the contrasting effects of amphetamine on the medial frontal and orbital frontal cortex. Abbreviations: UNTR, untrained; SUC, trained to bar press for sucrose; AMPH, trained to bar press for amphetamine (After Crombag et al., 2005). *Differs from control, $p < 0.05$; †differs from sucrose, $p < 0.05$.

on recovery in animal studies to humans with a rich lifetime of experiences that may modulate the action of the drugs.

The idea of metaplasticity is likely related to the concept of cognitive reserve, which refers to the differences in cognitive capacity in older people related to a lifetime of intellectual activities (e.g., Barulli and Stern, 2013). For example, Verghese et al. (2003) showed that extensive participation in leisure activities in older people (>75 year) is associated with a reduced risk of dementia. The hypothesis is that cognitive reserves stemming from previous learning experiences play a protective role in coping with neurodegenerative diseases. The idea can be extended to hypothesize that cognitive reserve might also have the benefit of enhancing the efficacy of rehabilitative strategies after brain injury, which would be an example of metaplasticity.

PLASTIC CHANGES ARE AGE-DEPENDENT

The developing brain is more responsive to experiences than the adult brain but there are qualitative differences in response to similar experience at different ages. It is known, for example, that stress in adulthood decreases spine density in medial prefrontal cortex but increases it in orbital frontal cortex (Liston et al., 2006). In contrast, however, prenatal stress produces just the opposite pattern in the adult brain, namely an increase in medial prefrontal cortex and a decrease in orbital frontal cortex (Muhammad et al., 2012). Similar age-dependent qualitative changes can be seen in the effects of complex housing (Kolb et al., 2003c). When adult animals are placed in such environments there is a general increase in spine density in cortical pyramidal cells whereas similar experience at weaning leads to a decrease in spine density. The age-dependent nature of synaptic change is clearly significant when we consider most effective treatments neurological disorders at different ages such as in pediatric vs. adult disorders.

BOTH PRENATAL AND PRECONCEPTUAL EXPERIENCE CAN ALTER THE ADULT BRAIN

Although the brain continues to develop long after birth, it has become clear in the last decade that prenatal and preconceptual experiences can profoundly alter the adult brain. Exposure to psychoactive drugs, including prescription drugs, stress, and complex housing all change synaptic organization of the prefrontal cortex (e.g., Kolb et al., 2012a,b; Muhammad et al., 2012; Mychasiuk et al., 2013). In addition, mild prenatal stress increases global methylation in prefrontal cortex and hippocampus (Mychasiuk et al., 2011a,b) and alters gene expression in a sexually-dimorphic and and region-specific manner in brains examined at weaning (Mychasiuk et al., 2011a,b). The frontal cortex changes were largely related to neurotransmitter function, whereas hippocampal changes were more prominent in females and concentrated around growth factors. It seems likely that the preconceptual experiences could influence the brain's later response to injury.

Although there have been many reports that parental experiences could influence epigenetics and subsequent health in the offspring (e.g., Barker, 1998; Kaati et al., 2002; Pembrey, 2004), it is only recently that experiences of the parents have been shown to affect epigenetics in the brain of offspring (Mychasiuk et al., 2011a,b, 2013). For example, Mychasiuk et al. (2013) found that paternal stress alters offspring behavior and DNA methylation patterns in a sexually-dimorphic manner, presumably because of experience-dependent effects on spermatogenesis. The authors suggest that brain development is influenced not only by postnatal experiences but is also influenced by earlier maternal and paternal experiences, which combine to produce the various phenotypes and individual differences that we perceive in closely related individuals. Once again, such differences are likely to influence how the brain responds to injury and rehabilitation much later in life.

NOT ALL PLASTICITY IS GOOD

Most studies of brain plasticity and behavior emphasize the idea that plastic changes can support improved cognitive and motor function. But plastic changes are not all good and can interfere with behavior. For example, it is likely that drug-related alterations in prefrontal cortex and other brain regions could underlie some of the maladaptive behavior observed in drug addicts. Another example can be seen in focal hand dystonia in musicians.

Focal hand dystonia refers to abnormal finger and hand positions, cramps, and difficulty in coordinating hand and finger movements. Dystonia can be so disabling that some musicians must give up their occupation. Dystonia is most common in instruments, such as stringed instruments, that require maximal fine finger movements. Although the precise cause of dystonia is still debated, one hypothesis is that it results from extensive practice of the more intensively used hand, which likely results from a distortion of the somatosensory and/or motor maps in the cortex (Candia et al., 2003). Another hypothesis is that the practice leads to abnormal activation patterns in the premotor cortex (Kadota et al., 2010), which in turn could be related to abnormal cortical maps. In either event, the dystonia symptoms appear to result from pathological plasticity.

There are many other examples of pathological plasticity including dementia (Mattson et al., 2001) epilepsy (Teskey, 2001), and pathological pain (Baranauskas, 2001). It is not known if there is pathological plasticity after brain injury but one suggestive example comes from Nudo et al. (1996) who showed that without rehabilitation after injury to the motor cortex, the area regulating hand movements becomes smaller—a phenomenon sometimes referred to as a disuse syndrome. With rehabilitation the hand area retains its cortical representation. The reversal of this type of pathological plasticity is presumably important in understanding the success of restraint-induced therapies.

HARNASSING PLASTICITY FOR NEUROREHABILITATION

The studies of neural plasticity in normal animals provide a launch pad to develop new strategies for designing rehabilitation programs (for an extensive review, see Cramer et al., 2011). Our basic assumption is that experiences that change the normal brain will likely produce similar, and hopefully even larger, changes in the injured brain. This is often the case but not always. For example we have shown that tactile stimulation in early development has a profound effect on neural organization and behavior (e.g., Richards et al., 2012) so we anticipated that tactile stimulation might be a good treatment for brain injury, which it is (see

Richards et al., 2012, below). What we had not expected, however, was that the synaptic changes in normal and brain-injured animals would be qualitatively different and that different injuries would respond with quite different changes. Thus, whereas there was a *decrease* in spine density in cortical pyramidal neurons in sham operates, which was correlated with enhanced motor and cognitive skills, there was an *increase* in animals with perinatal prefrontal injuries (see **Figure 3**). Nevertheless, the latter animals showed remarkable functional recovery in both cognitive and motor behaviors (e.g., Kolb and Gibb, 2010). Curiously, animals with posterior parietal injuries showed no effect of the treatment on spine density: they had an increase in dendritic length instead (not shown), which was also correlated with functional improvement. These results illustrate our ignorance of brain plasticity and behavior. In some ways we might think of brain injury followed by a treatment as a form of metaplasticity. The injury stimulates spontaneous reparative changes that then interact with our treatment.

As we consider the factors summarized in **Table 1**, and how they might be used to design rehabilitation treatments, we can group them into four somewhat arbitrary categories: postinjury experience, pharmacotherapy, cell-based therapy, and diet. Our focus here will be on studies of laboratory animals and mostly will use synaptic changes inferred from Golgi studies as a measure of brain plasticity.

BEHAVIORAL EXPERIENCE

There is a rich history of treatments on physiotherapy for skeletomuscular dysfunctions so it is reasonable to design treatments for brain injury based upon such treatments. For example, patients could practice impaired behaviors with the goal of relearning or improving functions. Historically, most such therapies have been based upon hunch and habit rather than RCTs or preclinical studies. The success of such studies is difficult to quantify but a large international meta-analysis by Teasell et al. (2006) reached five basic conclusions. First, interdisciplinary rehabilitation is beneficial over spontaneous recovery. Second, rehabilitation has no impact on mortality although it does influence quality of life. Third, there is conflicting evidence over which therapies are beneficial and many appear to have little direct benefit other than giving the patients a goal and social interaction. Fourth, there is strong evidence that greater intensity of therapies is beneficial over the short run. Fifth, there is no evidence regarding the timing and duration of therapy. In fact, one of the clear problems related to timing and duration is the willingness of insurers, whether they are private or government-based, to fund extensive rehabilitation. We can add one additional conclusion, which is that even if studies appear to be beneficial there is little known about why. That is, the mechanism whereby the treatment affects plasticity is usually unknown. It is here that the animal studies may inform us.

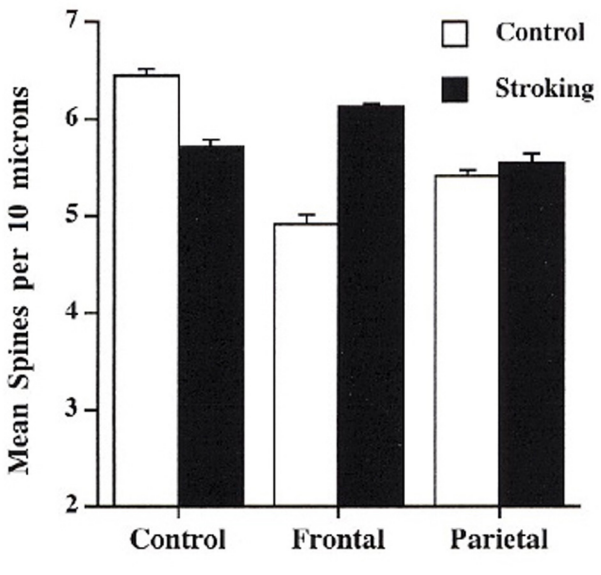

FIGURE 3 | Effect of tactile stimulation (stroking with a fine brush) on spine density in control, day 3 frontal injury, and day 3 parietal injury in rats (After Kolb and Gibb, 2010).

The single most effective postinjury experience in brain-injured laboratory animals is complex housing (for a review, see Kolb and Whishaw, 1998). This type of experience has been successfully used to facilitate functional restitution from experimental brain damage of varying etiologies (e.g., Kolb and Elliott, 1987; Will and Kelche, 1992; Johansson, 1996; Biernaskie and Corbett, 2001). The nature of the complex housing varies but the key features appear to be a novel and changing environment, a lot of exercise, and social interaction. The effects of this type of experience can be seen even in gross measures such as brain weight as well as dendritic measures such as length and complexity. In addition, there are non-neuronal changes too such as increases astrocyte number and complexity, and even in angiogenesis. The benefits of this experience is surprisingly general, including chronic improvements in both cognitive and motor functions.

Why is complex housing so beneficial? One clue comes from evidence of altered gene expression in the brain (Rampon et al., 2000). Changes in gene expression may alter the production of a variety of proteins, such as those involved in the synthesis of neurotrophic factors known to facilitate synaptic plasticity (e.g., Johansson, 2000).

How does this translate to humans? One constraint is on duration of the therapy: it is impractical to implement treatments that are 24 h/day for humans. This duration is likely not necessary in the animal studies, but it is convenient experimentally. Nonetheless, an effective treatment for humans would have to be intense, daily, and interdisciplinary including cognitive, social, and physical therapy. A useful laboratory animal experiment would be to titrate the amount of complex housing, perhaps comparing the effectiveness of 2, 4, 6, and 24 h/day.

A second type of effective experience in changing neural circuitry is tactile stimulation (e.g., Richards et al., 2012). Both adult and infant rats benefit from tactile stimulation using light touch with a fine brush several times daily for 15 min for 2–3 weeks after the brain injury. Both adult and infant rats with motor cortex or prefrontal injuries show marked functional improvements (e.g., Gibb et al., 2010; Kolb and Gibb, 2010). This is correlated with changes in dendritic length or spine density in cortex adjacent

to the injuries. As shown in **Figure 3**, the rats with infant lesions showed a reversal of the decrease in spine density related to the injury. In contrast, rats with adult lesions, who showed extensive atrophy of adjacent cortical pyramidal neurons, showed an increase in dendritic length, not shown by shams, related to the tactile stimulation.

Recently, Livingston-Thomas et al. (2013, 2014) devised a novel behavioral approach, which they called voluntary forced use movement therapy. Rats were placed in plastic pet activity balls for 30 min per day for 21 days beginning 5 days after ischemic stroke. The therapy resulted in small but consistent acceleration of forelimb performance in several behavioral tests. The functional improvement was associated with an increase in migrating neural precursor cells originating in the subventricular zone as well as increased expression of BDNF (brain-derived neurotrophic factor) that were presumed to be in microglia.

Few laboratory studies have examined the beneficial effects of physical therapy on recovery. One emerging strategy is to implement specialized training protocols, with the best known being constraint-induced movement therapy for the arm and hand (Wolf et al., 2002). More recently robotic devices (e.g., Hidler et al., 2009) behavioral shaping, bilateral arm training (Lin et al., 2010), body weight-supported treadmill training (Dobkin et al., 2006; Duncan et al., 2007),task oriented physical therapy (Jonsdottir et al., 2010), and music therapy (Schneider et al., 2007) have also proven effective. The reasons for the effectiveness of these treatments is unknown but they presumably lead to synaptic changes that may be identified in mapping studies using noninvasive imaging or intracortical transcortical magnetic stimulation (TMS). Indeed, a recent study by Amengual et al. (2013) used music-supported therapy followed by TMS and found improved motor functions correlated with plastic changes in the form of increased cortical excitability following the training.

PHARMACOTHERAPY

We showed above that many drugs are extremely effective in stimulating neuroplastic changes in cerebral neurons, and especially psychomotor stimulants. Early studies by Feeney and colleagues (e.g., Feeney and Sutton, 1987) used amphetamine as a postinjury treatment following unilateral motor cortex injury and found striking benefits provided that the animals received physical therapy while under the influence of the drug (see also Goldstein, 2003). One complication of the amphetamine treatment is that lesion size, location, and route of administration appear to influence efficacy. Moroz and Kolb (2005) compared the effect of amphetamine on focal and extensive unilateral ischemic injuries finding that whereas amphetamine was useful in the former condition, it was not helpful with the larger injuries. This may explain why clinical studies with amphetamine have had mixed success. Clinical studies tend to select patients most in need of therapy, which are those with larger injuries. (Alaverdashvili et al., 2007) made small lesions similar to those of Moroz and Kolb but administered the same dose (1 mg/kg) orally rather than subcutaneously. They found no beneficial effect of the drug.

In unpublished studies we used amphetamine after bilateral medial frontal lesions, finding no benefit on the performance of cognitive tests. Given that all of the positive preclinical studies using amphetamine have been with animals with motor cortex injuries, the jury is still out on whether it will be effective for cognitive improvement. As mentioned above, it is usually necessary to make bilateral injuries in rodents to obtain robust cognitive deficits. Thus, it may not be the motor/cognitive distinction that is important but rather the unilateral/bilateral difference.

Nicotine has also proven effective in stimulating postinjury functional improvement after ischemic motor cortex injury (Gonzalez et al., 2006). A low dose of nicotine was given subcutaneously twice daily for 12 days following the ischemia. Functional recovery was followed on several behavioral tasks for 7 weeks. All behaviors showed a benefit from the treatment, which was correlated with synaptic changes both ipsilateral and contralateral to the ischemia (see **Figure 4**). Once again, route of administration may be important. Lim et al. (2009) found no benefit of nicotine on skilled reaching after similar strokes to Gonzalez et al but they administered the same dose (0.3 mg/kg) orally rather than subcutaneously.

Other types of pharmacotherapies have taken advantage of compounds that enhance axonal sprouting after cerebral injury. One example is an antibody to NoGo-A, a myelin-associated inhibiting axonal regeneration after central nervous system injury (for a review, see Kempf and Schwab, 2013). Papadopoulos et al. (2006) showed that administration of an antibody to NoGo-A stimulated axon generation and increased synaptogenesis in cortical pyramidal neurons (see **Figure 5**). These morphological changes were correlated with functional recovery on measures of skilled reaching. Hamadjida et al. (2012) performed a parallel study of motor cortex injury in monkeys who received NoGo-A. They found functional improvement in the treated animals along with sprouting and/or sparing of callosal projections to premotor cortex on the lesion side compared to untreated monkeys.

Because NoGo-A is an endogenous molecule found in oligodendrocytes and some neurons, it is reasonable to wonder if the expression of NoGo-A might be affected by other therapies. Zhao et al. (2013) treated rats with motor cortex ischemia with constraint-induced therapy for 3 weeks poststroke. After removal of the constraint, the animals were significantly improved on a beam-walking test and this was correlated with increased crossings of contralateral corticospinal axons to the denervated cervical spinal cord and a reduction in the expression of Nogo-A receptor in the peri-infarct cortex.

Another compound that has proven effective is inosine, a compound that regulates axon outgrowth through changes in gene expression. Several studies have shown that inosine stimulates the projection of new axons from the undamaged side of the brain to denervated areas of midbrain and spinal cord (Chen et al., 2002; Smith et al., 2007; Zai et al., 2009).

Not only is inosine effective alone, it is synergistic in enhancing recovery of skilled reaching when given in combination with either antibodies to NoGo-A or complex housing (see **Figure 6**; Zai et al., 2011). The level of performance was one of the few examples of performance on a skilled task returning to preoperative levels following motor cortex injury plus treatment. Unfortunately, the authors did not do kinematic analysis so it is unclear how normal the actual movements were.

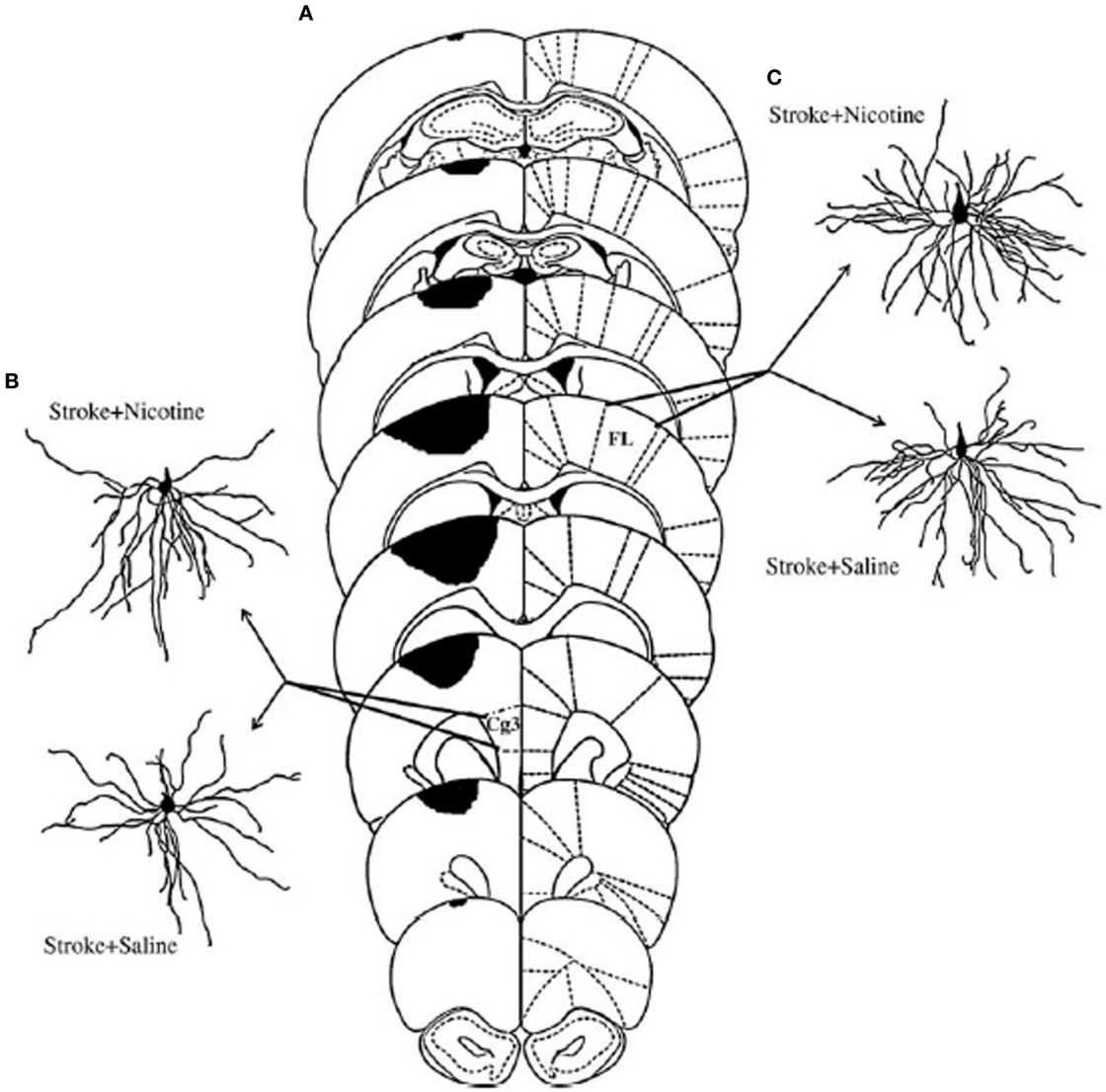

FIGURE 4 | (A) Extent of ischemic injury across 8 planes of measurement. **(C)** Illustrate representative camera lucida drawings of layer V pyramidal neurons in anterior cingulate cortex (Cg3) ipsilateral to the lesion **(B)**, or forelimb area (FL) in the contralesional hemisphere **(C)** from animals treated either with saline or nicotine. Increased dendritic length in the nicotine-treated animals was correlated with improved functional outcome (After Gonzalez et al., 2006).

CELL-BASED THERAPY

The possibility that mammalian brains can generate neurons after an injury can be traced to Altman (1962) but it was not until Reynolds and Weiss (1992) isolated and identified multipotent stem cells from the adult forebrain that more recent interest was spurred in the potential therapeutic value of stem cells. There are now many papers showing that following a stroke in rodents there is an enhanced proliferation within the SVZ followed by neuronal migration toward the ischemic regions (Arvidsson et al., 2002; Parent et al., 2002; Jin et al., 2003; Zhang et al., 2004). This spontaneous proliferation is not sufficient to restore functions, however. But what if it were possible to increase this spontaneous proliferation in some way? There appear to be two primary ways: have an early brain injury or infuse growth factors.

In the early 1990s we noticed that rats with medial frontal lesions around 7–12 days of age sometimes appeared to regenerate the lost tissue but this did not happen either before or after those ages. Following the paper of Reynolds and Weiss we hypothesized that perhaps the spontaneous regeneration was due to a redirection of newly generated neurons from the rostral migratory stream to the olfactory bulb into the region of injury (Kolb et al., 1998). This regrowth was associated with functional recovery. If the regeneration was prevented, the behavior did not recover (Kolb et al., 2012b) or if the tissue was removed in adulthood, the behavioral improvement was reversed (Dallison and Kolb, 2003). The timing of the critical period for this to happen correlated with the emergence of cells expressing a neurogrowth factor, Fibroblast Growth Factor-2 (Monfils et al., 2006). This led

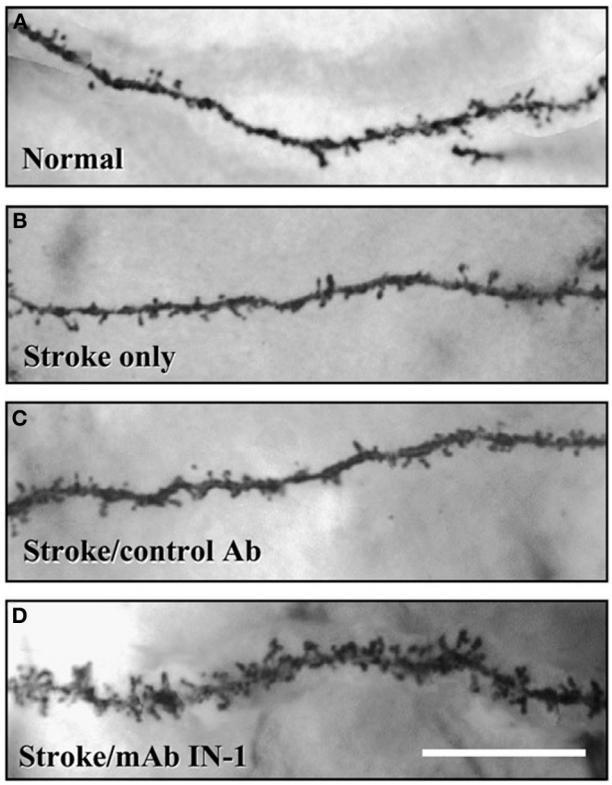

FIGURE 5 | Both dendritic length and spine density (shown here) in the contralateral forelimb cortex is enhanced by antibodies to NoGo-A (After Papadopoulos et al., 2006).

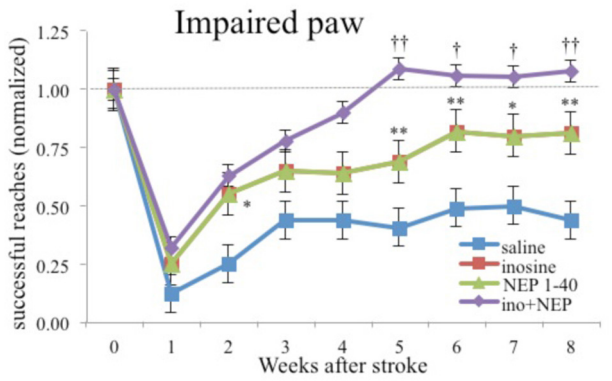

FIGURE 6 | Inosine combined with NoGo-A receptor blocker (NEP1-40) restores performance with the impaired paw to preoperative levels. Similar results were found with a combination of inosine and complex housing (after Zai et al., 2011). * and ** different from saline-treated controls significant at $p < 0.05$ and $p < 0.01$ respectively. † and †† different from rats treated animals with NEP1-40 at $p < 0.05$ and $p < 0.01$ respectively.

to the idea that adding a FGF-2 after an injury might stimulate the production of more neurons in brains that would not normally show this compensatory response, a possibility made even more likely by evidence that FGF-2 can stimulate proliferation of stem cells *in vitro*. Indeed, when rats with motor cortex lesions at postnatal day 10 received i.p. injections of FGF-2, it not only stimulated the proliferation of cells but these cells migrated to the site of injury, formed connections with the spinal cord, and supported apparently complete recovery of fine motor functions (e.g., Monfils et al., 2008). Unfortunately, this only appears to work in the developing brain and does not occur in adulthood, although the reason for this difference is not known.

Another powerful mitogen for adult neural stem cells is epidermal growth factor (EGF), either *in vitro* or *in vivo*. Intraventricular infusion of a combination of EGF and erythropoietin (EPO) following motor cortex strokes in adulthood and stimulates the growth of new neural precursor cells in the subventricular zone (Kolb et al., 2007). A plug of tissue containing both glia and immature neurons formed in the infarcted tissue. Cell tracking studies revealed that cells had migrated from the SVZ. Like the FGF-2 experiments, this was correlated with significant, but by no means complete, functional improvement on several motor tasks. In contrast to the FGF-2 studies there were also few direct connections into the original brain tissue. This led the authors to suggest that the plug of tissue was acting as some type of "factory" for chemicals that enhanced the function of the perilesional tissue. It is also possible that the EGF+EPO had modified existing neural circuits, which in turn led to enhanced behavioral outcome.

There are now many studies that have used a variety of growth factors to stimulate proliferation of neural stem cells after injury (for a review see Dibajnia and Morshead, 2013). Many of these factors have been shown to act directly on their respective receptors but they may also mediate proliferation of precursor cells but Dibajnia and Morshead point out that they may have indirect effects too via immunomodulation, neuroprotection, and angiogenesis.

There are significant challenges in moving to the clinic with compounds to increase the proliferation of neural precursor cells. Many of these compounds are powerful mitogens, leading to potential harmful effects. Another issue is the route of delivery of activation factors. Peripheral routes (intravenous, subcutaneous, intraperitoneal) may have widespread systemic effects and may not enter the brain in sufficient strength to produce the required number of neurons. Direct injection into brain tissue or ventricles is invasive and could lead to other complications. There is also the problem of white matter injury in larger brains that we mentioned earlier and whether the growth factors would affect white matter. In addition, there is the problem of the much greater distance that proliferating cells would have to migrate in humans vs. rodents. Finally, older brains have fewer neural precursor cells in the subventricular zone, likely making it more difficult to stimulate enough cells to make a difference. Clearly, transition to the clinic is some time away.

CONCLUSIONS

A key property of the nervous system is the capacity to change after experience, including injury. As we understand the principles that control plasticity in the normal brain novel treatment strategies are being developed to stimulate recovery after cerebral injury in people.

A significant challenge in the coming decade is to devise ways to apply the knowledge generated in laboratory animal studies to develop successful rehabilitation strategies. Recent results of rodent studies are especially encouraging and could be implemented fairly easily. The first type of treatment to prove successful in animal studies was complex housing but several pharmacological treatments (antibodies to NoGo-A, inosine), and their combinations, show promise to surpass complex housing as the most effective type of treatment, however. Further combinations of rehabilitation strategies and other treatments may provide even better outcomes.

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Recovery after brain injury: mechanisms and principles

Randolph J. Nudo*

Department of Molecular and Integrative Physiology, Landon Center on Aging, University of Kansas Medical Center, Kansas, KS, USA

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Athena Demertzi, University of Liège, Belgium
Gitendra Uswatte, University of Alabama at Birmingham, USA

****Correspondence:***
Randolph J. Nudo, Department of Molecular and Integrative Physiology, Landon Center on Aging, University of Kansas Medical Center, 3901 Rainbow Boulevard, MS 1005, Kansas, KS 66160, USA
e-mail: rnudo@kumc.edu

The past 20 years have represented an important period in the development of principles underlying neuroplasticity, especially as they apply to recovery from neurological injury. It is now generally accepted that acquired brain injuries, such as occur in stroke or trauma, initiate a cascade of regenerative events that last for at least several weeks, if not months. Many investigators have pointed out striking parallels between post-injury plasticity and the molecular and cellular events that take place during normal brain development. As evidence for the principles and mechanisms underlying post-injury neuroplasticity has been gleaned from both animal models and human populations, novel approaches to therapeutic intervention have been proposed. One important theme has persisted as the sophistication of clinicians and scientists in their knowledge of neuroplasticity mechanisms has grown: behavioral experience is the most potent modulator of brain plasticity. While there is substantial evidence for this principle in normal, healthy brains, the injured brain is particularly malleable. Based on the quantity and quality of motor experience, the brain can be reshaped after injury in either adaptive or maladaptive ways. This paper reviews selected studies that have demonstrated the neurophysiological and neuroanatomical changes that are triggered by motor experience, by injury, and the interaction of these processes. In addition, recent studies using new and elegant techniques are providing novel perspectives on the events that take place in the injured brain, providing a real-time window into post-injury plasticity. These new approaches are likely to accelerate the pace of basic research, and provide a wealth of opportunities to translate basic principles into therapeutic methodologies.

Keywords: motor cortex, stroke, traumatic brain injury, axonal sprouting, motor learning, recovery

INTRODUCTION

After injury to the cerebral cortex, as often occurs in stroke or traumatic brain injury (TBI), a large portion of the forebrain sensory-motor apparatus is affected, including the frontal and parietal cortex and/or subcortical structures in the striatum and thalamus, resulting in deficits in motor function in the contralateral musculature. However, substantial spontaneous recovery occurs in the weeks to months following injury. Understanding how the remaining sensory-motor structures can support the recovery of such functions has been a primary goal of recent neuroscientific research. This paper will review the current theoretical models and empirical evidence for functional plasticity in the cortical motor system. These data provide a basic understanding of plasticity principles needed to understand and optimize the effects of therapeutic interventions designed to promote adaptive plasticity.

MECHANISMS UNDERLYING EXPERIENCE-DEPENDENT PLASTICITY IN MOTOR CORTEX

Decades of experimentation in the cerebral cortex have demonstrated many physiological and anatomical examples of cortical plasticity. While such phenomena have now been observed in widespread cortical areas, the present article will focus exclusively on somatosensory and motor cortex, due to their importance in understanding motor recovery after brain injuries. Though these processes are triggered by several endogenous and exogenous events, one of the most potent modulators of cortical structure and function is behavioral experience (Nudo et al., 1996a; Karni et al., 1998; Kleim et al., 1998). Emergent properties of each cortical area are shaped by behavioral demands, driven largely by repetition, and temporal coincidence. For example, skilled motor activities requiring precise temporal coordination of muscles and joints must be practiced repeatedly. Such repetition is thought to drive the formation of discrete modules where the conjoint activity is represented as a unit.

Clues to understanding plasticity in adult brains can be found throughout the developmental neuroscience literature. During brain development, guidance cues for axonal sprouting are activity-dependent. There are two phases in the maturation of thalamocortical connections. In the first phase, thalamocortical axons are directed to their cortical targets by axonal guidance molecules. This process may involve spontaneous neural activity. In the second phase, cortical activity guides axonal sprouting within the cerebral cortex, determining topological connectivity patterns. Postnatal axonal branching patterns within cerebral cortex have also been shown to involve sensory related stimulus activity possibly by initiating molecular retrograde signals such as BDNF (Uesaka et al., 2006).

Though long-range axonal sprouting was once thought to be non-existent in adult animals, injury creates a particularly ripe

environment for axonal sprouting processes to be re-initiated. After a focal ischemic infarct in rats, synchronous neuronal activity is a signal for post-infarct axonal sprouting to be initiated from the intact cortical hemisphere to peri-infarct cortex and the contralateral dorsal striatum (Carmichael and Chesselet, 2002). Thus, evidence now supports the importance of cortical activity for axonal sprouting within the developing and adult brain. It follows that differences in post-infarct behavioral experience may influence which neurons become targets for both local and distant sprouting axons by differentially activating task-specific cortical areas.

It is important to point out that context-dependent reinforcement is critical for such plasticity to occur in cortical neurons of adult animals. That is, simple exposure to sensory stimuli causes little or no long-lasting change in receptive field properties. This principle was illustrated in a set of studies in which both somatosensory and auditory stimuli were presented to animals. The animals were rewarded for discriminating physical properties of only one of the modalities. Receptive field plasticity was seen in the cortex corresponding to the relevant sensory modality, but not the irrelevant modality (Recanzone et al., 1992).

Several general principles of motor map organization have been demonstrated that are thought to underlie the ability of the motor cortex ability to encode motor skills (Monfils et al., 2005). First, motor maps are fractionated, in that they contain multiple, overlapping representations of movements (**Figure 1**). Second, adjacent areas within cortical motor maps are highly interconnected via a dense network of intracortical fibers. Third, these maps are extremely dynamic and can be modulated by a number of intrinsic and extrinsic stimuli. Together, these characteristics provide a framework that facilitates the acquisition of novel muscle synergies, at least in part, through changes in the intracortical connectivity of individual movement representations (Capaday et al., 2013).

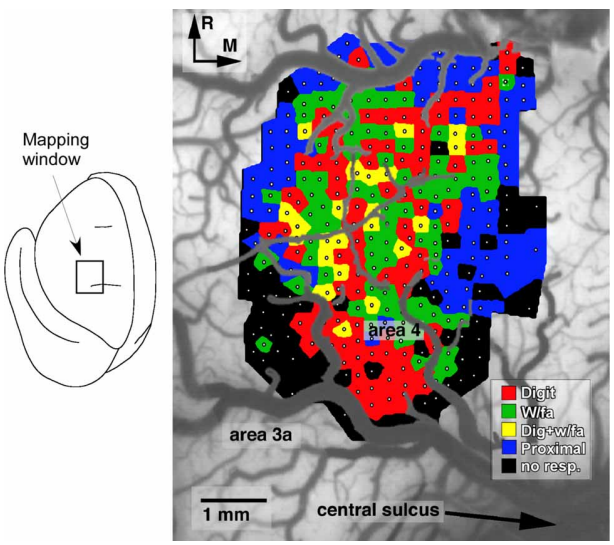

FIGURE 1 | Representation of distal forelimb movements in primary motor cortex (area 4) of a squirrel monkey. Under ketamine sedation, movements were evoked by intracortical microstimulation at each of 321 sites (small white dots) located approximately 250 μm apart. The distal forelimb representation is comprised of digit (red), wrist (w/fa; green), forearm (green) movements, as well as combinations of single-joint movements (yellow). This fractionated pattern of movement representations is due to the intermingling of corticospinal neurons that project to different subsets of motor neurons (Milliken et al., 2013).

However, the dynamic nature of motor maps belies the issue of stable neural connections that must be maintained to respond to environmental demands and retain acquired motor skills. In fact, using stimulus-triggered averaging of electromyographic activity in M1 of macaque monkeys, it appears that facilitation and suppression of individual muscles are surprisingly stable despite alterations in joint-angles, postural changes and various phases of a task (Griffin et al., 2009). Within the cortex, this balance is thought to be achieved through interactions of the excitatory and inhibitory connections of pyramidal cells and local inhibitory networks (Huntley and Jones, 1991; Aroniadou and Keller, 1993). This in turn requires an internal mechanism that is capable of shifting this balance toward strengthening relevant synaptic connections.

Horizontal fiber connections have been shown to arise from excitatory pyramidal neurons and allow for the co-activation of adjacent and non-adjacent cortical columns. In addition to activating excitatory pyramidal cells, they also generate inhibitory responses via the activation of GABAergic interneurons (Jones, 1993). Furthermore, the activity of these horizontal fibers has been shown to be mediated by both long-term potentiation (LTP) and long-term depression (LTD) between distant motor cortical areas (Hess et al., 1996). In slice preparations after motor learning, rats have larger amplitude field potentials in the motor cortex contralateral to the trained forelimb (Rioult-Pedotti et al., 1998). Thus, the synaptic strength of horizontal connections in the motor cortex is modifiable and may provide a substrate for altering the topography of motor maps during acquisition of motor skills. Together, these horizontal fiber characteristics provide a mechanism capable of both facilitating the activation of multiple novel muscle synergies that are required for motor skill acquisition, while likewise providing a mechanism, via inhibitory processes, of motor map stability that is required to maintain stable, neural representations in response to irrelevant (i.e., untrained) environmental events.

The hypothesis that Hebbian-like changes in intracortical synaptic connections link different cortical neurons to form functional modules gained further support by a study in the motor cortex of adult macaque monkeys. This study demonstrated that the output properties of motor cortex neurons can be altered by artificially coupling neuronal discharge patterns (Jackson et al., 2006). Electrodes were implanted in motor cortex of monkeys and two sites were selected on the basis of their response to ICMS. ICMS produced different movements at the two sites, which were located 1–2 mm apart. Then spike discharges were recorded from one site (Site 1) and used to stimulate the second site (Site 2) with a predetermined delay. When ICMS was used to determine the output properties of the two sites a few weeks later, it was found that Site 2 acquired the properties of Site 1—the ICMS-evoked movements were identical. This study provides further

support for the notion that temporal correlation of inputs and outputs drives the emergence of coupling among motor cortex modules.

Structural alterations also occur in adult animals as a consequence of experience. Dendritic and synaptic morphology of motor cortex neurons are altered by specific motor learning tasks (Jones et al., 1999; Kleim et al., 2002b). Both LTP-like processes and dendritic spine expansion has been demonstrated in the same preparation in the motor cortex during a skilled learning task (Harms et al., 2008). Dendritic spine formation occurs quite rapidly, within 1 h on pyramidal neurons in the motor cortex contralateral to the limb performing the task. Further, subsequent training stabilizes the expanded spines, presumably as a basis for long-term memory of skilled motor tasks (Xu et al., 2009). Structural changes are highly specific to the neurons relevant for the skilled task (Wang et al., 2011). Classes of genes associated with early phases of motor skill learning have been identified and include those known to regulate synaptic plasticity, synaptogenesis, and cytoskeletal dynamics (Cheung et al., 2013). However, it would appear that widespread synaptogenesis and motor map plasticity are only evident in relatively late phases of motor skill acquisition, when motor memories for the skill are well-established (Kleim et al., 2004; Xu et al., 2009).

Dopamine appears to play a significant role in acquisition of skilled motor tasks (Hosp and Luft, 2013). Motor cortex, at least in rat, receives a substantial number of dopamine terminals, most from the ventral tegmental area. Luft and colleagues found that after dopamine depletion (by injection of 6-hydroxydopamine), rats are impaired at learning a pellet retrieval task. However, if they had already learned the task, the performance was not impaired. The impairment is reversed by injection of levodopa. These and other studies by this group provide strong evidence that dopamine specifically plays a role in motor memory consolidation.

MOTOR SKILL LEARNING AND PLASTICITY IN MOTOR MAPS

The term "motor learning" is not rigidly defined in most experimental models, but instead thought of as a form of procedural learning that encompasses such elements as skill acquisition and motor adaptation. More specific is motor skill learning itself, which is often described as the modification of the temporal and spatial organization of muscle synergies, which result in smooth, accurate, and consistent movement sequences (Hammond, 2002). Paralleling animal experiments, functional magnetic imaging studies in humans have led to the hypothesis that motor learning is a two stage process (Ungerleider et al., 2002). The first stage is rapid, and results in within-session decreases in neural activity. The second, slower stage results in increases and expansion of activity in M1.

One technique that has provided valuable information regarding functional plasticity in motor maps in experimental animals is intracortical microstimulation (ICMS) (**Figure 1**). As employed in plasticity studies in motor cortex of non-human primates, the ICMS protocol utilizes glass microeletrodes (10–20 μm tips) filled with 3.5 M NaCl. Current is delivered through a platinum wire inserted in the stimulating electrode. The stimulus consists of 13, 200 μs cathodal, monophasic pulses delivered at 350 Hz, with a maximum current of 30 μA. The electrode is lowered perpendicular to the surface of the cortex to a depth of 1750 μm, which targets layer V of the cortex, the location of the corticospinal cell bodies. Current levels required for evoking overt movements in anesthetized animals are lowest at this depth. Electrode penetrations are made at 250 μm increments, and recorded on a digital picture of the cortical surface. This procedure allows for the derivation of high resolution maps of motor cortical movement representations with negligible damage to the tissue, thus allowing for repeated mapping procedures within the same subject. Typically, baseline maps are derived prior to behavioral training. These maps consist of digit and wrist/distal forelimb movement representations, bordered medially, rostrally, and laterally by proximal shoulder representations. The caudal border is composed of somatosensory cortex (area 3a), and thus movements are rarely evoked at the current intensities employed in these studies (max of 30 μA).

Based on ICMS results in non-human primates, the general topographic representation of specific body parts is quite consistent in M1, but substantial individual variability exists in the detailed topography on a more refined level, e.g., within the hand representation. The size of the hand representation can vary by over 100% in different monkeys, a difference that cannot be accounted for on the basis of the animal's size alone. It has been hypothesized that individual variation in motor maps is a result of each individual's sensorimotor experiences leading up to the motor mapping procedure (Nudo et al., 1992).

To examine the relationship between motor skill learning and changes in motor map representations, a manual dexterity task is often utilized. The typical apparatus consists of a plexiglas board that is attached to the front of the monkeys' home cage. The board contains food wells of different sizes: the largest is large enough to insert the entire hand, while only one or two digits can be inserted into the smallest well. Small, flavored food pellets are placed in the wells one at a time. While initial performance on the task is typically very poor, the monkeys are quite adept and came become very skilled on the task, retrieving 500–600 pellets per day within 1–2 weeks.

Utilizing manual dexterity training in combination with ICMS maps has been crucial in demonstrating the dynamic relationship between motor skill learning and cortical map plasticity. The first study to directly examine this relationship used varying behaviorally demanding tasks to selectively activate specific components of motor maps (Nudo et al., 1996a). This study used three monkeys trained on the manual dexterity task to retrieve food pellets using primarily using digit and wrist movements. A fourth subject was trained to use its wrist and forearm to receive pellets by turning a rotatable eye-bolt. Training continued for approximately 10–11 days, sufficient for an asymptotic level of performance to be reached. Post-training ICMS mapping revealed changes in motor map topography that directly reflected the demands of the particular behavioral task. Thus, monkeys trained on the manual dexterity task showed an increase in digit representations and corresponding reduction in wrist and shoulder representations compared to pre-training maps (**Figure 2**), while the monkey trained to turn the eye bolt exhibited the opposite

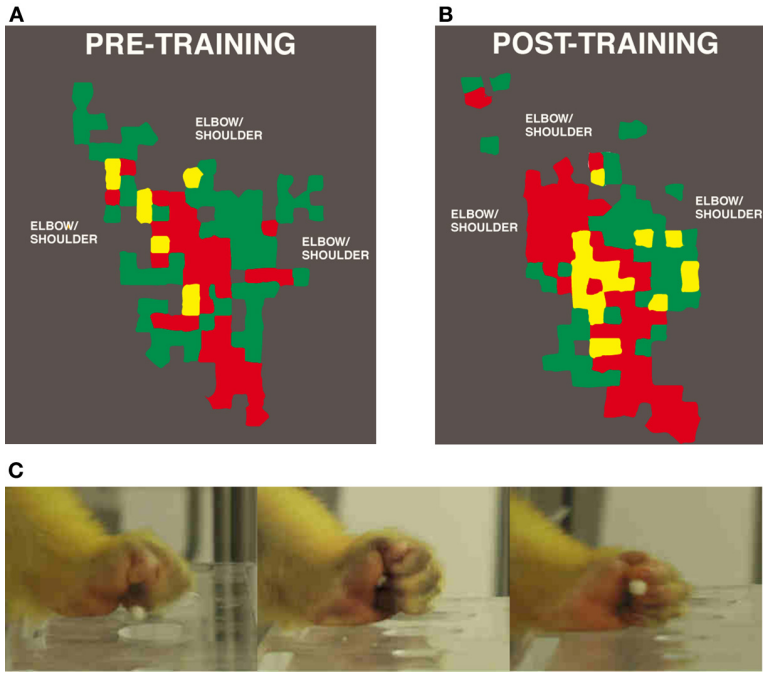

FIGURE 2 | Representation of distal forelimb representations in motor cortex after digit skill training as defined by intracortical microstimulation. Digit areas (red) expand after only 12 days of training. Combination movements that reflect the individual kinematics that the monkey employs also expand their representations. **(A)** Pre-training map. **(B)** Post-training map. **(C)** Still images of squirrel monkey retrieving food pellets from small wells (Nudo et al., 1996a).

effects—an increase in wrist and forearm representations at the expense of digit representations.

In addition, an increase in ICMS-evoked multi-joint movements was observed. These movements consisted of simultaneous executions of digit and wrist or proximal movements at low ICMS current levels, and were only observed after training on the digit-use intensive manual dexterity task. Both before and after training, thresholds for evoking multi-joint responses were significantly lower than single joint responses. These results imply that behaviorally relevant, simultaneous or sequential movements may become associated in the motor cortex through repeated activation. It is possible that temporal correlation of inputs and outputs in the motor cortex drives emergent properties, as it seems to do in somatosensory cortex. Thus, muscle and joint synergies used in complex, skilled motor actions may be supported by alterations in local networks within the motor cortex. As skilled tasks become more stereotyped in timing of sequential joint movements, functional modules emerge in the cortex to link the outputs of different motoneuron pools.

MOTOR SKILL LEARNING vs. MOTOR USE

These findings lead to the question of what aspects of motor skill learning drive the observed changes in map representations. Given that in the previous experiments, subjects were trained repeatedly on the same motor skills task, it is possible that increased muscle activity alone produced the observed changes in map representations. To address this issue, a group of monkeys was trained exclusively on either the largest or the smallest well in the digital dexterity task. The rationale in this design is that the largest well allows for simple multi-digit movements for pellet retrieval, which does not require the subject to develop novel skilled digit movements, since simply grasping for food is a normal part of their daily home cage behavior, and this is already part of their behavioral repertoire. Small well food pellet retrieval, in contrast, requires the monkey to manipulate 1–2 digits to retrieve the pellet, which is considerably harder given that squirrel monkeys lack monosynaptic corticospinal projections to motoneurons, which probably limits individuation of digit movements (Lemon and Griffiths, 2005).

Compared to pre-training maps, monkeys trained on the large well pellet retrieval did not show an expansion of the digit representation, while those trained on the small well did exhibit an expansion of the digit representation (Plautz et al., 2000). These findings strongly suggest that an increase in motor activity in the absence of motor skill acquisition is insufficient to drive neurophysiological changes in the motor cortex. Similar findings have been found in rodents examining pellet retrieval vs. bar pressing. Rats that learned to retrieve pellets from a rotating platform displayed more distal movements in their motor maps. This expansion was associated with significant synaptogenesis (Kleim et al., 1998, 2002a). Rats that simply pressed a bar showed no map changes or synaptogenesis (**Figure 3**). Thus, plasticity in motor cortex can be said to be skill- or learning-dependent, rather than strictly use-dependent. Tasks that require acquisition of new motor skill induce neurophysiologic and neuroanatomic changes in motor cortex, but simple repetitive motion or strength training tasks do not (Kleim et al., 1998; Plautz et al., 2000; Remple et al., 2001).

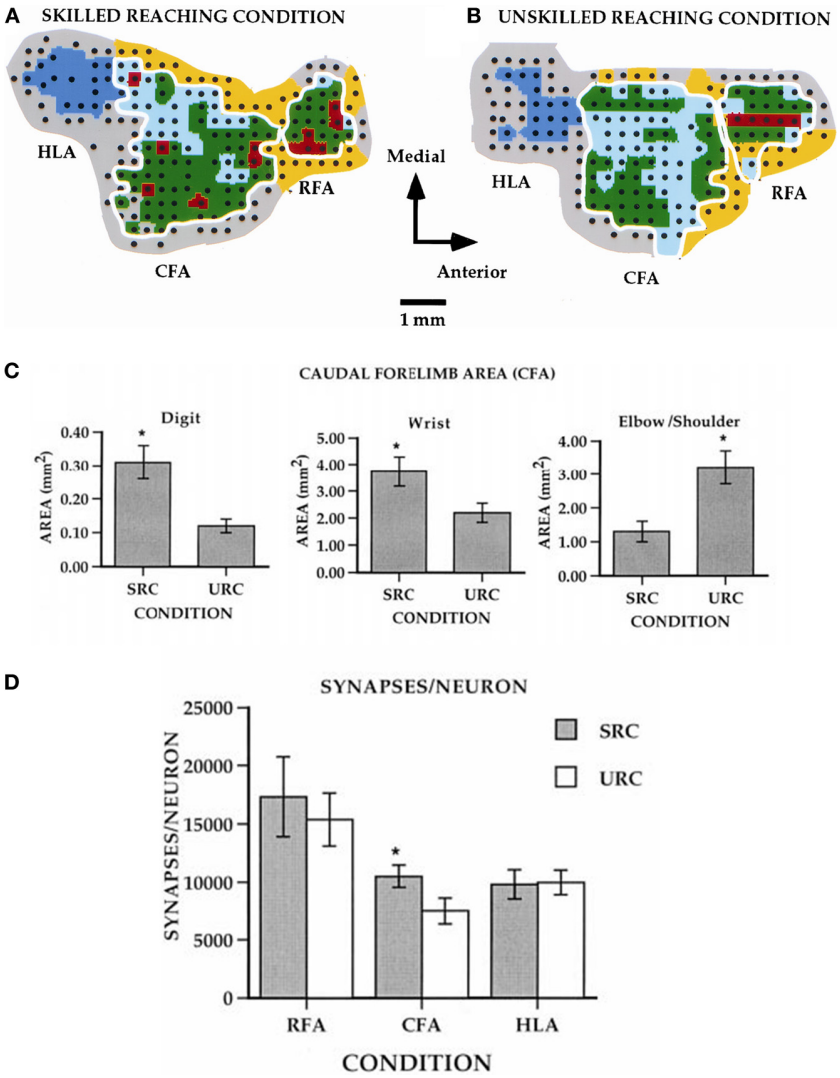

FIGURE 3 | Differential effects of skill vs. use. (A) ICMS-derived motor map (digit, red; wrist, green; elbow/shoulder, light blue) of a rat that learned a skilled reaching movement. **(B)** ICMS-derived motor map of a rat that learned to press a bar. The two forelimb areas are outlined in white. The caudal forelimb area (CFA) is separated from the rostral forelimb area (RFA) by a band of head/neck representations (yellow). The hindlimb area (HLA) is shown in dark blue and nonresponsive sites in gray. **(C)** Note the enlarged digit and wrist/forearm representations in the skilled reaching condition (SRC), and enlarged should representation in the unskilled reaching condition (URC, bar press). **(D)** In the CFA, synapses per neuron were significantly increased ($*p < 0.05$), but no changes occurred in RFA or HLA (Kleim et al., 2002a).

INJURY-INDUCED PLASTICITY IN MOTOR CORTEX

Deficits in motor function are common in numerous neurological conditions. However, the adult central nervous system retains an impressive capacity to recover and adapt following injury. Such so-called spontaneous (or natural) recovery occurs after spinal cord injury, TBI, and stroke. Therefore, a basic understanding of the mechanisms that underlie spontaneous recovery of function is the initial step in the development of modulatory therapies that may improve recovery rates and endpoints. While injury confined to one or another motor field occurs only in certain middle cerebral artery (MCA) strokes and focal TBI or neurosurgical resections, experimental data from animal models is extremely valuable in identifying the mechanisms underlying motor recovery after CNS injury.

While recovery on various outcome measures occurs spontaneously after injury, much of this recovery may be due to behavioral compensation (Whishaw et al., 1991). For example, it is well-known in human stroke that compensatory movements of the trunk are employed during reaching (Cirstea and Levin, 2000). In the case of the study above, the combination of the increased disuse of the impaired digits, with the increase use of proximal could explain shifts in map topography. In a rat model of focal TBI, the rat equivalent of M1, the caudal forelimb area, was injured. In the absence of rehabilitative training (spontaneous recovery), behavioral performance on a pellet-reaching

task improved over time, but at 5 weeks post-injury, and rats still have significant deficits (Nishibe et al., 2010). At this time point, the rat equivalent of premotor cortex (rostral forelimb area) contained a normal size forelimb representation. However, ICMS maps revealed a redistribution of forelimb representations: digit representations were reduced, while proximal representations were enlarged. Thus, at least in the absence of behavioral retraining, the plasticity in spared motor areas that occurs spontaneously may largely reflect the development of compensatory motor patterns, rather than true recovery of the original kinematic patterns (**Figure 4**).

The progression of recovery itself can be thought of a process of both reinstatement and relearning of lost functions, as well as adaptation and compensation of spared, residual function. Thus, it follows that the neurophysiological mechanisms that support learning in the intact cortex should mediate motor relearning and adaptation in the injured brain. Numerous studies over the last century and a half have provided substantial evidence to support the role of neural plasticity in functional recovery, both spontaneous and directed.

PLASTICITY IN ADJACENT, INTACT MOTOR CORTEX AFTER FOCAL INJURY

Direct evidence that adjacent regions of the cortex might function in a vicarious manner after injury can be traced to studies in the mid-20th century (Glees and Cole, 1949). Monkeys were subjected to focal injury to the thumb representation. When brains were remapped following behavioral recovery, the thumb area reappeared in the adjacent cortical territory. However, using ICMS techniques, somewhat different findings were observed by Nudo et al. in the 1990s. Small, subtotal lesions were made in a portion of the distal forelimb representation (DFL) in squirrel monkeys, and the animals were allowed to recover spontaneously (i.e., without the benefit of rehabilitative training) for several weeks. In contrast to earlier finding, the remaining DFL was reduced in size, giving way to expanded proximal representations (Nudo and Milliken, 1996). However, in animals that underwent rehabilitative training with the impaired limb, the DFL was preserved or expanded (Nudo et al., 1996b). In retrospect, it is quite possible that the re-emergence of thumb representations in the early study by Glees and Cole may have been driven by post-injury behavioral demands.

Post-ischemia reorganization of sensorimotor cortex has also been demonstrated recently in transgenic mice expressing the light-sensitive channelrhodopsin-2 in layer V pyramidal neurons (Harrison et al., 2013). As in other studies, the neuronal activation in the peri-infarct cortex was reduced (Jones et al., 2013). Motor cortex infarcts resulted in the somatosensory map to emerge in the undamaged motor cortex near the lesion. This study adds

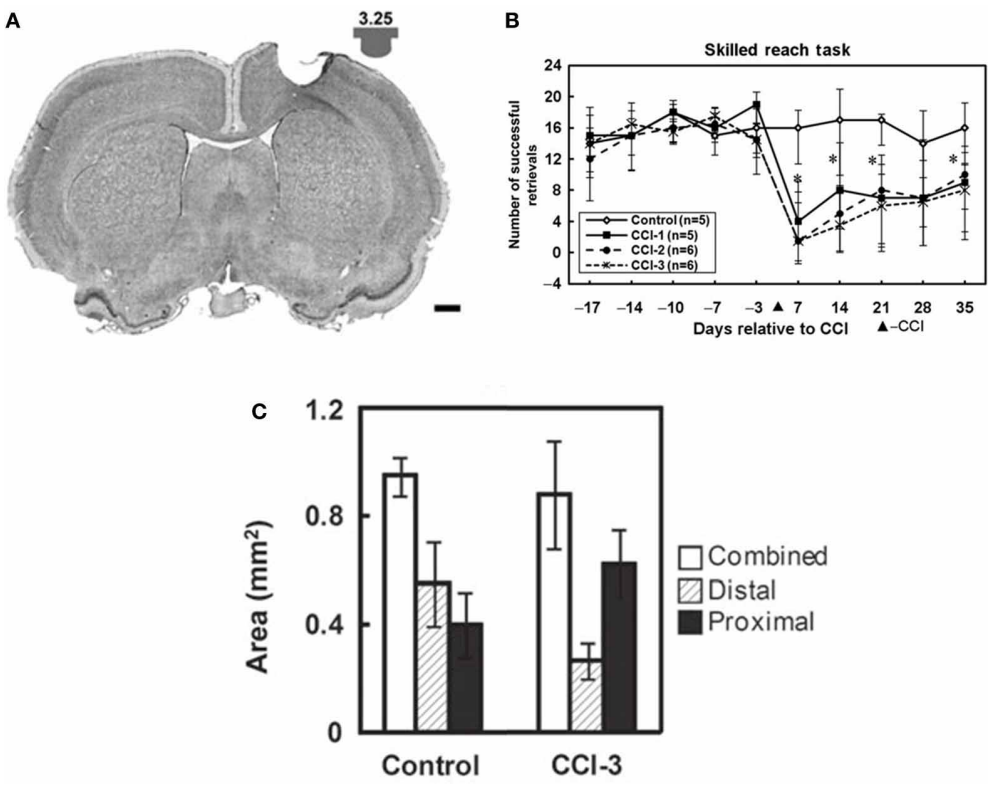

FIGURE 4 | Reorganization of the rat premotor cortex after controlled cortical impact in the primary motor cortex. (A) Coronal section through the primary motor cortex (caudal forelimb area, or CFA) of a rat approximately one month after a controlled cortical impact. Impactor tip dimension and shape is shown in the inset. **(B)** Behavioral performance on a single-pellet retrieval task before and after the injury (*$p < 0.05$). **(C)** Alteration in motor maps in the rat premotor cortex (rostral forelimb area, or RFA) approximately one month after a controlled cortical impact. In the premotor area that was spared by the lesion, digit representations contracted, while proximal representations expanded. This suggests that the behavioral recovery that was observed was due to compensatory kinematic patterns rather than true recovery (Nishibe et al., 2010).

additional credence to the notion that uninjured regions can play a vicarious function.

Studies in human stroke survivors also suggest that the intact, peri-infarct cortex may play a role in neurological recovery (Cramer et al., 1997; Jaillard et al., 2005; Teasell et al., 2005). Using transcranial magnetic stimulation (TMS) after stroke, it has been shown that the excitability of motor cortex is reduced near the injury, and the cortical representation of the affected muscles is decreased (Traversa et al., 1997; Butefisch et al., 2006). It is likely that this effect occurs from a combination of diaschisis-like phenomena and disuse of the affected limb (Liepert et al., 2000). Further, after several weeks of rehabilitation, motor representations in the injured hemisphere are enlarged relative to the initial post-injury map (Carey et al., 2002). Also, when goal-directed movement with the impaired hand is encouraged, a significant enlargement of the representation of the paretic limb is produced (Liepert et al., 1998), closely paralleling results in non-human primates.

Neuroanatomical changes occur in the peri-infarct cortex. Between 3 and 14 days post infarct, rats demonstrate increased GAP-43 immunoreactivity, suggesting significant neurite outgrowth in the peri-infarct region (Stroemer et al., 1995). Then, 14–60 days post-infarct, synaptophysin staining is elevated, signifying increased synaptogenesis. Local sprouting occurs in the peri-infarct area (Carmichael, 2006). Arteriolar collateral growth and new capillaries also form in the ischemic border (Wei et al., 2001). The picture is now emerging of an evolving peri-infarct environment in which growth inhibition is suppressed for about 1 mo post-infarct. This period is followed by "waves" of growth promotion which may modulate the brain's self-repair processes.

In the past decade, several new techniques have been applied in animal models of ischemia that are giving us a vivid new insight into the temporal dynamics of post-injury plasticity (Sigler and Murphy, 2010). In an elegant series of experiments, Murphy and colleagues used two-photon microscopy in live animals in the mouse somatosensory cortex during ischemia caused by either injection of endothelin-1 (a potent vasoconstrictor) directly into the cortex, or by venous injection of Rose Bengal followed by photoactivation (photothrombotic stroke model) in the cortex (Brown and Murphy, 2008). When local ischemia was severe, spines were lost in less than 10 min. Dendritic spine loss is independent of NMDA receptor activation (Murphy et al., 2008). However, mitochondrial depolarization that might lead to cell death occurs within 1–3 min (Liu and Murphy, 2009). Dendritic spine loss and mitochondrial depolarization were reversible if reperfusion occurred within 1 h, and spines were restored (Zhang et al., 2005; Liu and Murphy, 2009). Sensory-evoked hemodynamic responses obtained using intrinsic optical imaging showed that sensory responses were blocked in the region of damaged dendrites (Zhang and Murphy, 2007) (**Figure 5**). This group has also taken advantage of the recent advances in transgenic models that are now readily available. By employing the photothrombotic stroke model to transgenic mice whose layer V cortical neurons were tagged with yellow fluorescent protein, the dendritic structure could be examined in single layer V neurons (Enright et al., 2007). The additional use of the dextran, Texas Red, allowed the investigators to precisely determine the border of the ischemic core. The results confirmed the dendritic damage in the ischemic core found with two-photon imaging in live animals. However, dendritic damage was limited to only 300 μm around the ischemic border. Beyond this zone, dendrites were essentially intact. Therefore, at least in this photothrombosis model for producing ischemic infarct, a substantial substrate for structural and functional plasticity exists in the peri-infarct cortex.

These techniques transgenic mouse models have now demonstrated that dendrites are remarkably modifiable for several weeks after ischemia. A significant increase in dendritic spine formation continues peaks at 1–2 weeks post-ischemia and persists for up to 6 weeks (Brown et al., 2007). During this time frame, dendritic arbors near the stroke shorten, while those away from the stroke extend (Brown et al., 2010). Studies by Carmichael and colleagues have shown in a mouse photothrombotic stroke model that functional reorganization takes place in the same peri-infarct region as does axonal sprouting, suggesting an anatomical substrate for functional plasticity (Clarkson et al., 2013).

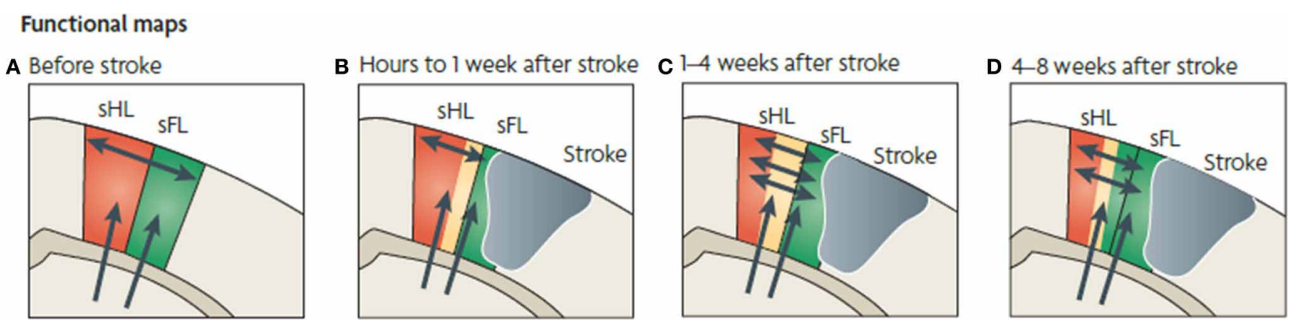

FIGURE 5 | Functional map changes in forelimb (sFL) and hindlimb (sHL) somatosensory cortex after a focal infarct in mouse. Thalamic projections (arrows) and intracortical connections (double arrows) are also shown. **(A)** Normal somatosensory representation of sFL and sHL. **(B)** Within hours after focal infarct (gray), yellow areas show reduced sensory specificity, responding to both FL and HL stimulation. **(C)** Over the ensuing weeks, growth-promoting processes are triggered. Local axonal sprouting (double-headed arrows), dendritic spine expansion, and synaptogenesis occurs in the peri-infarct cortex. **(D)** Several weeks after stroke, specificity in sensory responses returns. Neurons that were formerly responsive to stimulation of hindlimb become responsive to forelimb stimulation (Murphy and Corbett, 2009).

FUNCTIONAL AND STRUCTURAL PLASTICITY IN REMOTE REGIONS AFTER FOCAL DAMAGE TO M1

Primate brains are endowed with a rich intracortical network that allows reciprocal communication among the various sensory and motor areas. Injury to the motor cortex results in a potent disruption of integrated sensorimotor networks, resulting in loss of fine motor control. Upregulation of NMDA receptors and downregulation of $GABA_A$ receptors occurs in the ipsilesional and contralesional hemisphere (Redecker et al., 2000). Remote cortical neurons that project to the ischemic core express genes related to axonal growth and guidance, dendritic growth and branching and cytoskeletal organization (Urban et al., 2012). It follows that disruption of the cortical motor network triggers a major reassembly of inter- and intra-areal cortical networks.

Since the development of compensatory behaviors and involvement of uninjured M1 are thought to contribute to spontaneous recovery, it follows that intact, motor areas outside of M1 may also contribute to recovery. Thus, it is plausible that following an injury to M1, the remaining, intact motor areas provide some role in functional recovery, via intracortical connectivity with other cortical regions and/or their direct corticospinal projection pathways.

Experiments by Liu and Rouiller (1999) showed in non-human primates that inactivation of premotor cortex with the GABAergic agonist muscimol following an M1 ischemic lesion reinstated behavioral deficits. This reinstatement was not observed with inactivation of the peri-lesional, or contralateral cortex. Thus, it follows that if premotor cortex is capable of compensating for the loss of motor function following an M1 injury, there should exist physiological changes that accompany this recovery. In adult squirrel monkeys ICMS mapping techniques were used to characterize representational maps of both M1 and PMv, before and after experimental ischemic infarcts that destroyed at least 50% of the M1 hand representation (Frost et al., 2003). All subjects showed an increased hand representation in PMv, specifically in digit, wrist, and forearm sites. Further, the amount of PMv expansion was correlated with the amount of the M1 hand representation that was destroyed. In other words, the more complete the M1 hand area lesion, the greater the compensatory reorganization in PMv. TMS studies in stroke survivors also suggests that premotor areas may serve in a vicarious capacity after injury (Fridman et al., 2004).

Interestingly, when lesions in the monkey models were smaller than 50% of the M1 hand area, the PMv hand representation decreased in size. Thus, examining the entire spectrum of M1 infarcts of varying sizes, the linear relationship is maintained. This result occurred despite the fact that some of these subtotal M1 hand area lesions nonetheless destroyed nearly the entire terminal field of PMv-M1 connections. What possible compensatory changes in the neuronal network could account for proportional gains in premotor hand areas, but losses with very small lesions? This phenomenon is reminiscent of Lashley's classic description of the relationship between cerebral mass and behavioral change (Lashley, 1930). According to this hypothesis, lesion size is generally assumed to be associated with the severity of deficits, while lesion location is related to the specificity of deficits. Lashley also proposed the concept of equipotentiality suggesting that each portion of a given cortical area is able to encode or produce behavior normally controlled by the entire area. In that vein, after smaller lesions, the surviving M1 tissue could potentially subserve the recovery of function. In that case, reorganization in distant, interconnected cortical areas would be a more "*passive*" process resulting from the loss of intracortical connections. This reorganization could be compared to a "sustained diaschisis" of PMv. After larger lesions, reorganization of the adjacent tissue may not suffice for normal motor execution. Thus, learning-associated reorganization would need to take place elsewhere, resulting in greater PMv expansion. Accordingly, in rats, the contralesional cortex is thought to be involved in behavioral recovery only after large lesions (Biernaskie et al., 2005). Interhemispheric signal processing occurs very rapidly after stroke, so that sensory responses produced by stimulation of either the contralateral or ipsilateral pathways are enhanced in the intact, contralesional cortex within 1 h. This disinhibition could not be explained simply by a transcallosal process (Mohajerani et al., 2011). While clearly there are short-term changes, later neuroanatomical changes in the intact cortex also occur as a use-dependent or skill-dependent change due to increased use of the less-affected limb (Bury and Jones, 2004).

Since neuroanatomical changes are known to occur in the peri-infarct area, and neuronal networks are densely interconnected, it follows that many of the functional changes that have been observed in cortical remote regions may have structural correlates. Evidence now exists that cortical efferent fibers are alterable in adults after cortical injury. After cortical lesions in rats, corticostriatal fibers, which primarily connect various cortical motor areas with the ipsilateral striatum, sprout from the intact (contralesional) cortex, and terminate in the contralateral striatum (i.e., on the side of the lesion) (Napieralski et al., 1996). Such plasticity in crossed corticofugal fiber systems may provide one mechanism for the remaining intact hemisphere to participate in recovery.

Evidence that synaptogenesis and axonal sprouting occurs in the peri-infarct zone after a cortical injury was discussed above. Further, after an ischemic injury to the M1 hand representation in non-human primates, most intracortical connection patterns of the PMv remained intact (Dancause et al., 2005). However, when compared to uninjured control monkeys, after M1 lesions monkeys showed a remarkable proliferation of novel PMv terminal projections in primary sensory cortex (S1), specifically in the hand representations of areas 1 and 2 (**Figure 6**). Likewise, this somatosensory area had a significant increase in the number of retrogradely labeled cell bodies, indicating an increase in reciprocal projections from S1 to PMv. In addition, intracortical axonal projections from PMv significantly altered their trajectory near the site of the lesion. This finding is particularly interesting, given the direct intracortical connections between M1 and somatosensory cortex, as well as the presence of direct corticospinal projections originating from PMv. One hypothesis is that the post-injury sprouting represents a repair strategy of the sensorimotor cortex to re-engage the motor areas with somatosensory areas.

In intact brains, M1 receives input from various regions of the parietal lobe that supply cutaneous and proprioceptive

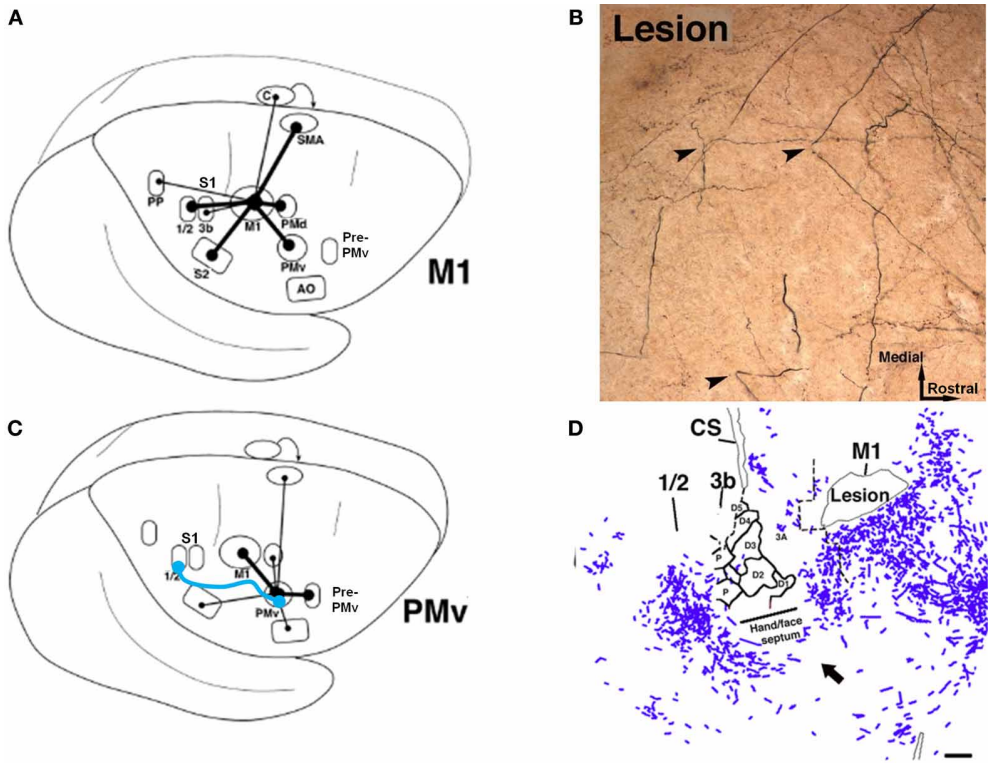

FIGURE 6 | Rewiring of corticocortical connections after ischemic infarct. **(A)** In normal healthy squirrel monkeys, the primary motor cortex (M1) has dense reciprocal connections with both the premotor cortex (PMv, PMd, SMA) as well as the primary somatosensory cortex (S1) and the second somatosensory area (S2). **(B)** In addition to M1, the ventral premotor cortex (PMv) has dense connections with a rostral area called pre-PMv. PMv has moderate connections with S2, but negligible connections with S1. **(C)** Several weeks after an ischemic infarct in M1, axons originating in PMv can be seen making sharp bends and avoiding the infarct area, as shown in this tract-tracing study. **(D)** A low-magnification plot of axons within the section show that the axons originating from PMv course around the central sulcus. Substantial terminal bouton labeling (not shown) appears in S1 (areas 1 and 2). The blue line in **(B)** signifies the *de novo* pathway that forms after the lesion (Dancause et al., 2005).

information that is largely segregated in the M1 hand area—cutaneous information arriving in the posterior portion of M1, and proprioceptive information arriving in the more anterior portion. The functional importance of this somatosensory input can be appreciated from studies employing discrete lesions in these subregions in M1. Lesions in the posterior M1 hand area lesions result in behavioral deficits akin to those seen after S1 lesions. These deficits appear to be similar to sensory agnosia, in which the animal reaches for food items, but does not appear to know whether the item is actually in the hand. In contrast, anterior M1 hand area lesions result in deficits in metrics of the reach, perhaps indicating the disruption of proprioceptive information in the motor cortex (Friel et al., 2005). One lesson from these studies is that the motor cortex cannot be considered solely as a motor structure. Deficits result from sensory-motor disconnection in addition to disruption of motor output. Thus, after M1 injury, there is a substantial reduction of somatosensory input to motor areas. Perhaps, the novel connection between PMv and S1 is an attempt by the cortical motor systems to reconnect with somatosensory input. However, it is not yet known if this connection is functional, or if it is, whether it is adaptive or maladaptive. An alternate hypothesis is that the new pathway represents an aberrant connection that interferes with behavioral recovery.

It is likely that this phenomenon of intracortical sprouting of remote pathways interconnected with the injured zone is not a unique event. It is more likely that many structures, both cortical and subcortical, that are normally connected with the injured tissue undergo substantial physiological, and anatomical aterations. For instance, each of the other cortical motor areas (PMd, SMA, cingulate motor areas) are likely to change their intracortical connectivity patterns since their targets are destroyed. If so, it follows that the brain with a focal injury is a very different system. It is not simply a normal system with a missing piece. If intracortical reorganization is a predictable process, as we think it is, then we may be able to begin to develop ways of enhancing adaptive, while suppressing maladaptive connection patterns.

After stroke in humans, widespread changes occur in activation patterns, associated with movement of the paretic limb, in both the ipsilesional and contralesional hemispheres (Chollet et al., 1991; Weiller et al., 1993; Nelles et al., 1999). Whether such bilateral activation is adaptive or maladaptive is still a matter of debate, but it appears that as recovery proceeds, activation of the various regions in the ipsilesional cortex increases (Nelles

et al., 1999; Carey et al., 2002). Increased ipsilateral activation after stroke is quite widespread, including spared premotor areas (Weiller et al., 1992; Seitz et al., 2005). In one longitudinal study, increased activation of SMA was correlated with better recovery (Loubinoux et al., 2003). Stroke survivors with MCA strokes that included lateral PM areas had poorer recovery (Miyai et al., 1999), while increased lateral PM activity was associated with better recovery (Miyai et al., 2003). In an experiment analogous to monkey secondary inactivation studies, the ipsilesional PMd of human stroke survivors was inactivated temporarily with repetitive TMS. This procedure resulted in reaction time delays that were not generated by inactivation of the contralesional PMd or the PMd of healthy subjects (Fridman et al., 2004). From the results to date, it is not possible to determine if any one motor area is more important in the recovery of motor abilities after stroke. We hypothesize that the entire cortical and subcortical motor system that is spared by the injury participates to varying degrees depending upon the extent and location of the injury and behavioral demands. At least some of the functions of the injured region(s) are thus redistributed across the remaining cortical and subcortical motor network.

INTERACTIONS OF INJURY AND EXPERIENCE: NEURAL CORRELATES OF LEARNED NON-USE

In the acute period immediately after neural injury, motor ability is often severely impaired. Taub and colleagues have suggested that during this stage, attempts to complete tasks with the impaired limb are unsuccessful, or effortful, both of which are conditions that punish the use of the more-affected extremity, making future attempts less likely (Taub et al., 1998). By counter-conditioning this so-called "learned non-use" that develops in the acute phase permits latent motor abilities to be expressed. From this assumption, it was stipulated that function could be improved in chronic stroke patients. In particular, this group developed an innovative approach known as constraint-induced movement therapy (CIMT). The idea behind the application of CIMT originates from fundamental experiments conducted in non-human primates following peripheral deafferentation. In these experiments, disuse of the affected upper limb was observed following the injury. This maladaptive behavior persisted if no manipulation was introduced, even after a 3–6 month spontaneous recovery period. At that point, the function of the deafferented limb could be greatly enhanced by forcing its use by restraining the non-affected limb (Knapp et al., 1963). This led to the "learned non-use" hypothesis which stipulates that non-use, or less than maximal use, of the deafferented limb results from punishment of attempts to use the affected limb. This negative feedback would consist of unsuccessful behavioral consequences of attempts to use the affected upper limb (e.g., absence of reward for goal-directed activity, or painful execution). After the initial recovery period, when the ability to use the affected limb is stable, behavioral sequelae caused by the learned non-use remain. Therefore, because of the phenomenon, the actual use of the affected limb is much less than its true potential.

Strong support for the learned non-use formulation came from a study where restraint was applied directly following the deafferentation of the upper limb of an animal for a 3-month duration, therefore preventing the learned non-use phenomenon to occur When the restraint was removed, the animals used their deafferented limb (Taub, 1977). In the ensuing years, the learned disuse/nonuse model was employed in the CIMT approach. A landmark Phase III clinical trial has demonstrated that stroke survivors experience significant gains in functional outcomes after CIMT even years after stroke (Wolf et al., 2006).

The procedures used for CIMT are strikingly similar to the techniques used by Nudo and colleagues to demonstrate reorganization of the peri-infarct cortex following a focal stroke in non-human primates (Nudo et al., 1996b). After stroke, monkeys in the rehabilitative training group wore a jacket with a long sleeve that extended to a closed mitt on the less-impaired distal forelimb. This was necessary simply to test motor skill in the monkeys, since, without the restraint of the impaired limb, the monkey would simply switch to the less-impaired limb. However, if disuse (or nonuse) occurs after brain injury, there is a potential confound in the interpretation of post-injury plasticity. Behavioral experience and neural injury interact, such that changes in spared cortex are a product of both injury-related mechanisms, and behaviorally-driven changes, such as disuse (Woodlee and Schallert, 2004). How much of the change in motor maps is due to the injury alone vs. the disuse that follows the injury? If motor map integrity is powerfully modulated by motor experience, then the lack of experience should result in similarly large changes in map organization.

Disuse independent of neural injury has rarely been examined using mapping techniques as described either in humans or non-human primates. While some human TMS studies exist following casting, these are typically performed on individuals with fractures (Zanette et al., 2004). In a unique longitudinal study, Milliken et al. examined the organization of motor cortex in otherwise normal, healthy squirrel monkeys. Detailed ICMS maps were derived longitudinally, before and up to 35 weeks after restriction of the preferred distal forelimb in a soft cast (Milliken et al., 2013). The casted forelimbs were occasionally used for support, but were not able to be used for skilled movements. The results demonstrated a progressive redistribution of digit and wrist/forearm representations in the M1 hand area. Digit area contracted, while wrist/forearm representations expanded (**Figure 7**). Furthermore, the changes were reversible After removal of the cast, behavioral skill generally returned within 1 week, and post-recovery maps returned to normal. Thus, disuse has neurophysiological consequences independent of the injury. However, as the effects of disuse in intact animals can be reversed, similarly, disuse after injury can also be reversed, at least if there is sufficient neural apparatus to support the execution of the motor task.

With respect to the integrity of the damaged hemisphere, the maladaptive effects go beyond simple disuse. Jones and colleagues have demonstrated in rat and mouse models that compensatory skill-learning by the less-affected limb impedes later functional recovery in the more-affected limb (Allred et al., 2005; Allred and Jones, 2008; Jones et al., 2013; Kerr et al., 2013). These maladaptive effects were not seen in animals that were trained on a bimanual task (Kerr et al., 2013).

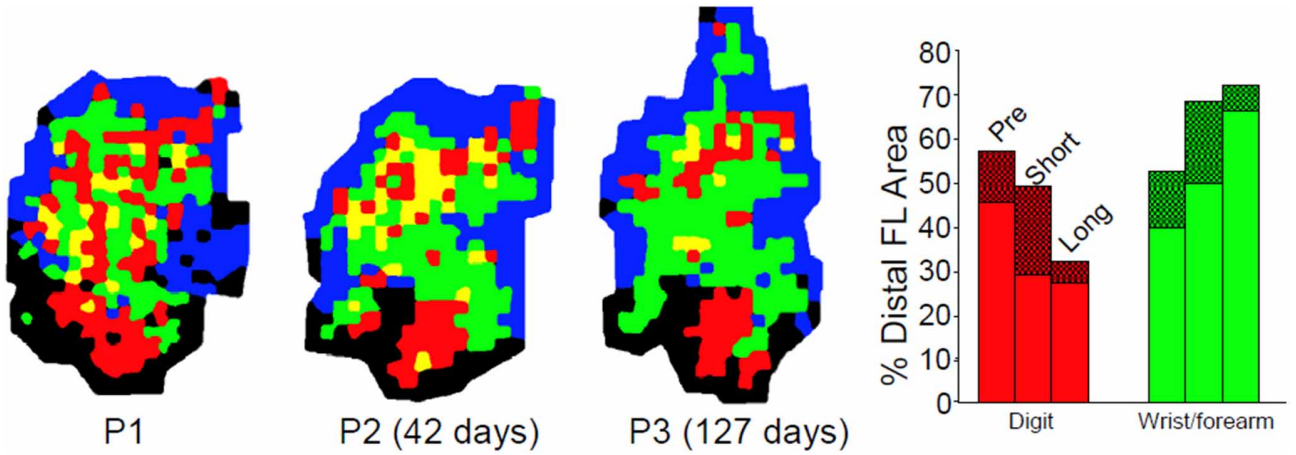

FIGURE 7 | Effects of disuse on motor maps in the absence of injury. The preferred forelimbs of normal, healthy adult squirrel monkeys were placed in soft, restrictive casts for periods up to 5 months. ICMS mapping studies showed a progressive decrease in digit representation and a progressive increase in wrist/forearm representation. These effects were reversible after removal of the cast. These studies demonstrate that disuse has a substantial impact on motor cortex representations independent of the injury-induced disuse and neuropathological changes associated with stroke or traumatic injury (Milliken et al., 2013).

WINDOWS OF OPPORTUNITY FOLLOWING BRAIN INJURY BASED ON NEUROPLASTICITY PRINCIPLES

As discussed above, following injury to the brain via trauma or stroke, a cascade of molecular and cellular events is set into motion in the surrounding tissue that results in both temporary and permanent changes in the anatomy and physiology of the affected structures. Many of these changes are pathological consequences of the injury (e.g., edema) and have potentially damaging results. However, many adaptive processes may begin early in the post-injury stage and result in reduction of pathophysiological events or in neuroplastic changes leading to at least some restoration of function (Witte and Stoll, 1997; Cramer, 2000). While a thorough understanding of these processes at the molecular, cellular and network levels is just beginning, sufficient knowledge is now available to begin testing hypotheses about the effects of specific post-injury interventions on functional recovery and its underlying neuroanatomical and neurophysiological bases.

The latent potential for enhancing neuroanatomical plasticity mechanisms after stroke has been demonstrated by the use of mutant mouse strains that lack the Nogo receptor. Nogo is a protein involved in the inhibition of axonal growth. Mice lacking the Nogo receptor recover motor function after stroke better than controls. Further, rats subjected to anti-No-go antibody treatment initiated 1 week after stroke resulted in better behavioral recovery compared with controls. Further, sprouting of contralateral corticorubral and ipsilateral corticospinal fibers was observed (Lee et al., 2004). Pharmacologic treatment with D-amphetamine after stroke has also been shown to enhance neocortical sprouting, synaptogenesis, and behavioral recovery after stroke in rats (Stroemer et al., 1998).

As a result of the abundance of evidence that has demonstrated that the brain is plastic after neuronal injury, and that behavioral experience can alter neuronal structure and function in both healthy and injured brains, it is now clear that principles of neuroplasticity can form the foundation for a wide range of therapeutic approaches to recovery. However, to develop effective, evidence-based rehabilitation protocols to promote recovery, two basic issues, timing and dose, still need to be addressed at molecular, cellular, and network levels of analysis.

Like many drug-based approaches to brain injury, there is most likely an optimal time period during which behavioral training paradigms are most effective. Upregulation of proteins involved in neural growth and guidance, many of which mimic events during neural development, occur over a relatively narrow window of time after injury. While recent clinical trials have demonstrated that outcome measures can be improved even years after stroke, the most optimal time may be during the period of maximum reorganization induced by the injury. Axonal sprouting takes place during a programmed process that is triggered, at least in animal models, by 1–3 days after stroke, and is fully mature by the end of 1 month (Carmichael, 2006). Neuronal growth-promoting and growth-inhibiting genes are turned on and off during similar post-injury time periods. In addition, events that trigger neurogenesis occur over a limited time period. Therefore, there is a critical need to understand how behavioral experience alters these reorganizational mechanisms differentially over time.

Likewise, any deleterious effects of behavioral interventions that are introduced too early in the process need further elaboration. Investigators and therapists in the field of neurorehabilitation became sensitized to this issue with the report in rats that early casting of the less-impaired limb resulted in exaggeration of neuronal injury (Kozlowski et al., 1996). The proposed mechanism for this use-dependent potentiation of the injury is that NMDA receptor-mediated processes are increased after brain injury, and that extreme overuse of the impaired limb causes further enhancement of these processes, ultimately resulting in

glutamate excitotoxicity (Humm et al., 1999). Other studies have demonstrated smaller infarct volume and improved recovery in rats with MCA occlusion followed by treadmill running for 28 days (Matsuda et al., 2011). The authors also found increases in neurotrophic factors and decreases in apoptotic factors in the treadmill group. Thus, it is possible that the type and amount of motor activity may be critical in modulating the neural environment, and in determining whether regenerative processes or neuronal death cascades predominate during the early stages following brain injury. However, it should be noted that since rats are quadrupeds, the casting of the less-impaired limb constitutes a relatively severe form of overuse. The rat must use the impaired limb for postural support, grooming, feeding and locomotion. Whether this severe overuse phenomenon could occur simply via intense, repetitive training is not completely known.

These studies of early use raise the second issue of optimal "dose" of the behavioral experience. This factor is important not only for understanding the margin of safety for acute rehabilitation, but also the dose-response relationship for rehabilitation protocols across the continuum of the post-stroke intervention period. Animal models of experience-dependent recovery after injury have the advantage of utilizing highly motivated subjects that are on controlled feeding schedules. Thus, it has been common to implement protocols with very high levels of repetition compared with the equivalent therapy in human studies (Birkenmeier et al., 2010). Thus, it is important to define what human stroke patients can tolerate and whether more is better. A recent randomized controlled trial in 18 chronic stroke survivors demonstrated that doubling the number of repetitions in an upper-limb robot-assisted therapeutic intervention resulted in significant improvement in motor function (Hsieh et al., 2011). A recent meta-analysis also found limited evidence for a dose-response relationship in effecting motor recovery after stroke (Cooke et al., 2010). However, meta-analyses to examine dose-response relationships are complicated by the differences in how dose is defined (number of repetitions, number of days, number of sessions), and the various outcome measures that are employed. Studies that directly assess these relationships in both the acute and chronic stages after stroke are critically needed.

A rational, mechanistically-based approach to dose-response relationships can only be fully realized with analogous non-human animal studies to determine what molecular and cellular events are driven by increased dose. Does greater repetition simply result in increased neurotrophic factors? Is rehabilitation intensity related to greater synaptic number? At what point do these processes saturate? While ultimately, results of clinical trials, practicality and economics will drive our decisions regarding dose, developing a neurobiological model for this important factor will define the rules that govern limitations of therapy.

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Use it and/or lose it—experience effects on brain remodeling across time after stroke

Rachel P. Allred[1], Soo Young Kim[2] and Theresa A. Jones[1]*

[1] *Department of Psychology and Institute for Neuroscience, University of Texas at Austin, Austin, TX, USA*
[2] *Department of Integrative Biology, University of California Berkeley, Berkeley, CA, USA*

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Randolph J. Nudo, University of Kansas Medical Center, USA
Christopher J. Steele, Max Planck Institute for Human Cognitive and Brain Sciences, Germany

****Correspondence:***
Theresa A. Jones, Department of Psychology and Institute for Neuroscience, University of Texas at Austin, 1 University Station A8000, Austin, TX 78712, USA
e-mail: tj@austin.utexas.edu

The process of brain remodeling after stroke is time- and neural activity-dependent, and the latter makes it inherently sensitive to behavioral experiences. This generally supports targeting early dynamic periods of post-stroke neural remodeling with rehabilitative training (RT). However, the specific neural events that optimize RT effects are unclear and, as such, cannot be precisely targeted. Here we review evidence for, potential mechanisms of, and ongoing knowledge gaps surrounding time-sensitivities in RT efficacy, with a focus on findings from animal models of upper extremity RT. The reorganization of neural connectivity after stroke is a complex multiphasic process interacting with glial and vascular changes. Behavioral manipulations can impact numerous elements of this process to affect function. RT efficacy varies both with onset time and its timing relative to the development of compensatory strategies with the less-affected (nonparetic) hand. Earlier RT may not only capitalize on a dynamic period of brain remodeling but also counter a tendency for compensatory strategies to stamp-in suboptimal reorganization patterns. However, there is considerable variability across injuries and individuals in brain remodeling responses, and some early behavioral manipulations worsen function. The optimal timing of RT may remain unpredictable without clarification of the cellular events underlying time-sensitivities in its effects.

Keywords: upper extremity function, restorative plasticity, motor skill learning, learned non-use, motor cortex

INTRODUCTION

Stroke is a leading cause of chronic disability worldwide (Johnston et al., 2009). Upper extremity (hand and arm) impairments are especially prevalent lasting post-stroke disabilities (Lai et al., 2002; Kwakkel et al., 2003). Compensatory reliance on the nonparetic hand exacerbates impairments in the paretic side by encouraging its disuse (i.e., "learned nonuse," Taub et al., 2006). Motor rehabilitative training (RT) approaches are the main tools for treating these impairments, but they are typically insufficient to normalize function. A better understanding of the mechanisms of RT efficacy could help optimize its therapeutic potential.

Ischemic injury triggers prolonged periods of neuroanatomical reorganization (Li and Carmichael, 2006; Wieloch and Nikolich, 2006; Cheatwood et al., 2008). This reorganization unfolds over months or longer, but is particularly dynamic early after stroke (Anderson et al., 1986; Carmichael, 2006; Murphy and Corbett, 2009). There are likely to be windows of opportunity for driving functionally useful brain remodeling with RT, as well as windows of vulnerability for promoting suboptimal neural changes. When is early enough? When is it safe? What should be done in these windows? The answers to these questions remain unclear. Considerable variability in neural remodeling time courses can be expected between individuals and across brain regions (e.g., Hsu and Jones, 2006; Krakauer, 2007; Riley et al., 2011). Furthermore, earlier is not better for everything. Peri-infarct tissue is vulnerable to use-dependent excitotoxicity in very early periods (Humm et al., 1998) and there is potential to ingrain maladaptive behavioral strategies (Allred and Jones, 2008a,b; Jones and Jefferson, 2011).

Motor RT relies on mechanisms of skill learning, as does compensatory learning with the nonparetic hand. In intact brains, manual skill learning depends on practice-dependent synaptic structural and functional reorganization of motor cortex (Monfils et al., 2005; Kleim et al., 2006; Xu et al., 2009; Dayan and Cohen, 2011). These learning mechanisms are likely to interact with regenerative responses to stroke, many elements of which are sensitive to behavioral manipulations, as reviewed previously (Jones and Adkins, 2010). Optimally timing and tailoring RT requires a better understanding of how it interacts with post-stroke remodeling processes as they unfold over time. Below we review a framework for understanding these interactions, progress in unraveling them and ongoing knowledge gaps surrounding time-sensitivities for experience-driven plasticity after stroke.

A DEVELOPMENTAL FRAMEWORK FOR UNDERSTANDING SENSITIVE TIME WINDOWS AFTER STROKE

Greenough et al. (1987) introduced the term "experience-expectant plasticity" to refer to the role of experience in brain development during early sensitive periods. The developing brain depends on external stimuli to shape neural circuitry patterns via mechanisms of synaptic competition, in which the most effectively activated neural connections are selectively maintained and matured, and those less well activated are eliminated (Black et al., 1997; Jones et al., 1998). A well-known example is the maturation

of ocular dominance columns in visual cortex, which is driven by competitive activity of inputs from either eye. In the absence of visual stimulation of one eye, thalamocortical afferents of the remaining eye claim a disproportionate share of cortical territory, a pattern that is difficult to reverse (Hubel and Wiesel, 1965; Berardi et al., 2003; Wright and Bourke, 2013). This developmental process is contrasted with "experience-dependent" plasticity, i.e., the mechanism of learning. The two processes have overlapping cellular mechanisms, but vary in the magnitude and persistence of brain changes instigated by them (e.g., Zuo et al., 2005; Xu et al., 2009; Yu et al., 2013). In essence, experience-expectant plasticity establishes the major connectivity patterns of the brain and experience-dependent plasticity continuously refines this connectivity across the lifespan.

Mechanisms of experience-*dependent* plasticity clearly contribute to post-stroke brain reorganization (Williams et al., 2006; Kerr et al., 2011) and to the efficacy of RT (Nudo, 2003; Adkins et al., 2006), and they should be able to do so at any time. An unresolved question is to what extent early neural remodeling events after stroke rely on experience-*expectant* mechanisms resembling those of brain development. The regenerative responses to stroke are highly sensitive to behavioral manipulations (Jones and Adkins, 2010). The early pro-growth environment is reminiscent of development (Cramer and Chopp, 2000; Carmichael, 2006; Murphy and Corbett, 2009) and some neural restructuring events resemble those typical of brain development (Jones and Jefferson, 2011). To the degree that these responses also behave in an experience-expectant manner, one would predict early periods after stroke in which it is not only (1) relatively easy to drive neural remodeling into functionally beneficial directions using manipulations of experience and neural activity, but also (2) easy for any experiences that dominate the time window to stamp in suboptimal or maladaptive circuitry patterns that are difficult to reverse.

The first prediction above is reasonably supported, though there are still knowledge gaps that hamper its usefulness for clinical decisions, as described below. The second prediction has received less attention, but its potential implications seem equally important (Jones et al., 2013). Even with early interventions, most of the experiences of stroke survivors occur outside of the treatment context (Bernhardt et al., 2004, 2007; West and Bernhardt, 2013), creating a potential for these experiences to dominate reorganizational patterns. The existence of experience-expectant mechanisms after stroke would also raise the possibility of facilitating RT with treatments that prolong or reinstate these mechanisms (e.g., as demonstrated in visual system by Morishita and Hensch, 2008).

It is reasonable to draw upon brain development to understand brain reorganization after stroke, as cellular mechanisms for growing and re-growing neural connections overlap. However, unlike development, the adult post-stroke brain must remodel in a matrix of mature, dying, traumatized and dysfunctional structure. Stroke damages glia and vascular cells, as well as neurons, and substantially alters the intricate interactions among them. The creation of new stable patterns of neural connectivity after stroke depends on the coordinated plasticity of neurons, glia and vasculature.

NEURAL, GLIAL, AND VASCULAR REMODELING: MOVING TARGETS FOR NEUROREHABILITATION

The loss of neurons in the core of ischemic injury leaves connected regions partially denervated and efferent neurons stripped of postsynaptic targets. The counteroffensive is the induction of a growth permissive environment that promotes axonal sprouting, synaptogenesis and dendritic remodeling (Kelley and Steward, 1997; Carmichael, 2006; Brown et al., 2010). Synapse densities around an infarct decline and then recover over time to varying degrees depending on proximity to the infarct core (Brown et al., 2008; Sigler and Murphy, 2010). Remaining projections to denervated regions sprout collateral axons and form new synapses (Cotman and Anderson, 1988; McNeill et al., 2003; Dancause et al., 2005). The axons that most prominently contribute to reinnervation tend to be the most abundant (Raisman and Field, 1990) and the most active (in firing) of the surviving projections (Carmichael and Chesselet, 2002; Carmichael, 2003; Cesa and Strata, 2005; Brus-Ramer et al., 2007). The latter property helps make the remodeling processes sensitive to manipulations of neural activity (Brus-Ramer et al., 2007; Adkins et al., 2008; Carmel et al., 2010) and behavior (Jones and Jefferson, 2011; Overman et al., 2012). There are also persistent alterations in excitatory and inhibitory activity patterns that present potential targets for combining RT with other treatments (Carmichael, 2012; Zeiler et al., 2013).

Post-ischemic reactions of neurons, astrocytes and vasculature are tightly coordinated. For example, factors expressed by glia and neurons stimulate the formation of blood vessels, and new vessels release neural growth and survival factors (Wieloch and Nikolich, 2006; Hermann and Chopp, 2012). Glia have diverse roles in mediating neuroregenerative responses (Kelley and Steward, 1997; Mack and Wolburg, 2013). Astrocytes are intricately involved in synaptic plasticity (Murai et al., 2003; Haber et al., 2006; Eroglu and Barres, 2010). Astrocytes release thrombospondins to promote synapse formation (Christopherson et al., 2005; Eroglu et al., 2009), cholesterol to promote synapse maturation (Mauch et al., 2001; Goritz et al., 2005) and D-serine to regulate synaptic potentiation and depression (Panatier et al., 2006). Astrocytic behavior is neural activity- and experience-dependent (Jones and Greenough, 2002; Theodosis et al., 2008), e.g., astrocytic reactions to denervation in motor cortex are elevated by forced forelimb use (Bury et al., 2000). After cortical infarcts, quantities of perisynaptic astrocytes and synapses vary together with injury severity (Kim and Jones, 2010), and behavioral outcome is altered by pharmacological manipulation of astrocytic glutamate transport (Kim and Jones, 2013).

There are multiphasic vascular responses after stroke. Ischemic stroke results in expanses of reduced cerebral blood flow (CBF) and capillary density (Gjedde et al., 1990; Anderson et al., 1999; del Zoppo and Mabuchi, 2003), as well as major elevations in pro-angiogenic factors (Zhang and Chopp, 2002; Hayashi et al., 2003; Carmichael, 2006; Beck and Plate, 2009), the levels of which are predictive of functional outcome in stroke patients (Slevin et al., 2000; Sobrino et al., 2007). Angiogenic microenvironments also are supportive of neurogenesis (Ohab et al., 2006). However, new vessels tend to be transient and leaky (Yu et al., 2007; Hayward et al., 2011), and there is a variable degree of

recovery of CBF and vessel densities in humans (Gjedde et al., 1990; Krupinski et al., 1994; Szpak et al., 1999) and rodent models (Marti et al., 2000; Biernaskie et al., 2001; Lin et al., 2008; Mostany et al., 2011). Because sufficient blood flow is essential for activity-dependent neural remodeling, RT efficacy could depend on the success of vascular remodeling, and it might promote or accelerate it depending on its timing. For example, sensory stimulation starting 3 days after cortical ischemia promotes angiogenesis and CBF recovery (Whitaker et al., 2007).

Vascular and glial responses to injury and to behavioral experience are potentially major sources of variability in RT efficacy and its optimal timing. For example, time courses and magnitudes of astrocytic and vascular reactions to injury are altered with age (Gao et al., 2009; Brown and Thore, 2011; Popa-Wagner et al., 2011), injury severity (Kim and Jones, 2010) and diabetes (Prakash et al., 2013; Tennant and Brown, 2013). Neuroregeneration time courses and magnitudes also vary with age (Anderson et al., 1986), injury severity (Kim and Jones, 2010), injury modality (Napieralski et al., 1996; Phillips and Reeves, 2001; Voorhies and Jones, 2002; Jones et al., 2012) and other conditions (Hermann and Chopp, 2012). Thus, while there are many potential targets for treatment in stages of neurogliavascular remodeling after stroke, there is also much potential for variability in the optimal timing of these treatments.

EARLIER CAN BE MUCH BETTER FOR REHABILITATIVE TREATMENTS

Motor RT after stroke can drive structural and functional reorganization of the injured motor cortex of humans (Taub et al., 2003; Mark et al., 2006; Dong et al., 2007; Sterling et al., 2013) and other animals (Jones et al., 1999; Biernaskie and Corbett, 2001; Frost et al., 2003; Dancause et al., 2005). In animal models, training the paretic limb in skilled reaching after cortical infarcts (**Figure 1**) increases its movement representation area (Castro-Alamancos and Borrel, 1995; Nudo et al., 1996) and synaptic densities (Adkins et al., 2008) in residual motor cortex of the injured hemisphere. Blocking the reorganization prevents the functional gains (Ramanathan et al., 2006). In the absence of RT, representations of the paretic limb are reduced, even well outside of infarct borders (Nudo et al., 1996).

Several studies support that RT is more effective if initiated earlier after stroke. RT beginning within 1 week of motor cortical infarcts in monkeys spares the paretic hand representation in motor cortex compared with controls (Nudo et al., 1996), but this effect is lost if training is delayed until 30 days post-infarct (Barbay et al., 2006). In rats, greater improvements in the paretic forelimb, and less compensatory reliance on the nonparetic limb, result from RT initiated within 5, vs. 14 or 30, days post-ischemia (Biernaskie et al., 2004). In humans, early (within 4 days post-stroke) interventions are associated with reduced disability at

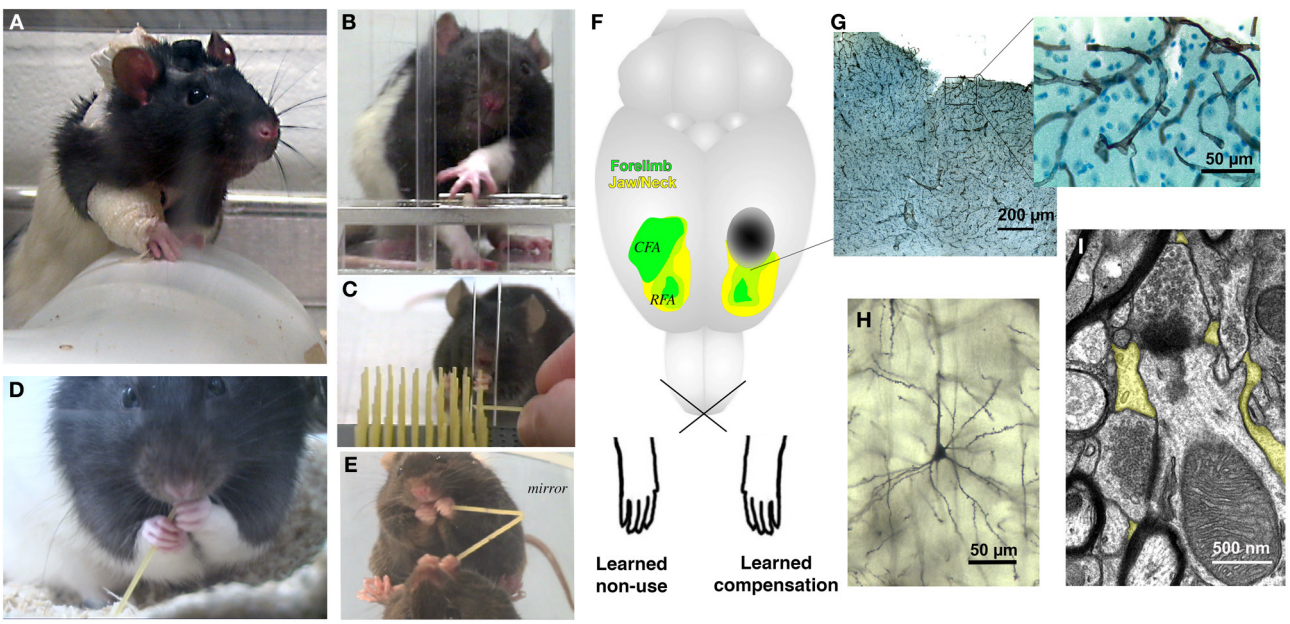

FIGURE 1 | Rodent models of upper extremity impairments after stroke used to study forelimb experience effects on brain and behavioral outcomes. Examples of behavioral manipulations in rats and mice include **(A)** forelimb constraint, used to force greater use of the paretic limb, **(B,C)** skilled reaching tasks, used to model both rehabilitative training (RT) focused on the paretic limb and compensatory skill learning with the nonparetic limb and, **(D,E)** pasta handling tasks, used to provide coordinated bimanual experience. **(F)** Approximate motor cortical infarct location (dark oval) used in several studies, as shown relative to head (yellow) and forelimb (green) movement representation regions of motor cortex. The caudal forelimb area (CFA) is in primary motor cortex and the rostral forelimb area (RFA) is in premotor/supplementary motor cortex. Motor cortical samples showing **(G)** vasculature (collagen IV immunolabeled), **(H)** a pyramidal neuron dendritic arbor (Golgi stained) and **(I)** synapses surrounded by peri-synaptic astrocytic processes (yellow highlights). The functional efficacy of motor RT has been linked with the reorganization of movement representations in peri-infarct motor cortex, but the influence of RT over time on the remodeling of surviving neurons, glia and vasculature has not yet been well examined.

the time of hospital discharge compared with later interventions (Matsui et al., 2010). Patients receiving RT within 1 month post-stroke have greater functional improvements and require shorter RT duration to achieve them compared to those with delayed RT (Salter et al., 2006). Constraint induced movement therapy (CIMT), initiated within 3–9 months post-stroke enhances performance in several fine motor tasks compared to delayed (>9 months) CIMT (Lang et al., 2013).

In the studies above, RT timing was a categorical variable (earlier vs. later, **Table 1**), as is logical for determining if timing matters at all, but this does not lend precise information to the question of when, exactly, is optimal for RT onset. We also lack a precise understanding of the brain mechanisms of these time sensitivities. Our present understanding of RT mechanisms is based primarily on endpoint measures. We lack knowledge of how RT interacts with neuroremodeling responses as they unfold over time, and of the roles of vascular and glial plasticity in RT efficacy.

EARLIER IS NOT BETTER FOR EVERYTHING

Schallert and colleagues were the first to discover that forced use of a paretic limb, via constraint of the nonparetic limb, can be detrimental to functional outcome if done too early (Schallert et al., 2003). In rats, forelimb impairments are worsened, and injury size increased, by constraining the nonparetic limb for 2 weeks beginning immediately after motor cortical lesions (Kozlowski et al., 1996; see also Risedal et al., 1999; Farrell et al., 2001). If constraint is delayed for 7 days, there is no detrimental effect (Humm et al., 1998). These constraint manipulations were dissimilar to the clinical application of CIMT, e.g., rats did not engage in RT and the constraint was continuous (24 h/day). In contrast, RT efficacy is improved by its combination with less intensive constraint (8 h/day) beginning 7 days after intracerbral hemorrhage in rats (DeBow et al., 2003). In humans, high intensity CIMT when initiated very early (∼10 days) after stroke lessens functional improvement compared with lower intensity treatments (Dromerick et al., 2009).

Early intense exercise also can also be detrimental in rodent models of traumatic brain injury. Voluntary wheel running enhances cognitive performance if initiated *after* an acute (0–6 days) post-injury time period. However, exercise during the acute period impairs cognitive performance and prevents the normally seen up-regulation of BDNF (Griesbach et al., 2004, 2007; Griesbach, 2011).

Together, these findings support that *highly intense* physical activity *very* early after injury onset can be risky. We know of no evidence that less intense activity is detrimental. However, some types of RT might benefit from a delay, e.g., to allow resolution of metabolic dysfunction or target specific remodeling stages. Consistent with this possibility, intense cognitive training in rats is effective if it is initiated at 30 days, but not at 10 days, after hippocampal system lesions (Mala et al., 2012).

Table 1 | Time-sensitive effects of behavioral manipulations on functional outcome after brain damage in animal models.

Post-injury experience(onset time)			Functional outcome	References
Very early	**Early**	**Delayed**		
	Motor RT (Day 5–7)		↑↑	Nudo et al., 1996; Biernaskie et al., 2004
		Motor RT (Day 14)	↑↑	Biernaskie et al., 2004
	NPT (Day 5–7)	Motor RT (Day17–22)	↑	Allred et al., 2005, 2010; Allred and Jones, 2008b; Kerr et al., 2013
	NPT (Day 5–7)		↓↓	Allred et al., 2005, 2010; Allred and Jones, 2008b; Kerr et al., 2013; Maclellan et al., 2013
Exercise (Day 0)			↓↓	Griesbach et al., 2004
		Exercise (Day 14)	↑↑	Griesbach et al., 2004
	Cognitive RT (Day 7)		=	Mala et al., 2012
		Cognitive RT (Day 21)	↑↑	Mala et al., 2012
CNP (immediate)			↓↓	Kozlowski et al., 1996; Humm et al., 1998
	CNP (≥ Day 7)		=	Kozlowski et al., 1996; Humm et al., 1998
	CNP + motor RT (Day 7)		↑↑	DeBow et al., 2003

Onset time is relative to the time of injury. Behavioral manipulations continued for days to weeks after onset. Functional outcome direction is relative to no-behavioral-manipulation-controls with the same injury. RT, rehabilitative training; NPT, nonparetic limb training; CNP, constraint of the nonparetic limb.

TIMING-DEPENDENCIES—EFFECTS OF LEARNING TO COMPENSATE WITH THE NONPARETIC LIMB

The typical response to upper extremity impairments is to learn to rely on the better functioning limb to perform daily activities. This compensatory strategy contributes to learned nonuse of the paretic side (Taub et al., 2006) and, because it begins early after stroke, it is also likely to interact with neural remodeling events. We've studied this in rodents with motor cortical infarcts, using training on reaching tasks to examine effects of skill learning with either forelimb (Jones et al., 2013). Skill training of the nonparetic forelimb (NPT) increases dendritic growth in the contralesional cortex, but this appears not to benefit the paretic limb (Jones and Jefferson, 2011). NPT also exacerbates disuse of the paretic forelimb, impairs the efficacy of subsequent paretic limb RT (Allred et al., 2005, 2010; Kerr et al., 2013) and reduces RT-driven neuronal activation of peri-lesion cortex (Allred and Jones, 2008a,b). Thus, NPT alters a neural substrate for RT efficacy. Maclellan et al. (2013) found that paretic limb function was worsened even when tested 30 days after an earlier period of NPT. Bilateral and unskilled limb use are not detrimental to paretic limb function, but learning new unimanual skills with the nonparetic limb is detrimental, at least after motor cortical infarcts (Allred and Jones, 2008a).

The influence of the nonparetic limb could vary with infarct territories. The disruptive effects of NPT depend on contralesional motor cortex and its transcallosal projections. They are blocked by callosal transections and absent after bilateral motor cortical lesions (Allred et al., 2010). Thus, injuries that leave little remaining territory for transcallosal projections are potentially immune from maladaptive effects of compensating with the nonparetic limb.

These findings suggest that experiences of the nonparetic body side may contribute to abnormal interhemispheric interactions after stroke (Murase et al., 2004; Calautti et al., 2010). They also indicate that RT efficacy can vary, not only with its timing after stroke, but also its timing relative to the development of compensatory skills with the nonparetic body side.

CONCLUSIONS

There are clearly early sensitive periods after stroke for the influence of RT and other behavioral experiences on functional outcome. It seems reasonable to assume that the early dynamic period of neural remodeling contribute to these time-sensitivities, but the remodeling process is complex and multiphasic, and the events or stages within it that are most important for RT efficacy have yet to be identified. For example, RT efficacy might benefit from coinciding with early stages of axonal sprouting, so that it shapes patterns of synaptic re-connectivity and effectively competes with maladaptive compensatory strategies in doing so. It could also depend on whether it is timed to coincide with stages of new vessel formation and/or stabilization, so that it can benefit from blood flow recovery or help promote it. These and other possibilities have yet to be directly tested, but it is feasible to do so in animal models of chronic stroke (**Figure 1**). It is also possible that, once events that contribute to heightened sensitivity to RT are identified, imaging or other assays could be used to reveal them in clinical populations. This could be essential to efforts to optimize RT, because the cellular conditions that create sensitive windows are likely to vary in time and magnitude with brain region, age, injuries and premorbid conditions. An "early" that is reliably *best* for RT in a reasonable portion of the clinical stroke population could be elusive in the realm of time, as measured by hours and days, but there is potential for it to be found within stages of sequential brain events. Knowledge of the events that create sensitive windows for experience-driven plasticity after stroke also could lead to treatments that promote these windows when they are deficient or reopen them after they have passed.

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Non-invasive brain stimulation in neurorehabilitation: local and distant effects for motor recovery

Sook-Lei Liew[1]*, Emilliano Santarnecchi[2], Ethan R. Buch[1,3] and Leonardo G. Cohen[1,3]*

[1] *Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA*
[2] *Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy*
[3] *Center for Neuroscience and Regenerative Medicine, Uniformed Services University of Health Sciences, Bethesda, MD, USA*

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

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Jack Van Honk, Utrecht University, Netherlands
Jane Allendorfer, University of Alabama at Birmingham, USA

***Correspondence:**
Sook-Lei Liew and Leonardo G. Cohen, Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, 10 Center Drive, Bethesda, MD 20892, USA
e-mail: lei.liew@nih.gov; cohenl@ninds.nih.gov

Non-invasive brain stimulation (NIBS) may enhance motor recovery after neurological injury through the causal induction of plasticity processes. Neurological injury, such as stroke, often results in serious long-term physical disabilities, and despite intensive therapy, a large majority of brain injury survivors fail to regain full motor function. Emerging research suggests that NIBS techniques, such as transcranial magnetic (TMS) and direct current (tDCS) stimulation, in association with customarily used neurorehabilitative treatments, may enhance motor recovery. This paper provides a general review on TMS and tDCS paradigms, the mechanisms by which they operate and the stimulation techniques used in neurorehabilitation, specifically stroke. TMS and tDCS influence regional neural activity underlying the stimulation location and also distant interconnected network activity throughout the brain. We discuss recent studies that document NIBS effects on global brain activity measured with various neuroimaging techniques, which help to characterize better strategies for more accurate NIBS stimulation. These rapidly growing areas of inquiry may hold potential for improving the effectiveness of NIBS-based interventions for clinical rehabilitation.

Keywords: non-invasive brain stimulation, transcranial direct current stimulation (tDCS), transcranial magnetic stimulation, neurorehabilitation, stroke

INTRODUCTION

Stroke is a leading cause of serious long-term adult disability around the world. Recovery of motor function remains highly variable despite standardized rehabilitation programs (Kwakkel et al., 2003; Go et al., 2013). The study of the mechanisms underlying recovery of motor function after stroke has been difficult due to the heterogeneity among individual lesion profiles, the severity of motor impairment and the differences in plasticity processes depending on the time passed since the ictal event.

Non-invasive brain stimulation (NIBS) has been explored as a possible technical adjuvant of customarily used neurorehabilitative treatments. NIBS, which employs electrical or magnetically-induced currents to stimulate the brain through the scalp, can temporarily excite or inhibit activity in target brain regions. In this review, we first introduce the use of NIBS in basic science and clinical neuroscience, focusing on the two most commonly used NIBS techniques (transcranial magnetic stimulation, *TMS*, and transcranial direct current stimulation, *tDCS*). We then delve into recent work exploring the effects of local application of NIBS on activity under the stimulating site and in distant brain regions. We discuss the evidence for the application of NIBS techniques in motor rehabilitation and provide a map of possible future research directions, including the combined use of NIBS with neuroimaging techniques, and the use of transcranial random noise stimulation and transcranial alternating current stimulation, among others.

BACKGROUND

Early studies of "therapeutic electricity" can be traced back to the late 1800s. Since then, NIBS applications have been used in a variety of settings (for reviews, see Priori, 2003; Wagner et al., 2007a,b; Schlaug and Renga, 2008). Scientific research and public awareness of these techniques has increased greatly over the last few decades. While only a handful of papers were published on the topic in 1988, almost 1400 papers were published in 2012 alone (see **Figure 1**).

In contrast, the use of NIBS in neurorehabilitative settings has more recently taken off, starting in the mid-2000s (Elbert et al., 1981; Ward and Cohen, 2004; Hummel et al., 2005; see **Figure 1**). Currently, the most common NIBS techniques are TMS and transcranial electric stimulation (tES; for a recent review, see Dayan et al., 2013). NIBS is thought to modulate neural activity via differing mechanisms, including the induction of LTP-like protocols (Ziemann and Siebner, 2008; Fritsch et al., 2010; Muller-Dahlhaus et al., 2010; Ziemann, 2011). It has been proposed that modulation of these mechanisms induce motor plasticity, contributing to motor learning (Reis et al., 2009; Censor et al., 2010; Fritsch et al., 2010; Buch et al., 2011; Dayan and Cohen, 2011; Schambra et al., 2011; Conde et al., 2013) and secondarily impacting neurorehabilitative processes (Dimyan and Cohen, 2010, 2011).

TYPES OF NIBS

NIBS techniques have been tested in a wide array of research and clinical settings (Dayan and Cohen, 2011; Song et al., 2011;

Ziemann, 2011; Censor et al., 2013; Sandrini and Cohen, 2013; Vidal-Dourado et al., 2014), and the testing of NIBS to modulate learning and memory processes has attracted particular attention in the last few years (for reviews, see Tanaka et al., 2011; Kandel et al., 2012; Sandrini and Cohen, 2013). While there is wide variation in stimulation protocols, traditional TMS and tDCS mechanisms and protocols are discussed briefly here (see **Figure 2** for a summary diagram; see **Box 1** for safety considerations).

TMS

First introduced by Barker et al. (1985), TMS used within international safety guidelines is safe and non-invasive (Kobayashi et al., 2003; Rossini and Rossi, 2007). TMS produces a time-varying magnetic field at that flows perpendicular to the stimulating coil, which then induces electric currents that are generally parallel to the coil in the underlying cortical tissue. The specific protocol and magnetic coil design allows TMS to

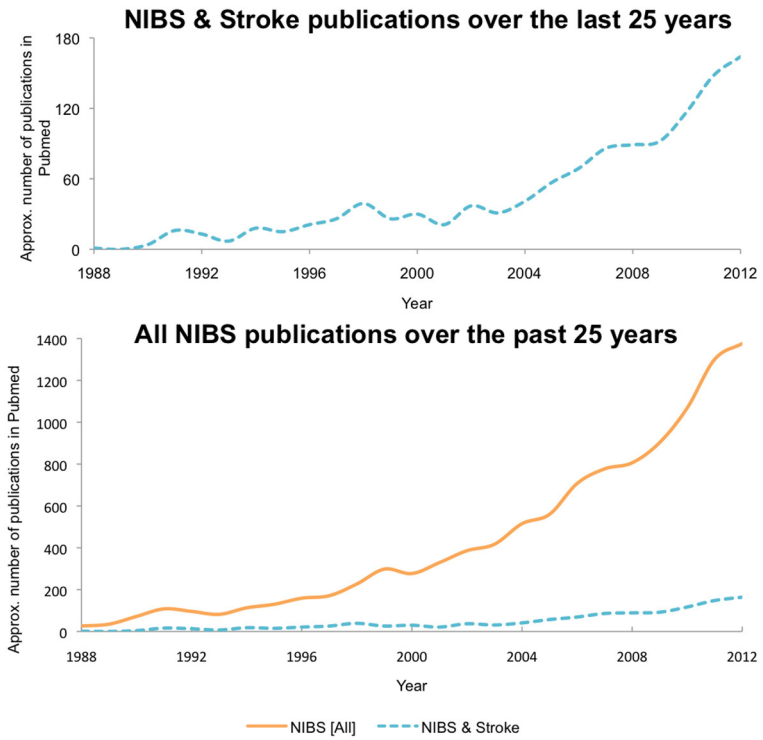

FIGURE 1 | NIBS publications. Graph depicting exponential growth in the number of publications on NIBS from 1988 to 2012, with NIBS publications specific to stroke depicted at the top, and NIBS publications specific to stroke shown in the context of the general NIBS field at bottom.

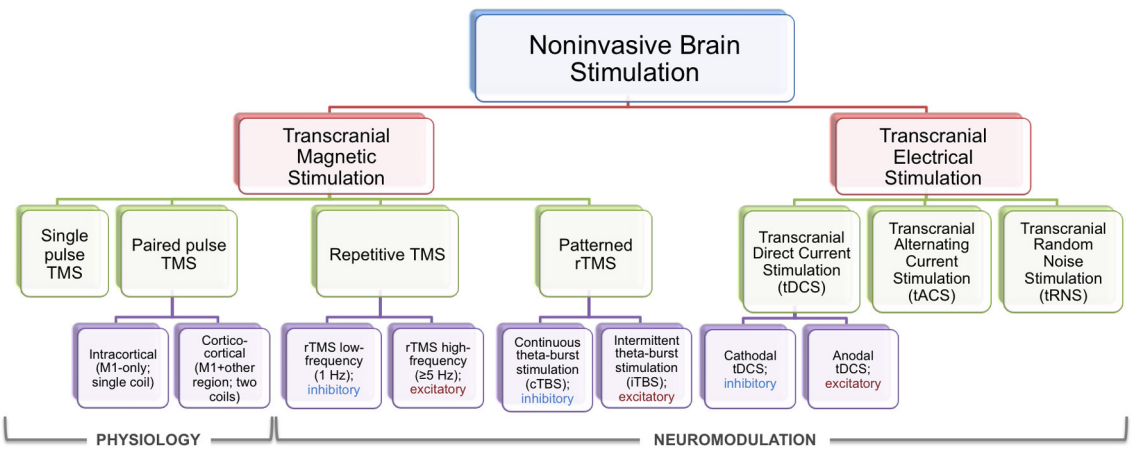

FIGURE 2 | NIBS schematic. Chart depicting the general breakdown of NIBS techniques, focusing on TMS and tES. Types of TMS and tES paradigms are describe, and the divide between physiology and neuromodulatory functions is depicted. Inhibitory and excitatory neuromodulatory techniques are also labeled.

Box 1 | Safety Considerations for TMS and tDCS

Safety considerations for TMS

Apart from general safety considerations regarding tissue heating, magnetization of ferromagnetic objects, and magnetic field exposure for both subjects and operators, consideration must be given to potential side effects of TMS, which consist primarily of the rare induction of seizures, as well more common effects like local transient pain, headaches, and discomfort (Rossi et al., 2009). Consequently, while there are no specific concerns about single and paired pulse TMS applications, rTMS and patterned rTMS deserve specific attention in terms of the number of stimuli delivered per unit time. Generally speaking, the safety of high-frequency rTMS protocols is usually assured by including periods of no stimulation between shorter periods of rTMS. TBS protocols are usually applied by replicating the original protocol published by Huang et al. (2005), consisting of 3 pulses at 50 Hz applied at 5 Hz for 20 or 40 s, in the case of cTBS. In contrast, iTBS is obtained by conducting 2-s periods of cTBS, each separated from one another by 8 s. It must be noticed that there are almost an infinite number of combinations for such protocols, with even small changes possibly having strong impacts on both the effects and safety of such protocols. Thus, general guidelines for rTMS delivery should be always checked, particularly when applied in clinical settings. Additionally, it should be considered that the effects of these techniques present interindividual differences.

Safety considerations for tDCS

Compared to TMS, tDCS is relatively safer and easier to use. A vast literature supports the use of low-intensity transcranial stimulation as safe for use in humans, with only rare and relatively minor adverse effects, such as mild tingling of the scalp, minor fatigue, or itching of the scalp (Poreisz et al., 2007) and no effects over serum levels of molecular markers of neuronal injury such as neuron-specific enolase (Nitsche et al., 2003b) or N-acetyl-aspartate (Rango et al., 2008). It must be noticed that all the aforementioned effects of tDCS are strongly dependent on current density, electrode positioning, and stimulation duration. While differences in such parameters may be of interest for their consequences over observed behavioral responses, they must also be taken into account for safety purposes. For instance, caution should be used during monopolar stimulation with extracephalic references due to the hypothetical stimulation of brainstem regions, thus possibly modulating sympathetic outflow (Cogiamanian et al., 2010). However, such findings are still a matter of debate. Most importantly, anatomical changes due to central nervous system pathology can significantly modify the current distribution induced by tDCS. For instance, in subjects with stroke, the affected cortical area is usually replaced by cerebrospinal fluid, which has a high conductance, and current can accumulate on the edges of cortical stroke lesions (Wagner et al., 2007a,b).

stimulate cortical tissues at variable depths beneath the scalp (Cohen et al., 1998).

TMS can be used to assess neurophysiological processes and influence brain function via application of single, paired, or repetitive stimulation. In single-pulse TMS (*spTMS*), one single stimulus is applied, for example, over the primary motor cortex (M1; Reis et al., 2008). When the intensity of the stimulus is strong enough (suprathreshold), it will induce a measurable electromyographic (EMG) response in target hand muscles contralateral to the stimulated M1, known as a motor-evoked potential (MEP). spTMS may be used to map M1 corticospinal outputs, study central motor conduction time, and investigate causal chronometry in brain-behavior relations (for a review, see Dayan et al., 2013). Due to the relative simplicity of recording with surface EMG electrodes, spTMS-induced MEPs have become a routine procedure in clinical neurophysiology for assessing the functional integrity of corticospinal and corticobulbar motor pathways in a wide range of neurological disorders (Rossini and Rossi, 2007). Paired (ppTMS) or triple-pulse TMS (tpTMS) utilize one or more conditioning stimuli applied prior to a suprathreshold M1 (test) stimulus that induces a measurable MEP (Groppa et al., 2012). This technique can be used to investigate intra- or cortico-cortical neuronal interactions depending on the precise latency and intensity (sub- or supratheshold) of the conditioning pulses, and depending whether they are applied to the target region or to an interconnected brain region. For example, ppTMS applied to M1 has been used to investigate different aspects of local interneuron dynamics with the resulting effect of the conditioning pulse on the output MEP demonstrating intracortical facilitation (ICF) or inhibition (ICI), depending on the latency of stimulation (Chen et al., 1998; Cohen et al., 1998; Boroojerdi et al., 2000). ppTMS can be applied to different sites to evaluate the effects of a stimulus on one region over the excitability of a different brain region. In this form, ppTMS can test cortico-cortical connectivity between two different regions. For example, connectivity can be assessed between homologous regions of both M1s (with this effect referred to as interhemispheric inhibition; Di Lazzaro et al., 1999; Murase et al., 2004; Duque et al., 2005), between premotor cortex and M1, between dorsolateral prefrontal cortex (DLPFC) and M1, between the posterior parietal cortex and M1, and between the cerebellum and M1 (Oliveri et al., 2005; Koch et al., 2007; Daskalakis et al., 2008; Buch et al., 2010). This work provides insight into the causal relationship of prefrontal, frontal, and parietal inputs on M1 corticospinal output within motor behavioral contexts such as prehension, action selection, and action reprogramming. Investigations of these dynamics in patient groups, such as chronic stroke, have revealed relationships between altered cortico-cortical interactions and behavioral deficits (for example, see Murase et al., 2004; Nowak et al., 2009).

Repetitive TMS (*rTMS*) can also be used as a neuromodulatory tool. Low-frequency rTMS ($\leq$1 Hz) can be used to transiently perturb the stimulated brain region inducing a so-called "virtual lesion" (Pascual-Leone et al., 1999). This form of inhibitory rTMS represents an *in-vivo* non-invasive method available for demonstrating the causal influence of a given cortical region or its interconnected network on specific behaviors (Chen et al., 1997; Cohen et al., 1997; Walsh and Cowey, 2000). rTMS induces frequency- and intensity-specific after-effects, with low-frequency stimulation ($\leq$1 Hz; Chen et al., 1997) inducing a decrease in cortical excitability as described previously, while high-frequency stimulation ($\geq$5 Hz) results in an increase

in MEP amplitude, increasing activation within the region for at least 30 min (Rossi et al., 2009). Depending on the specific stimulation protocol used, the neuromodulatory effects of rTMS can outlast the stimulation period by several minutes to hours. Paired associative stimulation (PAS) is a related technique that involves application of a peripheral nerve stimulus followed by a TMS pulse at varying interstimulus intervals. Pairs are applied at very low-frequency (0.1 Hz) to M1 and to a peripheral nerve (Stefan et al., 2000, 2002; Wagner et al., 2007a,b). By varying the inter-stimulus intervals, PAS can induce potentiation or inhibition of M1 corticospinal output lasting for up to 90 min. A modified version of this protocol has been developed to investigate the induction of associative plasticity within cortico-cortical pathways (Rizzo et al., 2009; Buch et al., 2011).

Another form of rTMS is patterned rTMS. It consists of the repetitive application of short rTMS bursts at a high stimulation frequency. The most common paradigm is theta burst stimulation (TBS, continuous cTBS or intermittent iTBS), in which short bursts of 50 Hz rTMS are applied at a rate in the theta range (5 Hz) (Huang et al., 2005). As with low and high-frequency rTMS, cTBS, and iTBS induce cortical depression and facilitate corticospinal excitability, respectively, in healthy subjects for up to 70 min. When applied to prefrontal areas, it may influence memory processes like reconsolidation of episodic memories (Sandrini et al., 2013). Of note, the effects of these different techniques on motor cortical excitability present substantial interindividual differences, the origin of which are under investigation. The use of this technique in clinical populations thus requires further work and a careful approach (Ridding and Rothwell, 2007).

tDCS

tDCS is applied using a battery-powered direct current (DC) generator connected to two relatively large anodal and cathodal sponge-enclosed rubber electrodes (20–35 cm^2 in area) positioned over the scalp. It is thought that low amplitude currents (ranging from 0.5 to 2.0 mA) applied at the scalp can partially penetrate and reach cortical tissues (Datta et al., 2009). In contrast to TMS, tDCS does not result in the induction of action potentials. tDCS seem to modify the threshold for discharge of cortical neurons (Nitsche and Paulus, 2001; Priori, 2003). As a reference point, the magnitude of tDCS stimulation (0.079–0.20 A/m2) is far below the range of action potential thresholds (22–275 A/m2).

tDCS can modulate cortical excitability in a polarity-dependent fashion. While anodal stimulation increases cortical excitability, cathodal stimulation is thought to decrease it. It should be noted though that these effects, as those of facilitatory and inhibitory TMS, exhibit high interindividual variability (Ridding and Rothwell, 2007) and depend on the activity levels of the stimulated tissues (Silvanto et al., 2008). Both produce after-effects lasting 30–40 min, following 15–30 min of stimulation, with the after-effects strongly dependent on the duration and intensity of the stimulation (see Nitsche and Paulus, 2001). In addition, the direction of such polarization strictly depends on the orientation of axons and dendrites in the induced electric field. While tDCS has been initially shown to modulate activity in both the motor and visual cortices (Nitsche and Paulus, 2011), recent evidence has suggested that it is also efficacious in modulating higher-order cognitive processes through its applications over prefrontal and parietal regions (Nitsche et al., 2012; Monti et al., 2013; Santarnecchi et al., 2013).

Special consideration should be given to the placement of the electrodes and the focality of tDCS interventions. Newer tDCS montages include bipolar and monopolar scalp stimulation, with the former consisting of both cathode and anode placed on the scalp surface, while the latter positions the "active" electrode on the scalp, with the "reference" placed on an extracephalic target (shoulder, leg, arm, etc.; Schambra et al., 2011). Different electrode configurations may result in different patterns of current spreading over the scalp and consequently on the cortex; it is feasible that the typical "reference" position over the supraorbital region may produce undesired stimulation in non-target regions, thus newer monopolar stimulation montages attempt to avoid this problem (DaSilva et al., 2011). In addition, it has been proposed that high-resolution tDCS may improve this form of stimulation's focality (high-definition tDCS, or HD-tDCS; Datta et al., 2009). From an instrumental point of view, HD-tDCS uses multiple sites of anodal and cathodal stimulation to target a specific region. While substantial work is under way to model the fields induced by these different montages, clear behavioral or physiological data is lacking on the differences between these approaches.

While tDCS-induced changes in cortical excitability have been related to changes in the underlying cortical neuronal activity, less is known about the specific mechanisms mediating these effects. It has been reported that carbamazepine, dextromethorphan, and the calcium channel blocker flunarizine diminish the effects of anodal tDCS on motor cortical excitability (Nitsche et al., 2003a). On the contrary, the partial NMDA agonist D-cylcoserine prolongs the effects of anodal tDCS on cortical excitability (Nitsche et al., 2004). Anodal tDCS applied to a slice preparation of rodent M1 induced LTP-like effects. This effect was NMDA-receptor dependent and mediated by secretion of brain-derived neurotrophic factor (BDNF; Fritsch et al., 2010). Overall, these findings suggest that the magnitude of membrane polarization, the conductance of sodium and calcium channels, the magnitude of NMDA receptor activity as well as BDNF secretion contribute to different extents to the tDCS after-effects. These findings open the possibility of pharmacologically modulating tDCS effects.

tDCS has also been tested in small clinical trials evaluating corticospinal excitability, neurophysiological changes, and the modulation of behavioral variables in neurological and psychiatric diseases such as depression, chronic pain, epilepsy, neuropsychiatric disorders, and stroke, with mixed results (for reviews, see Nitsche and Paulus, 2011; Rothwell, 2012).

NETWORK EFFECTS OF NIBS

Recently, a wealth of studies have begun to demonstrate that brain stimulation leads not only to local changes in activity under the stimulated coil or electrodes, but also to distant changes in interconnected brain regions throughout the brain (for reviews, see Siebner et al., 2009; Siebner and Ziemann, 2010).

Successful behavior requires the concerted action of multiple brain regions. Neuroimaging studies started to provide important information on the activity of these different networks. In this setting, regions in communication with one another are thought to be highly synchronized (Biswal et al., 1995; Fries, 2005). Interregional connectivity can be analyzed as simple correlations between regions' activations and phase-locked coherence in neural oscillations, or can be modeled with more complex approaches that include *a priori* hypotheses (e.g., using dynamic causal modeling, *DCM*). It is now known that patterns of functional connectivity are predictive of successful motor behaviors and motor recovery in healthy individuals and in patients with stroke (for reviews, see Grefkes and Fink, 2011, 2012). Thus, while individual regions perform specific functions, the sharing of this information amongst a wide array of interconnected regions is critical for successful behavior. Given this information, the ability of NIBS to modulate activity locally and in interconnected networks seems valuable. There is substantial research activity in this area.

TMS AND CONNECTIVITY

Early studies demonstrated it is possible to evaluate changes in brain activity after TMS using single-photon emission computerized tomography (SPECT) (Shafran et al., 1989; Dressler et al., 1990). Several groups performed similar evaluations using positron emission tomography (PET) while participants underwent TMS stimulation (Fox et al., 1997; Paus et al., 1997; Paus, 1999). Other studies evaluated neurophysiological rather than blood flow changes induced by TMS using electroencephalogram (EEG) (Amassian et al., 1992). In the late 1990s, Bohning et al. (1997, 1998) demonstrated the feasibility of recording blood-oxygen-level dependent (BOLD) signal activity changes using fMRI in close temporal proximity to TMS. This early work documented local and distant changes in regional cerebral blood flow and in physiological activity associated with focal TMS stimulation. In the two decades since this pioneering work, researchers have developed new paradigms of combined brain imaging and brain stimulation to explore the effects of focal stimulation on global brain activity (see **Table 1**).

In healthy volunteers, Bestmann and colleagues demonstrated that suprathreshold high-frequency rTMS stimulation over M1 induces BOLD signal changes in distant cortical and subcortical regions, including the primary sensorimotor, supplementary and premotor cortices, as well as in the putamen and thalamus (Bestmann et al., 2004). Consistently, high-frequency suprathreshold rTMS over M1 enhanced connectivity with the supplementary motor area (SMA) (Bestmann et al., 2003). More recently, it was shown that low-frequency inhibitory rTMS over M1 also modified connectivity between M1, SMA, and the anterior cerebellum, and more importantly, showed that modulation of such connectivity correlated with the ability of healthy humans to modify a previously consolidated motor memory (Censor et al., 2013).

Application of rTMS over regions other than M1 also modulates functional activity. Suprathreshold rTMS over the left dorsal premotor cortex (PMd) for example increases BOLD signal locally, under the stimulating coil, and in distant regions like the right PMd, bilateral ventral premotor cortex, SMA (Bestmann et al., 2005).

In patients with chronic stroke, subthreshold rTMS over the ipsilesional M1 modulates interhemispheric and effective connectivity between this region, the basal ganglia and the thalamus (Chouinard et al., 2006). Inhibitory rTMS over the contralesional M1 resulted in increased connectivity between the ipsilesional M1 and SMA (Grefkes et al., 2010). These results suggest that reducing excitability and connectivity of the contralesional M1 may result in increased connectivity of the ipsilesional M1. The finding that modulation of ipsilesional and contralesional M1 effective connectivity correlated with motor function in these patients (Grefkes et al., 2010), in concordance with Chouinard et al. (2006) work, is suggestive of a causal link between changes in connectivity and behavior.

Stimulation of the contralesional PMd in chronic stroke patients induced stronger connectivity between this region and the ipsilesional primary sensorimotor cortex in individuals with greater motor impairments (Bestmann et al., 2010), suggesting that contralesional influences from regions other than M1 are also relevant to behavior, particularly for patients with greater motor impairment. Future work is needed to examine these effects in greater detail.

Altogether, these studies suggest that facilitatory stimulation of ipsilesional M1 increases M1-SMA functional connectivity while inhibitory stimulation of contralesional M1 decreases contralesional but strengthens ipsilesional connectivity—a pattern that is associated with improved motor performance (Ward et al., 2003; Rehme et al., 2011). Additionally, stimulation of regions other than M1 also induces substantial connectivity changes in interconnected brain regions. See Bestmann et al. (2008), Ruff et al. (2009), Ferreri and Rossini (2013) for additional information on this issue.

tDCS AND CONNECTIVITY

tDCS also induces changes in connectivity between different brain regions, both at rest and during task performance. Initial evaluations of the influence of tDCS on cortical connectivity have primarily focused on the primary motor cortex (M1) and the DLPFC in healthy individuals (see **Table 2**). Functional connectivity before, during, and after tDCS application has been studied with EEG (for a review, see Miniussi et al., 2012), fMRI, arterial spin labeling (ASL) and, most recently, magnetoencephalography (MEG) (see **Figure 3**, Soekadar et al., 2013a,b).

Polania and colleagues demonstrated that tDCS applied over M1 influences cortical connectivity measured with EEG, with effects more evident when studying connectivity during voluntary hand movements than during rest (Polania et al., 2011a,b, 2012b). Anodal tDCS over left M1, with the cathode positioned over the contralateral supraorbital area increased synchronization in alpha and lower frequency bands in frontal and parieto-occipital regions, and in the high gamma frequency (60–90 Hz) band in motor-related regions (Polania et al., 2011a) during voluntary hand movements, with fewer changes during rest (Polania et al., 2011a). The same group studied the influence of tDCS on activity measured with fMRI, which consistently was more evident during hand movements than at rest (Antal et al.,

Table 1 | Studies showing the effects of TMS on neural connectivity.

Stimulation site	Stimulation type	Neuroimaging technique	Population	Connectivity increases	Connectivity decreases	Behavioral result	References
Left M1	High-frequency rTMS (3.125 Hz), suprathreshold	fMRI	Healthy volunteers	M1/S1, SMA, dorsal premotor cortex, cingulate motor area, putament, thalamus			Bestmann et al., 2004
Left M1	High-frequency rTMS (3.125 Hz), subthreshold	fMRI	Healthy volunteers	SMA, dorsal premotor cortex, cingulate motor area, putamen, thalamus (but at a lower intensity)			Bestmann et al., 2004
Left M1	High-frequency rTMS (4 Hz), subthreshold	fMRI	Healthy volunteers	SMA, bilateral premotor cortex	Right M1/S1		Bestmann et al., 2003
Left M1	High-frequency rTMS (4 Hz), suprathreshold	fMRI	Healthy volunteers	Left M1/S1, SMA	Right M1/S1		Bestmann et al., 2003
Right M1	Low-frequency rTMS (1 Hz)	fMRI	Healthy volunteers		Decreased connectivity between right M1 and SMA, bilateral anterior cerebellum, right dorsal striatum, and left M1	Decreased SMA activity corresponded with decreased motor memory modificiation	Censor et al., 2013
Left dorsal premotor cortex (PMd)	High-frequency rTMS (3 Hz), suprathreshold	fMRI	Healthy volunteers	Left PMd, left premotor ventral (PMv), right PMd, bilateral PMv, SMA, somatosensory cortex, cingulate motor area, left posterior temporal lobe, cerebellum, caudate nucleus			Bestmann et al., 2005
Left dorsal premotor cortex (PMd)	High-frequency rTMS (3 Hz), subthreshold	fMRI	Healthy volunteers	Bilateral PMv, SMA, bilateral auditory cortex, bilateral thalamus, bilateral cingulate gyrus			Bestmann et al., 2005
Contralesional PMd	High-frequency rTMS (11 Hz), suprathreshold	fMRI	Chronic stroke patients	Increased activity in ipsielsional sensorimotor cortex		Greater ipsilesional sensorimotor cortex activity after rTMS to contralesional PMd correlated with greater motor impairment	Bestmann et al., 2010
Ipsilesional M1	High-frequency rTMS (10 Hz), subthreshold	PET	Chronic stroke patients	Altered effective connectivity between ipsilesional M1, basal ganglia, thalamus; altered interhemispheric connectivity		Ipsilesional TMS response covaries with improvement after movement therapy	Chouinard et al., 2006
Contralesional M1	Low-frequency rTMS (1 Hz)	fMRI	Subacute stroke patient	Increased coupling between ipsilesional SMA and M1		Inhibitory contralesional TMS improved motor performance of paretic hand; decreased influences of contralesional M1 after rTMS correlated with motor improvement	Grefkes et al., 2010

Table 2 | Studies showing the effects of tDCS on neural connectivity.

Anodal stimulation site	Cathodal stimulation site	Neuroimaging technique	Task	Population	Connectivity increases	Connectivity decreases	Behavioral result	References
Left M1	Right frontopolar cortex	EEG	Voluntary hand movements	Healthy volunteers	Increased intrahemispheric connectivity; increased connectivity patterns in left premotor, motor, sensorimotor regions in high-gamma 60–90 Hz range; increased synchrony in frontal and parieto-occipital regions in low-frequency (alpha and below) bands	Decreased interhemispheric connectivity		Polania et al., 2011a
Left M1	Right frontopolar cortex	EEG	Resting	Healthy volunteers	Increased synchronization within frontal electrodes in theta, alpha, and beta bands			Polania et al., 2011a
Left M1	Right frontopolar cortex	fMRI	Resting	Healthy volunteers	Increased coupling between left thalamus and MI; increased connectivity between left caudate nucleus and parietal cortex			Polania et al., 2012b
Right frontopolar cortex	Left M1	fMRI	Resting	Healthy volunteers		Decreased coupling between left M1 and right putamen		Polania et al., 2012b
Left M1	Right frontopolar cortex	fMRI	Resting	Healthy volunteers	Increased nodal minimum path length in left sensorimotor cortex (less distant functional connectivity); increased coupling between left sensorimotor cortex and premotor and superior parietal areas			Polania et al., 2011b
Left M1	Right frontopolar cortex	fMRI	Voluntary hand movements	Healthy volunteers		Decreased activity in SMA during finger tapping with anodal tDCS compared to no stimulation		Antal et al., 2011
Left M1	Right frontopolar cortex	fMRI	Resting	Healthy volunteers	No significant effects	No significant effects		Antal et al., 2011

(Continued)

Table 2 | Continued

Anodal stimulation site	Cathodal stimulation site	Neuroimaging technique	Task	Population	Connectivity increases	Connectivity decreases	Behavioral result	References
Left M1	Right frontopolar cortex	EEG	Resting	Healthy volunteers	Increase in power density of low frequency oscillations (theta, alpha)		Increased corticospinal excitability as indexed by MEP amplitude, and increased cortical reactivity	Pellicciari et al., 2013
Right frontopolar cortex	Left M1	EEG	Resting	Healthy volunteers	Increase in power density of low frequency oscillations (theta, alpha)		Decreased corticospinal excitability as indexed by MEP amplitude, and decreased cortical reactivity	Pellicciari et al., 2013
Right M1	Left M1	fMRI	Resting	Healthy volunteers	Increased connectivity between right MI, PMd, bilateral SMA, and prefronal cortex			Sehm et al., 2012
Right M1	Right frontopolar cortex	fMRI	Resting	Healthy volunteers	Increased connectivity in left frontotemporal, bilateral pareital, and right cerebellar regions			Sehm et al., 2012
Right M1	Left M1	fMRI	Resting	Healthy volunteers	Increased intracortical connectivity	Decreased interhemispheric connectivity		Sehm et al., 2013
Left dlPFC	Right frontopolar cortex	fMRI	Resting	Healthy volunteers	Increased connectivity in the frontal component of the default mode network and bilateral frontoparietal networks			Keeser et al., 2011
Left dlPFC	Right frontopolar cortex	fMRI	Resting	Healthy volunteers	Increased functional connectivity between prefrontal and parietal regions	Decreased spatial robustness of default mode network		Pena-Gomez et al., 2012

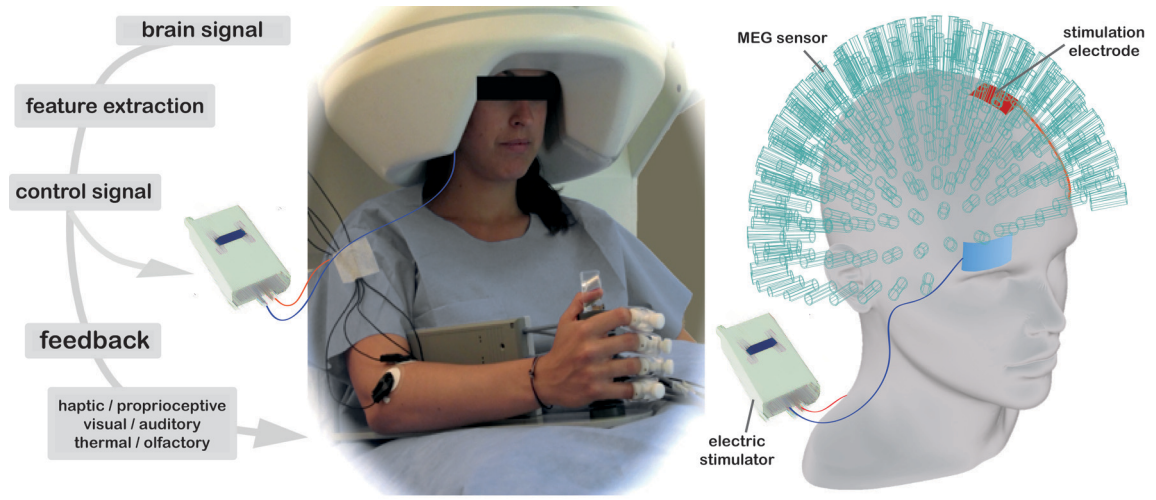

FIGURE 3 | Example of combined tDCS, MEG, and BCI experimental setup. This design uses a 275-sensor whole-head MEG to record neuromagnetic brain activity during tDCS stimulation, with electrodes placed in the classic unilateral M1 montage (anode placed above the area of the right M1 and reference electrode above the left supraorbital area). This set-up is used in conjunction with BCI visual feedback in the form of a computer game and sensorimotor feedback via a robotic hand orthosis that opened as target oscillations increased. Image courtesy of S. Soekadar (Soekadar et al., under review).

2011). Another EEG study showed that anodal tDCS over left M1 during rest in healthy volunteers only increased the power density of low frequency oscillations (theta, alpha; Pellicciari et al., 2013). These results suggest that substantial changes in brain activity associated with tDCS are augmented by its combination with performance of an active behavioral task, as predicted from basic science studies (Fritsch et al., 2010).

The effects of tDCS on fMRI connectivity have also been studied using a graph theoretical approach. This analytical tool showed that anodal tDCS over M1 reduced the functional connectivity between the stimulated M1 and more distant regions but increased connectivity between the stimulated M1 and premotor and superior parietal regions (Polania et al., 2011b). In a different study, these authors demonstrated that anodal tDCS over M1 also increases connectivity between the stimulated region and subcortical structures on the same hemisphere, including the ipsilateral thalamus (Polania et al., 2012a,b). These findings are supported by Stagg et al. (2013), who demonstrated increases in perfusion MRI during anodal tDCS in regions anatomically-interconnected to the stimulated site. Thus, tDCS likely increases blood perfusion in the target site as well as in anatomically interconnected networks. While still speculative, these studies suggest that increasing M1 excitability through anodal tDCS exerts its greatest effects in high frequency bands during active task performance, and reduces distant connectivity, increasing local, intrahemispheric connectivity (both cortical and subcortical). Stimulation during rest appears to primarily influence low frequency bands, such as theta and alpha bands, while stimulation during active movement may additionally influence high gamma bands (Polania et al., 2011a, 2012b; Pellicciari et al., 2013).

Studies of effects of tDCS on cortical connectivity also examined the use of different stimulating montages. A direct comparison of the effects of bilateral (with the anode over right M1 and cathode over left M1) vs. unilateral tDCS (with the anode over over right M1 and cathode over left supraorbital region) with fMRI was done in healthy volunteers (Sehm et al., 2012). Bilateral tDCS resulted in resting state changes in both primary and secondary motor areas, as well as in the prefrontal cortex, while unilateral M1 stimulation (with the anode over right M1 and cathode over the left supraorbital region) only influenced prefrontal, parietal, and cerebellar areas. Using seed-based connectivity metrics with a seed in the stimulated right M1, Sehm et al. (2013) showed that bilateral tDCS resulted in increased intracortical connectivity with right M1 after stimulation, which did not occur with unilateral stimulation. Both bilateral and unilateral tDCS resulted in decreased interhemispheric connectivity, however. This suggests that while tDCS over bilateral M1 (e.g., anode over left M1, cathode over right M1) increases connectivity within and between primary motor regions of the stimulated hemisphere, unilateral tDCS stimulation of only one hemisphere (e.g., anode over M1, cathode over a supraorbital region) only increases connectivity with other regions, such as parietal cortex and cerebellum. Some studies started to examine the effects of tDCS over other cortical areas, such as the left DLPFC. Results from these investigations are to some extent contradictory and require further exploration (Keeser et al., 2011; Pena-Gomez et al., 2012).

In summary, tDCS applied over a specific region induces distant effects on network connectivity, which may conceivably impact behavior. Modulation of distant neural regions via location-specific stimulation holds intriguing possibilities. However, caution is urged when interpreting these preliminary results, since within this handful of studies, there is great variability in the experimental designs used (e.g., in the stimulation

montage, period of stimulation, recording method, time of recording, type of analysis performed). In addition, there is significant interindividual variability in results depending on the state of the subject's or network's activity (state-dependency), and the task performed. Evaluation of connectivity effects of tDCS in clinical populations may contribute to the understanding of behavioral deficits in these patients (for example, O'Shea et al., 2014). To this end, there is a need for studies that examine connectivity effects of tDCS in stroke patients at different time points (acute, subacute, chronic), with different lesion locations (cortical, subcortical), and with different levels of impairment.

It is possible that new NIBS stimulation paradigms using time-varying waveforms, periodical as in the case of alternating current stimulation (tACS) (Herrmann et al., 2013), or random as for random noise (tRNS) (Terney et al., 2008) may contribute in the future to more effective neurorehabilitative efforts. Preliminary studies show similar modulation of excitability in the sensorimotor cortices (Kanai et al., 2008; Feurra et al., 2011) and on cognitive functions (Polania et al., 2012a; Cappelletti et al., 2013; Santarnecchi et al., 2013) using these techniques.

While tDCS influences neuronal firing rates in a bimodal manner depending on its polarity, tACS seems to up- and down-regulate the firing rate affecting neuronal spike timing (Reato et al., 2010). tACS generates an alternating current at a specific frequency, with the potential to synchronize or desynchronize activity between targeted brain regions. tACS follows models of phase-locking communication and communication through coherence that suggest that neural populations communicate through time-locked oscillations (Fries, 2005), making it a potential way to modulate neural communication across brain regions. Such a feature may be used for tailoring individualized interventions aimed at coupling or decoupling activity between specific brain regions depending on the subject/patient if this is proved at some point to be desirable or therapeutically useful.

In contrast, tRNS involves the application of alternating currents at different, random frequencies to the scalp. Due to its oscillatory, rather than direct current, nature, it has been proposed that tRNS ensures the application of stimulation is polarity-independent (i.e., neither anodal or cathodal; Miniussi et al., 2013). High-frequency tRNS (100–640 Hz) has been shown to elicit powerful cortical excitability modulations with even longer after-effects than tDCS, reaching 70 min following 10 min of stimulation (Chaieb et al., 2011). These newer methods provide promising new ways to modulate excitability in the brain both locally and across neural networks.

NIBS AND CORTICAL REORGANIZATION AFTER STROKE

Following stroke, patients with the most successful recovery of motor function are those whose patterns of brain activity as measured by fMRI most resemble those present in healthy volunteers (Johansen-Berg et al., 2002; Ward et al., 2003; Lotze et al., 2006; Nair et al., 2007; Grefkes and Fink, 2011). While healthy individuals show greater activity in the hemisphere contralateral to the hand they are moving, individuals with chronic stroke show in general a more bilateral pattern. Patients with greater motor impairment display increased fMRI activity in the contralesional hemisphere during attempted movement of the impaired hand (Johansen-Berg et al., 2002; Ward et al., 2003; Fridman et al., 2004; Lotze et al., 2006). In contrast, patients with better motor function show more normal patterns of ipsilesional motor activity, similar to the patterns one might see in healthy controls (Ward et al., 2003; Rehme et al., 2011). However, it is unclear which patients could benefit more from contralesional activity, if it serves an adaptive role (see for example Lotze et al., 2006).

Given these neuroimaging patterns after stroke, it has been proposed that upregulation of activity in the ipsilesional M1 or downregulation in the contralesional M1 might contribute to improved motor control (Ward and Cohen, 2004). Numerous proof of principle studies have now been done with some reporting that increasing excitability in ipsilesional M1 through high-frequency rTMS or anodal tDCS may yield improvements in motor performance or motor learning in healthy subjects (for example, Nitsche et al., 2003a,b,c; Reis et al., 2009) and small clinical studies have demonstrated modest, yet variable, improvements in individuals with stroke (Hummel and Cohen, 2005, 2006; Khedr et al., 2005; Kim et al., 2006; Pomeroy et al., 2007; for a review, see Sandrini and Cohen, 2013). Importantly for rehabilitation, it has been proposed that some of these changes outlast the period of stimulation (Khedr et al., 2010; Krawczyk, 2012).

Similarly, downregulating excitability in the contralesional motor cortex in chronic stroke patients was also associated with improvements in motor function, along with increased cortical motor excitability in the ipsilesional M1 and decreased cortical excitability in the contralesional M1 (Fregni et al., 2005; Takeuchi et al., 2005, 2008). Consistently, low-frequency rTMS or cathodal tDCS applied to downregulate excitability in the contralesional hemisphere resulted in motor gains. When applied for this purpose, single sessions of 10–25 min of rTMS over the contralesional M1 were reported to induce improvements in movement kinematics (Mansur et al., 2005; Takeuchi et al., 2005; Boggio et al., 2006; Liepert et al., 2007; Dafotakis et al., 2008; Nowak et al., 2008). When applied over several days, with or without motor training, some improvements were reported in grip strength and upper extremity function as measured by the Fugl-Meyer score and other assessments (Kirton et al., 2008; Kakuda et al., 2011).

It is also possible to use simultaneous stimulation of the ipsilesional cortex, with inhibition of the contralesional M1. This appears to also produce motor gains when combined with physiotherapy which last for 1 week (Lindenberg et al., 2010), but which seem to plateau after 2 weeks (Lindenberg et al., 2012). Bilateral stimulation over M1 with constraint-induced movement therapy also led to reported functional gains in the Fugl-Meyer test and handgrip strength. However, one recent study compared the differences between anodal, cathodal, and bilateral stimulation in stroke patients and demonstrated that anodal and cathodal stimulation had greater effects on motor output (via MEPs) than bilateral stimulation (O'Shea et al., 2014). Moreover, the effects of high-frequency rTMS over M1 may be more pronounced in individuals with subcortical, compared to cortical, stroke, suggesting that different patients may be differentially susceptible to beneficial effects of these techniques (Ameli et al., 2009).

To this end, it should be kept in mind that there is by no means agreement on the extent or universality of these beneficial effects and that well-controlled multicenter clinical trials are required

to assess this issue (Wallace et al., 2010; Talelli et al., 2012). Further research should be done to determine the most effective paradigms for brain stimulation and to factor in the lesion location, specific genetic markers if any (e.g., BDNF), levels of motor or cognitive impairment or neuroimaging patterns as predictors of responsiveness to NIBS. More insight into this topic and great caution is required until results from well-designed multicenter clinical trials are available (Ridding and Rothwell, 2007; Kandel et al., 2012; Rothwell, 2012; Sandrini and Cohen, 2013).

FUTURE DIRECTIONS FOR NIBS RESEARCH IN NEUROREHABILITATION

NIBS represents a novel and exciting tool to modulate cortical excitability, in specific local and distant brain regions and has been shown to alter connectivity with areas interconnected with the stimulated site. One exciting new application of NIBS is this ability to modulate functional connectivity between different interconnected regions and its proposed impact on behavior. For instance, dual-site stimulation paradigms, such as paired pulse stimulation applied repetitively could potentially modulate connectivity between two specific regions (Buch et al., 2011).

Another line of research is based on the ability of NIBS to modulate brain intrinsic oscillatory activity as in the framework of brain-computer interface applications (Soekadar et al., 2011), through the use of frequency-specific entrainment (Thut et al., 2012). To this effect, paradigms can be designed to enhance or decrease activity within the range of physiologically-relevant, region-specific frequencies, for instance, resonance phenomena with endogenous brain rhythms. Newer methods of brain stimulation, such as tACS and tRNS mentioned previously, may also prove useful toward this effort.

A recent feasibility study demonstrated that it was possible to combine tDCS with MEG recording, and in addition, provide a chronic stroke patient with neurofeedback about her brain activity in motor regions in the form of a visual stimulus and a robotic orthosis that opened and closed as her hand moved (**Figure 3**; Soekadar et al., 2013a,b, 2014a,b). This preliminary work showed that stimulation with online neural recording and feedback was feasible in the MEG environment, and results in enhanced performance after stimulation. The use of NIBS with other forms of brain-computer interfaces, robotic prosthetics, or with enhancement of pharmacological treatment may yield greater gains, due to the influence of NIBS by specific neurotransmitters as mentioned previously.

The use of NIBS in conjunction with other methods like neuroimaging or genetic analyses may prove particularly useful, not only to study what NIBS does to distributed brain activity, but also to identify predictors of response to NIBS interventions. For instance, O'Shea et al. (2014) used MR spectroscopy and behavioral measures to identify who responders to tDCS interventions. They found that GABA concentration in the stimulated region could predict the magnitude of behavioral changes after anodal tDCS.

Finally, emerging combinations of new methods are afforded by improvements in technology, computing, and mathematical modeling, such as simultaneous tDCS stimulation with MEG recordings (Soekadar et al., 2013a,b). NIBS in conjunction with biofeedback training designed to help individuals control their own brain activity may also contribute to neurorehabilitation (Buch et al., 2012). Using these methods in conjunction with brain-computer interfaces, virtual reality displays, or other feedback paradigms may contribute new insights to improve neurorehabilitative efforts using NIBS.

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Computational anatomy for studying use-dependant brain plasticity

Bogdan Draganski[1,2]*, Ferath Kherif[1] and Antoine Lutti[1]

[1] LREN – Department for Clinical Neurosciences, CHUV, University of Lausanne, Lausanne, Switzerland
[2] Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Victor W. Mark, University of Alabama at Birmingham, USA
Gitendra Uswatte, University of Alabama at Birmingham, USA

***Correspondence:**
Bogdan Draganski, LREN – Department for Clinical Neurosciences, CHUV, University of Lausanne, Mont Paisible 16, CH-1011 Lausanne, Switzerland
e-mail: bogdan.draganski@chuv.ch

In this article we provide a comprehensive literature review on the *in vivo* assessment of use-dependant brain structure changes in humans using magnetic resonance imaging (MRI) and computational anatomy. We highlight the recent findings in this field that allow the uncovering of the basic principles behind brain plasticity in light of the existing theoretical models at various scales of observation. Given the current lack of in-depth understanding of the neurobiological basis of brain structure changes we emphasize the necessity of a paradigm shift in the investigation and interpretation of use-dependent brain plasticity. Novel quantitative MRI acquisition techniques provide access to brain tissue microstructural properties (e.g., myelin, iron, and water content) *in-vivo*, thereby allowing unprecedented specific insights into the mechanisms underlying brain plasticity. These quantitative MRI techniques require novel methods for image processing and analysis of longitudinal data allowing for straightforward interpretation and causality inferences.

Keywords: magnetic resonance imaging, computational anatomy, brain plasticity

INTRODUCTION

Groundbreaking research in the last two decades brought strong evidence of the lifelong capacity of the mature mammalian brain to respond to alterations in the environment or individual's homeostasis. Although the concept of brain plasticity was initially coined in a different neuroscientific context, we here adopt the definition of plasticity as an intrinsic brain property change driven mainly by a mismatch between existing functional supply and environmental demand or caused by primary changes in functional supply (Lovden et al., 2010). Brain plasticity can be studied at different scales—from molecular and cellular up to the systems level through the perspective of either brain structure and/or function. Building on pioneering studies in rodents based on *post mortem* assessment of experience-induced brain volume changes in rodents (Rosenzweig et al., 1962) and non-human primates (Wang et al., 1995) most recent *in vivo* works demonstrate at the cellular level the complex dynamics of use-dependant dendritic spine plasticity (for review Holtmaat and Svoboda, 2009) and at the systems level—the extent of cortical reorganization after lost of peripheral input (Flor et al., 1995).

In humans, the advancement of magnetic resonance imaging (MRI) and the development of sophisticated computer-based analytical methods capturing the complex patterns of brain shape, volume and surface characteristics—computational anatomy, opened new possibilities for *in vivo* studies of use-dependant plasticity. A steadily growing number of studies confirm the notion of a remodeling of brain anatomy, but fail to provide further insight into the underlying neurobiological processes. The main reason for this is the fact that current state-of-the-art imaging assessments of structural brain plasticity rely mostly on relative changes in gray matter volume, density, and cortical thickness derived from MRI data which are the result of multiple microstructural factors which cannot be disentangled.

Here we review the published literature on use-dependant plasticity of the adult human brain studied with MRI-based computational anatomy methods. The next section outlines novel theoretical frameworks for studying brain plasticity, followed by a section on the accumulated scientific evidence on use-dependant plasticity. Recent advances in MRI acquisition techniques are presented that offer promising prospects for the exploration of the neurobiological basis of brain plasticity. Considering the most recent reviews on the topic (Lovden et al., 2010; Zatorre et al., 2012; Thomas and Baker, 2013), we focus on the necessity for investigation and interpretation of training-induced brain anatomy changes based on the quantification of specific tissue properties rather than metrics such as gray matter volume or density which are rather loosely defined in neurobiological terms. Further, we stress the specific need for longitudinal studies with multiple time points of data acquisition before, during, and after behavioral intervention allowing for inferences about causality.

THEORETICAL CONCEPTS

The concept of mutual link between modification of behavior and ability of the human brain for profound functional and structural reorganization throughout the whole lifespan is well established in modern neuroscience. However, after decades of research on the topic and empirical evidence for ongoing plasticity in the mature human brain we are still far away from understanding the basic neurobiological principles underlying plasticity (for review see Buonomano and Merzenich, 1998; Dayan and Cohen, 2011; Zatorre et al., 2012). An overwhelming number of studies at the cellular, synaptic, and systems level spanning a wide methodological spectrum confirm the notion of experience- induced

interaction between complex processes embedded in a somewhat blurry theoretical framework. The conceptualization of use-dependant human brain plasticity across the lifespan turned out to be particularly challenging—both in terms of definition when differentiating between development and experience or learning as well as when looking for straightforward neurobiological interpretation of plasticity-associated brain MRI findings (Galvan, 2010; Zatorre et al., 2012; Draganski and Kherif, 2013; Thomas and Baker, 2013).

Paradoxically, one of the first neuroscientists suggesting a possible impact of exercise on the brain is Ramon y Cajal, better known for his view on the brain as fixed and immutable tissue (Ramon Y Cajal, 1894). More than 80 years later—in the late 1970s Paillard formulated Ramon y Cajal's assumptions in a theoretical framework postulating that plastic brain changes emerge only when experience-associated anatomy alterations have functional consequences rather than the opposite—when functional changes occur within established anatomical networks (Paillard, 1976). The framework acknowledged the importance of both structural brain connectivity and changes in its constitutive elements—the neurons, considering the fundamental principle of "lasting" plastic changes long after the triggering event (see also Will et al., 2008a,b).

More recently, this much needed theoretical framework was not only refined in terms of semantic precision (Lovden et al., 2010), but also amended with the clearer concept of an interaction between brain and environmental demand for plastic changes. The core of this concept is the notion of functional supply—demand mismatch triggering the system's ability for plastic change. Here, demand is used in the sense of requirement to perform a task whereas functional supply denotes the individual's capacity to function within a certain range of performance and is defined mainly by brain anatomy constraints. Supply—demand mismatch could either be due to primary changes in environmental demand—in the simple case adaptation to a new condition—or to primary changes in functional supply—injury after stroke or chronic degenerative process. The assumption of functional supply—demand mismatch requiring "lasting" plasticity changes is embedded in the frame of the "sluggishness" of the system's response, which integrates different manifestations of plasticity and the corresponding underlying physiological mechanisms—long lasting neuro-/ and angiogenesis vs. rapid long-term potentiation (LTP)—based modulation of dendritic spines dynamics (Lovden et al., 2010).

The evolution of these current theoretical concepts provides a flexible framework to integrate recent findings of use-dependant brain plasticity at various scales and across different analytical techniques. Inherent part of these concepts is the notion of causal pathways, which, translated in the field of computational anatomy, motivates the need for longitudinal studies with multiple data acquisition time points allowing for inferences about causality. The newly proposed model (Lovden et al., 2010) addresses in a holistic way pertinent questions about causes, consequences and dynamics of use-associated brain plasticity leading to new ideas for future research.

BRAIN PLASTICITY—MACROSTRUCTURAL CHANGES

The emergence of structural and functional MRI has provided a unique opportunity for non-invasive *in vivo* investigation of plasticity-associated changes across the whole-brain. According to the experimental design, studies on brain plasticity can be divided in two types—cross-sectional studies with a single data acquisition time point and longitudinal studies with multiple time points of data acquisition. While inferences from cross-sectional studies remain at the descriptive level of correlation analysis, longitudinal studies bring the potential for revealing how behavioral changes result from the temporal dynamic of interaction between brain regions.

CROSS-SECTIONAL STUDIES OF BRAIN PLASTICITY

Cross-sectional computational anatomy studies investigate the correlation between brain structural features and training-/learning abilities by comparing cohorts of experts and non-experts in a particular field. Milestone previous studies reported associations between e.g., navigational experience and volume of the posterior hippocampus (Maguire et al., 2000), musical proficiency and volume increase in motor and auditory areas (Gaser and Schlaug, 2003; Hyde et al., 2009). The supposition here was that local brain volume changes would represent the use-dependant plasticity of a particular region or system implicated in the specific function of interest—assumption supported by the correlation between magnitude of change and individual performance.

Identically to computational anatomy studies on gray matter volume, voxel-based analysis of water diffusion indices from diffusion-weighted data demonstrated differences associated with piano practice (Bengtsson et al., 2005), bimanual coordination skills (Johansen-Berg et al., 2007) and grammar learning abilities (Floel et al., 2009). The major limitation of correlation analyses using brain imaging data obtained at a single time point is the inability to infer causality and to distinguish between the independent use-associated effects on brain structure from the impact of environmental and pre-existing intra-individual factors (Draganski and Kherif, 2013).

LONGITUDINAL STUDIES OF BRAIN PLASTICITY

Addressing the major limitation of cross-sectional studies in use-dependant plasticity—inability to distinguish between cause and consequence (i.e., "nature" vs. "nurture"), we aimed to infer causality using a prospective study design with multiple data acquisition time-points (Draganski et al., 2004). Led by the assumptions that neurogenesis drives use-dependant brain structure changes and considering findings reporting a 3 months period needed for differentiation of a pluripotent stem cell to mature neuron (Cummings et al., 2005), we carried out a longitudinal study involving juggling training. Young volunteers were scanned at three time points—before starting to learn how to juggle, 3 months after, followed by another 3 months period with restriction from juggling. We observed transient gray matter increases in the extra-striate motion specific area hMT/V5 bilaterally and in the left inferior parietal cortex. The demonstrated regional gray matter changes reversed nearly to baseline paralleled by decrease in juggling

performance at the third time point. No significant structural changes were detected in the control group where data was acquired at the same time points as in the intervention group.

Following the same neurobiological assumption of neurogenesis underlying use-dependent plasticity in the adult human brain we monitored morphometric changes related to memory and learning over three time points each 3 months apart (Draganski et al., 2006). The behavioral intervention consisted of intensive preparation for the German preliminary medical exam including both oral and written exams in biology, chemistry, biochemistry, physics, social sciences, psychology, human anatomy, and physiology, which demanded high level of encoding, retrieval, and content recall. Our findings demonstrated differential effects of learning regarding dynamic temporal characteristics on cortical structures. Besides the predicted changes in medial temporal lobe structures where hippocampal and parahippocampal gray matter expanded continuously through the three time points we detected initial gray matter increase in posterior parietal cortex between the first and second time point without further change toward the third time point.

Subsequent longitudinal studies confirmed the notion of use-dependent anatomy remodeling both in the brain gray and white matter (Golestani and Pallier, 2007; Scholz et al., 2009; Taubert et al., 2010; Bezzola et al., 2011; Herholz and Zatorre, 2012; Meyer et al., 2012; Sagi et al., 2012; Steele et al., 2013). Neuroimaging studies on juggling, acquisition of auditory skills, balance, and musical training and spatial navigation showed strong effects of behaviorally relevant interventions on local brain volume and white matter microstructure. White matter plasticity studies reported differential directionality of use-dependent changes in water diffusion indices—fractional anisotropy and mean diffusivity, most likely due to local differences in underlying white matter architecture.

Clearly, many principled questions on the topic of use-dependant plasticity studied with computational anatomy remain unanswered. Our assumptions on the temporal dynamics and linearity of exercise-associated brain anatomy changes next to the often-neglected impact of intra-individual variability (Kanai and Rees, 2011) are important points to be considered in future studies. The potential interaction between these factors and the effects of ageing motivate the investigation of training-induced plasticity across different age groups and particularly the interaction with ongoing developmental processes during puberty or progressive neurodegeneration associated with ageing and brain disorders (Sehm et al., 2014). Finally, we also cannot ignore the fact that neuroimaging techniques—while overcoming the limitations of animal studies regarding invasiveness and restricted volume of investigation—suffer from a coarse spatial resolution, which only allows for inferences at the macroscopic level.

BRAIN PLASTICITY—CELLULAR MECHANISMS

Brain plasticity can be studied at different scales ranging from sub-cellular to macroscopic brain systems level, which is mirrored in the abundance of invasive methods to investigate *in vivo* structural and functional aspects of brain remodeling. The dynamic link between use-dependant modulation of behavior and brain structure requires specific anatomical changes enabling optimal information processing. Theoretically, the underlying physiological mechanisms could be due to alterations in the number and morphological properties of neuronal and glial cells, synaptic connectivity, axons, and myelin as well as angiogenesis (Zatorre et al., 2012).

At the macroscopic level animal studies conducted in the 1960s demonstrated correlations between enriched environment and increases in cerebral cortex volume and total brain weight (Rosenzweig et al., 1962; Bennett et al., 1964). The experimental setting consisted of frequent changes and rearrangement of toys in the animals' cage building on the Hebbian idea of complex enrichment (Hebb, 1949). Follow-up studies in rodents in enriched environment reported experience-associated brain plasticity changes in the range of 3-20% (Black et al., 1990; Anderson, 2011) in line with observations in birds (Clayton and Krebs, 1994). In the following decades neurobiology research focused on investigations at the sub-cellular level using sophisticated analytical tools (e.g., immuno-histochemistry, quantitative electron microscopy, two photon laser microscopy, opto-physiological recordings) to capture *in vivo* or *post mortem* the modulation of synaptic strength and the turnover of synaptic and dendritic spines (for review Holtmaat and Svoboda, 2009). The Hebbian postulates of LTP, long-term depression and the spike-timing dependant plasticity were considered as basic physiological mechanisms underlying synaptic remodeling (Raymond, 2007). More recent studies revealed the plasticity-dependant role of glia associated with synapses (for review Haber and Murai, 2006; Henneberger et al., 2010). The supposition here was that use-dependant synaptic plasticity mediated by glutamatergic synaptic transmission and guided by neuronal activity represents the structural basis of learning and memory (for review Holtmaat and Svoboda, 2009). Converging evidence supported the notion that use-dependant functional remodeling is not only the result of connectivity changes associated with structural plasticity at the synaptic level (Kleim et al., 2007). Adding another layer of complexity, studies attributed an important role to the myelinated perineuronal nets as structural and functional "brakes" limiting morphological changes associated with use-dependant plasticity in the mature brain (Pizzorusso et al., 2002). Along these lines, combined *in vivo* MRI imaging and *ex vivo* histological studies in mice subjected to different versions of the water maze task demonstrated convincingly not only the anatomical specificity of the reported training- induced brain structure changes, but also strong evidence for associated axonal growth rather than changes in neuronal cell size or number (Lerch et al., 2011).

A strong argument against the assumption that neocortical neurogenesis outside the hippocampal dentate gyrus could underlie use-dependant changes in the mature human brain comes from a *post mortem* study measuring the integration of human DNA with (14)C isotopes generated by nuclear bomb tests during the Cold War. The main finding of this study is that neocortical neurogenesis outside the hippocampal dentate gyrus is restricted to the developmental period rather than a lifelong property of the human brain (Bhardwaj et al., 2006). Using the same (14)C isotopes assessment method the same group confirmed the functional relevance of hippocampal neurogenesis in the

mature human brain with estimated 1.75% annual turnover of hippocampal neurons (Spalding et al., 2013). Nevertheless, interpretations about specific role of neurogenesis in use-dependant brain plasticity could still be based on the assumption that newly generated hippocampal neurons can migrate to distant anatomical sites (Uchida et al., 2000; Pereira et al., 2007).

The main drawbacks of the abovementioned studies are their invasiveness and their limited observations of single synapses or restricted areas of single neurons. Aiming to bridge structure and function at the theoretical level, recently developed computational models started implementing rules for anatomical modifications at the cellular level to look for dynamic network property changes over time (Butz et al., 2008).

COMPUTATIONAL ANATOMY OF BRAIN PLASTICITY—INTERPRETATIONAL ISSUES

In the field of imaging neuroscience the introduction of state-of-the-art mathematical algorithms (i.e., computational anatomy) for automated data analysis in the spatial and temporal domains enabled unbiased feature reductions and statistical parametric mapping in standardized space. Despite the overwhelming variability of existing software solutions for the computation of volume, surface, and shape characteristics of the brain rely on the very same basic principles of data processing and statistical analysis. Although computational anatomy studies on use-dependant brain plasticity raised hopes to answer pertinent questions about neurobiological processes underlying the remodeling of brain anatomy, only few attempts have been made to validate measures of relative changes in gray matter volume, density and cortical thickness derived from MRI data with "gold standard" histology assessment.

GRAY MATTER CHANGES

The most widespread computational anatomy technique—voxel-based morphometry—VBM (Ashburner, 2009), provides automated brain volume and cortical thickness estimation for statistical inference on a population of interest. VBM includes an iterative algorithm combining voxel-by-voxel classification of each participant's MRI data into different tissue classes based on class-specific priors with the spatial registration to a common anatomical space. Following this step, the brain tissue classes are low-pass filtered by convolution with an isotropic Gaussian kernel (i.e., smoothing) to enter classical mass-univariate or multivariate statistical analyses. The inherent divergence of VBM based on MRI images from histological analysis of brain tissue properties is confirmed by a recent study, which failed to show any correlation between tissue classification probabilities estimated with computational anatomy algorithms and histological measures of neuronal density (Eriksson et al., 2009).

Automated estimation of voxel-based cortical thickness uses gray matter, white matter and CSF tissue partitions created in the classification step of VBM to extract cortical gray matter boundaries (Hutton et al., 2009). Subsequently, the voxel-based cortical thickness maps are registered to a common standardized space using the deformation fields applied for gray matter warping. The surface-based method for cortical thickness estimation implemented in Freesurfer (http://surfer.nmr.mgh.harvard.edu) relies on atlas information to detect the boundaries of gray matter followed by projection of the thickness values on a surface mesh (Dale et al., 1999; Fischl et al., 1999). Estimation of cortical thickness is assumed to provide more straightforward interpretation of computational anatomy results but is strongly affected by spatial and temporal changes in the brain tissue properties underlying MRI contrast changes (Salat et al., 2009; Lutti et al., 2014).

Despite a steadily growing number of computational anatomy studies a detailed insight into the neurobiological processes underlying use-dependant brain plasticity is still lacking. One reason for this is that most acquired MRI data is a mixed contribution of multiple brain tissue properties (e.g., myelin, iron, and water protons bound to macromolecules; Tofts, 2003) which cannot be disentangled at the analysis stage. Also, the specific effects of exercise and brain development and ageing on the microstructural properties affected by brain plasticity remain largely unknown and could lead to significant tissue classification bias and misinterpretation of the detected volume and cortical thickness changes. Additionally, commonly acquired MRI data is known to be severely affected by scanner-related effects leading to increased inter-scanner variability and reduced sensitivity which might explain some of the discrepancies observed across study sites (Weiskopf et al., 2013).

WHITE MATTER CHANGES

The investigation of use-dependant brain white matter changes using computational anatomy and T1-weighted MRI images is reduced to only few VBM studies from the past (Golestani et al., 2002; Golestani and Pallier, 2007). With the emergence of diffusion-weighted imaging research focused on plasticity-associated white matter microstructure changes by inferring directionality and magnitude of water diffusion (i.e., fractional anisotropy and mean diffusivity) (Pierpaoli and Basser, 1996). However, the currently existing computational anatomy analytical frameworks are not fully adapted for the analysis of parameter data (e.g., fractional anisotropy, mean diffusivity value etc.), which hampers the straightforward interpretation of results. Exemplified by VBM in the SPM framework, statistical inferences about local gray matter volume changes are based on tissue probability estimates derived from the MR signal in a Bayesian framework using anatomically informed tissue priors (see above). Subsequent neurobiological interpretation is enabled by adjustments for linear and non-linear interpolation effects of spatial transformation, which preserve the total signal. In the case of fractional anisotropy or mean diffusivity parameters this framework is not readily applicable without modifications. Recent work provided potential solution for quantitative multi-parameter data, however the concept should be validated for the special case of DWI derived parameters (Draganski et al., 2011). In more general terms, the lack of specificity of the underlying biophysical model carries the risk of overinterpreting inferences made on the basis of diffusion-weighted data (Jones et al., 2013). The theoretical and empirical resolution of these issues is subject of recent attempts to correlate *in vivo* obtained diffusion-tensor derived indices with histology results (Concha et al., 2010; Sagi et al., 2012), resolve interpretational ambiguities (Douaud et al., 2011) and propose novel biophysical models (Zhang et al., 2012).

CURRENT METHODOLOGICAL ADVANCES AND OUTLOOK

Addressing the specificity limitations of commonly used MRI anatomical data, novel multi-parameter quantitative mapping protocols disentangle the contribution of each MRI parameter (e.g., T1, T2,. . .) to the acquired signal. Quantification of these MRI parameters, which correlate with brain tissue microstructural properties such as myelin, iron and water content allows for an unprecedented insight into the mechanisms underlying brain plasticity. This represents a paradigm shift in how we estimate and analyse brain anatomy features, which can be used to relate plasticity-associated brain tissue property alterations to changes in behavior and to estimate their specific impact on the currently used gray matter volume, density and cortical thickness. A tipping point for future advances in understanding the principles of human brain plasticity will be the creation of causal generative models based on longitudinal studies with multiple data time points acquired before, during, and after behavioral intervention. Finally, the biophysical mechanisms linking tissue microstructure and the MRI signal are still under investigation. Accurate modeling of these mechanisms will be essential to produce estimates of the microstructural properties relevant in brain plasticity studies from the quantitative MRI data. The development of biophysical models will require validation, most preferably in the form of correlation studies with "gold standard" histology assessment.

QUANTITATIVE STRUCTURAL MRI

Quantitative structural brain imaging allows differentiating between plasticity-associated tissue property changes and local brain volume/density or cortical thickness changes. Our approach includes whole-brain multi-parameter mapping at high resolution (Helms et al., 2008; Helms and Dechent, 2009) correction for radio frequency transmit inhomogeneities (Lutti et al., 2010, 2012), and an established analytical framework—voxel-based quantification (VBQ) (Draganski et al., 2011). The ability of this approach to deliver robust and sensitive biomarkers of brain tissue properties has been demonstrated in a number or recent studies (Dick et al., 2013; Sereno et al., 2013; Lutti et al., 2014). Using VBQ we showed parameter-specific distribution patterns in healthy ageing and suggested a biophysical interpretation, which corroborates with histological studies showing age-dependant iron accumulation and rate of de−/remyelination (Draganski et al., 2011).

STATISTICAL INFERENCES ON CAUSALITY

The investigation of causal relationships between use-dependant plasticity changes in the mature human brain has not been approached systematically yet. Researchers suggested a combination of brain stimulation techniques (transcranial magnetic stimulation—TMS and transcranial direct current stimulation—TDCS) and computational anatomy studies to lend support to causality assumptions (Kanai and Rees, 2011). Similarly, attempts to combine neural activity with brain anatomy measurements to study brain plasticity remained at the descriptive level due to a lack of prior knowledge about causal and temporal dynamics (Ilg et al., 2008; Haier et al., 2009). Future analytical strategies could capitalize on novel methods in a Bayesian generative model framework that provides the causal link between behavior, neural activity and tissue property changes. The explanatory and predictive power of the model could be tested in a data-driven approach integrating temporal and spatial scales of imaging data in parallel with histology assessment to facilitate biological interpretation.

COMPARATIVE HISTOLOGICAL STUDIES

Despite the steadily growing number of longitudinal computational anatomy studies reporting use-dependant brain structure changes, there has been no attempt to date to link macrostuctural morphometric findings with microstructural information (for review (Fields, 2009). We still face a very limited number of systematic comparative studies in animals demonstrating use-dependant regional volume changes assessed with established computational anatomy methods to correlate these with *post mortem* histological findings at higher spatial resolution. Though scientifically highly advantageous, the concept of looking for brain plasticity correlates at the microscopic level has its limitations—results from animal models cannot readily be extrapolated to humans and many cognitive processes cannot be studied in animals. There are also practical limitations such as shrinking of the specimen due to fixation, artifacts of staining or difficult localization of investigation/recording sites (Sincich and Horton, 2005), which hamper the comparison of histological results with computational anatomy studies.

In conclusion, the overwhelming number of computational anatomy studies on use-dependant brain plasticity has provided major contributions to our understanding of the characteristic spatial and temporal patterns of brain structure changes at the macroscopic scale. First animal studies applying in parallel computational anatomy methods and histological investigation provide important findings at unprecedented fine granularity. Newly emerging MRI acquisition techniques hold promising prospects that might allow the detection for the first time of the brain tissue microstructural changes associated with use-dependant plasticity beyond the vague neurobiological observation of concomitant volume or density changes. Not only will this advance our scientific understanding of brain plasticity, it will also provide an empirical basis for the creation of reliable non-invasive biomarker for the rehabilitation progress after brain function loss. A generative model capturing spatial and temporal characteristic of use-dependant changes could help in unraveling causal pathways between exercise-induced behavioral changes and remodeling of brain structure to make an important contribution to our scientific understanding of brain function and dysfunction.

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Neurorestorative therapy for stroke

***Jieli Chen*[1]*, *Poornima Venkat*[1,2], *Alex Zacharek*[1] and *Michael Chopp*[1,2]**

[1] Department of Neurology, Henry Ford Hospital, Detroit, MI, USA
[2] Department of Physics, Oakland University, Rochester, MI, USA

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Victor W. Mark, University of Alabama at Birmingham, USA
Gitendra Uswatte, University of Alabama at Birmingham, USA

***Correspondence:**
Jieli Chen, Neurology Research, Henry Ford Hospital, E & R Building, Room 3091, Detroit, MI 48202, USA
e-mail: jieli@neuro.hfh.edu

Ischemic stroke is responsible for many deaths and long-term disability world wide. Development of effective therapy has been the target of intense research. Accumulating preclinical literature has shown that substantial functional improvement after stroke can be achieved using subacutely administered cell-based and pharmacological therapies. This review will discuss some of the latest findings on bone marrow-derived mesenchymal stem cells (BMSCs), human umbilical cord blood cells, and off-label use of some pharmacological agents, to promote recovery processes in the sub-acute and chronic phases following stroke. This review paper also focuses on molecular mechanisms underlying the cell-based and pharmacological restorative processes, which enhance angiogenesis, arteriogenesis, neurogenesis, and white matter remodeling following cerebral ischemia as well as an analysis of the interaction/coupling among these restorative events. In addition, the role of microRNAs mediating the intercellular communication between exogenously administered cells and parenchymal cells, and their effects on the regulation of angiogenesis and neuronal progenitor cell proliferation and differentiation, and brain plasticity after stroke are described.

Keywords: BMSC, HUCBC, neurorestoration, microRNA, niaspan

INTRODUCTION

Stroke is one of the leading causes of mortality, long-term disability, and morbidity. Current stroke treatments have mostly targeted early neuroprotection, utilizing therapeutic agents designed to prevent or reduce cell damage from ischemia. These agents have provided promising results in animal stroke models. However, except for thrombolysis with tissue plasminogen activator (tPA), to-date, all Phase III clinical trials utilizing them have failed to provide therapeutic benefit. For now, tPA is the only FDA approved pharmacological therapy for acute ischemic stroke. Based on the European Cooperative Acute Stroke Study (ECASS III) (Cronin, 2010; Carpenter et al., 2011), tPA was therefore approved for thrombolytic therapy of acute ischemic stroke for selected patients when therapy starts within 4.5 h of stroke onset. However, treatment with tPA is limited by this narrow time window, as well as by an increased risk for intracranial hemorrhage. Consequently, only 4–7% of patients in US suffering a stroke receive thrombolysis with tPA (Katzan et al., 2004; Schwammenthal et al., 2006; Weimar et al., 2006). After decades of research focused on acute neuroprotection and the failure of clinical trials to overcome this barrier, the National Institutes of Neurological Disease and Stroke (NINDS) Stroke Progress Review Group in 2006 and in 2011 identified delayed neurorestoration after stroke as a major priority for stroke research (Grotta et al., 2008; NINDS, 2012).

Cell-based and pharmacological restorative therapies are promising approaches for the treatment of stroke (Modo et al., 2002a,b; Savitz et al., 2003; Watson et al., 2003; Willing et al., 2003). Cell-based therapy, e.g., bone marrow stromal cells (BMSC) or human umbilical cord blood cells (HUCBCs), when administered intravenously after stroke improves neuroplasticity and neurological outcome through the up-regulation of restorative processes such as: neurogenesis, angiogenesis, and oligodendrogenesis in the post-ischemic brain (Chen et al., 2001a). Among pharmacological agents, which promote neurological recovery when administered subacutely, Niaspan, a slow-releasing vitamin B3 drug has shown promise as a neurorestorative agent (Chen et al., 2007). In addition, Niaspan extends the therapeutic window for tPA therapy (Chen et al., 2001a; Shehadah et al., 2011). Recently, microRNAs (miRNAs), each of which may regulate the translation of hundreds of genes and thereby act as molecular master switches, have been shown to mediate BMSC cell therapy for stroke (Juranek et al., 2013) and control neuronal progenitor cell proliferation and differentiation, and will therefore be discussed here (Lim et al., 2010; Wang et al., 2013). This review paper will also focus on molecular mechanisms promoting neurorestorative effects (angiogenesis, neurogenesis, and oligodendrogenesis) after cerebral ischemia, which underlie the restorative effects of cellular and experimental pharmacological approaches for the treatment of stroke.

BIOLOGICAL BASES FOR NEURORESTORATIVE THERAPY POST-ISCHEMIA

Neurorestoration post-stroke is achieved by enhancing neurogenesis, angiogenesis, and oligodendrogenesis, which in concert promote neurological recovery (Chen et al., 2003b, 2006, 2010; Chopp et al., 2009; Hermann and Chopp, 2012). Neurogenesis, the generation of new parenchymal cells from neural stem and progenitor cells, stimulates plasticity, oligodendrogenesis, restores neuronal signal transduction, and promotes myelination (Skihar et al., 2009), Vascular remodeling (angiogenesis and arteriogenesis) increases cerebral blood flow (CBF) perfusion and mediates the generation of important restorative trophic factors and proteases and thereby helps to establish a hospitable environment for neurite outgrowth, remyelination, and in general for the resident cells (Chen et al., 2009).

VASCULAR REMODELING (ANGIOGENESIS AND ARTERIOGENESIS)

Recent findings concerning the pathophysiological events following acute ischemic stroke suggest that angiogenesis plays a critical role in improving long-term recovery of patients (Arenillas et al., 2007; Navarro-Sobrino et al., 2011). Typically, elderly patients tend to have lower levels of new vessel formation following stroke, which may be associated with lower rates of functional recovery (Allen, 1984; Granger et al., 1992). Maintenance of neural function is critically dependent upon regulation of CBF (Pratt et al., 2004). Angiogenesis and arteriogenesis reestablish functional microvasculature in the ischemic border zone (IBZ), creating a microenvironment hospitable for neuronal plasticity, which can lead to functional recovery (Plate, 1999; Chen et al., 2003b; Renner et al., 2003). After stroke, patients who have a higher cerebral blood vessel density do better and survive longer than patients having lower vascular density (Krupinski et al., 1994; Wei et al., 2001). Clinical improvement shortly after stroke also correlates with the presence of arteriolar collaterals (arteriogenesis). Stroke mortality increases in the absence of significant collateralization (Christoforidis et al., 2005). It is therefore reasonable that stimulating angiogenesis and arteriogenesis may be an effective treatment strategy for stroke patients.

Angiogenesis leads to mature and functional blood vessels, which is a therapeutic goal. Angiogenesis refers to the biological process resulting in the growth of new blood vessels, branching off from pre-existing vessels. Hypoxia and tissue ischemia are the main physiological stimuli for angiogenesis (Dor and Keshet, 1997). Endothelial cell proliferation, migration, and sprouting of new vessels from existing vessels toward the site of ischemic brain injury are stimulated when angiogenic factors bind to specific receptors located on brain endothelial cells (Greenberg, 1998). This sprouting of endothelial cells leads to the formation of tube-like vascular structures. The initial vascular plexus during angiogenesis forms mature vessels via branching, pruning, sprouting, as well as the promotion of differential growth of endothelial cells, and the recruitment of supporting cells, such as pericytes and smooth muscle cells (SMCs) (Folkman and D'Amore, 1996; Risau, 1997). Angiogenesis and vascular maturation are regulated by many factors, such as, Angiopoietin-1 (Ang1)/Tie2 system (Patan, 2004), basic fibroblast growth factor (bFGF), endothelial nitric oxide synthase (eNOS), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) (Greenberg, 1998; Lutsenko et al., 2003; Matsui and Tabata, 2012). To determine the optimal window for the initiation of angiogenic therapies, a rigorous timetable of angiogenic event steps must be drawn. Angiogenesis takes place in the penumbra of human ischemic brain hours after initial onset and continues to be present weeks after ischemic onset (Krupinski et al., 1994). Nitric oxide (NO) initiates vasodilation and is considered the first step in angiogenesis (Carmeliet, 2000). Combining the vasodilation effect of NO with the increase in VEGF expression, which increases vascular permeability allows extravasation of plasma proteins that lay down a provisional scaffold for the migration of endothelial cells for vascular sprouting. The second step involves the dissociation of SMCs and loosening the extracellular matrix, which enwraps the mature vessel. Angiopoietin-2 (Ang2), an inhibitor of Tie2 signaling, may be involved in facilitating the detachment of pericytes from endothelial cells, while the matrix metalloproteinase (MMP) family of proteinases degrade matrix molecules and further weakens vascular integrity (Feng et al., 2009). Once the path of sprouting has been established, proliferating endothelial cells migrate to distant sites. During this time, an array of molecular signals, including VEGF, VEGF receptors, and placental growth factor, work in synchrony to guide this process. Once new blood vessel networks are formed, Ang1, which activates Tie2 receptors, helps to stabilize networks initiated by VEGF. The angiogenic process is tightly regulated by growth factors and the up-regulation of specific growth factors that dictate event progression (Greenberg, 1998; Lutsenko et al., 2003).

Arteriogenesis adapts existing systems of vessels into functional ancillary conduits for blood flow to tissues distal to the site of occlusion of large, peripheral conduit arteries (Buschmann and Schaper, 1999; Schaper and Buschmann, 1999). This process shares characteristics with angiogenesis, though different pathways lead to arteriogenesis. The main differences between arteriogenesis and angiogenesis reside in the development of collaterals from existing arterioles, which are activated by the large pressure differences between perfusion territories, which produce high intravascular shear stress (Erdo and Buschmann, 2007). The attraction, adhesion, and activation of circulating cells such as monocytes, T-cells, and basophils, are responsible for a large percentage of the vascular–arteriolar growth and remodeling (Schaper and Buschmann, 1999). The majority of growth factors and proteolytic enzymes are secreted by monocytes, and they allow SMCs to migrate and divide (Scholz et al., 2001). Arteriogenesis results in the formation of new arterioles, which are believed to occur when SMCs coat pre-existing capillaries, which are then transformed into larger diameter channels (Buschmann and Schaper, 2000; van Royen et al., 2001). Arteriogenesis is a decisive process of altering existing vessels into functional collateral conduits to bypass occlusions of large peripheral conduit arteries and restore the supply of oxygen-enriched blood to tissues (Seetharam et al., 2006).

Angiogenesis and arteriogenesis foster functional restoration after neurological injury by inducing brain plasticity (Risau, 1997; Plate, 1999; Cramer and Chopp, 2000; Cairns and Finklestein, 2003; Renner et al., 2003; Landers, 2004). Vascular remodeling promotes neurorestoration, which is the goal of all neurorestorative therapies (Hurtado et al., 2006). Generating new blood vessels post-ischemia promotes neurorestorative processes such as neurogenesis and synaptogenesis, which foster improved functional recovery.

WHITE MATTER AND AXONAL REMODELING AND OLIGODENDROGENESIS

In humans, after stroke or any acute injury to the central nervous system (CNS), functional recovery is highly limited, leaving many survivors with life-long neurological deficits. The inability to completely restore function in these individuals can be partially attributed to inadequate axonal regeneration and neuroplasticity (Walmsley and Mir, 2007). Preclinical studies demonstrate that remodeling of the axons starts 2–3 weeks post-stroke (Liu et al., 2009, 2010). Successful axonal outgrowth in the adult CNS is critical to brain repair processes after stroke (Hou et al., 2008). Surviving cortical neurons in the peri-infarct motor cortex experience

axonal sprouting, which can restore connections in the brain (Carmichael et al., 2001; Carmichael, 2003; Dancause et al., 2005). Thus, axonal remodeling in the corticospinal system may contribute to spontaneous functional recovery following stroke (Liu et al., 2009).

Oligodendrogenesis and remyelination play a crucial role in behavior and functional restoration post-ischemia. Oligodendrocytes (OLs) produce the myelin sheaths, which wrap around axons, facilitating nerve conduction. Oligodendrocyte progenitor cells (OPCs) differentiate into mature OLs. OLs are highly vulnerable to ischemic stress, because white matter has lower blood flow than gray matter, and deep white matter has little collateral blood supply (Back et al., 2002). OL damage leads to demyelination, which contributes to neurological and behavior function deficits. Mounting evidence suggests that inflammatory response post-ischemia is especially detrimental to white matter cohesion through the up-regulation of matrix MMPs (Chen et al., 2011). MMP-9 and MMP-2 have both been shown to increase white matter lesions (Chen et al., 2011). In the rodent transient middle cerebral artery occlusion model (MCAo), the number of OLs decreased between 24 and 48 h after ischemia and increased 1–2 weeks after reperfusion in the peri-infarct cortex (Tanaka et al., 2001). Ischemic damage of OL results in demyelination, contributing to neurological and behavioral functional deficits. Although mature OLs are considered post-mitotic and unable to proliferate, the white matter contains an abundance of OPCs that respond to ischemic injuries (Gensert and Goldman, 1997; McTigue and Tripathi, 2008). Evidence of oligodendrogenesis post-ischemic stroke has been demonstrated (Iwai et al., 2010).

NEUROGENESIS AND SYNAPTOGENESIS

Neurogenesis and synaptogenesis contribute to post-stroke functional improvement. Adult mammalian neurogenesis takes place in the subgranular zone (SGZ) and subventricular zone (SVZ) of the hippocampus (Shehadah et al., 2010a). Under normal conditions, the neural progenitor cell (NPC) population is maintained through tightly regulated cell apoptosis and proliferation (Shehadah et al., 2010b). After ischemic stroke onset in rats, the population of NPCs significantly expands in the SVZ, and neuroblasts are systematically recruited and differentiate into mature neurons, astrocytes, and OLs in the migratory target of the ischemic penumbra region (Parent et al., 2002). Forty-eight hours post-ischemic stroke, NPC proliferation is up-regulated in the SVZ in the adult rodents by shortening the cell-cycle length from 19 h in non-stroke SVZ cells to 15.3 h in stroke SVZ cells (Zhang et al., 2006). The decrease in the duration of the cell cycle is mainly due to a reduction in the G1 phase (Zhang et al., 2006). A larger percentage of progenitor cells from the stroke SVZ re-enter the cell cycle after mitosis than cells from the non-stroke SVZ (Zhang et al., 2006). Fourteen days post-ischemia, cell-cycle lengthening results in daughter cells, which exit the cell cycle and differentiate into neural cells (Zhang et al., 2008). Above evidence suggests that neurorestoration follows a tightly regulated sequence of events with the initial step of NPC proliferation followed by differentiation of these progenitor cells into mature neural cells. Following an ischemic insult, neurogenesis in the SVZ is enhanced and precursors migrate to the IBZ and differentiate into region-specific neural phenotypes (Parent et al., 2002). Cell therapies enhance this endogenous neurogenesis, migration, and differentiation of neural cells and promote neurological recovery (Chen et al., 2003a, 2013; Munoz et al., 2005; Bao et al., 2011; Zhang et al., 2012b; Gutierrez-Fernandez et al., 2013).

Synaptogenesis, the process of formation of new synapses, can be enhanced by angiogenesis, as there is enhanced oxygen supply to tissue via blood vessels (Zhang and Chopp, 2009). Increased expression of Synaptophysin, a pre-synaptic vesicle protein and an indicator of synaptogenesis (Stroemer et al., 1995; Ujike et al., 2002), has been observed in cell therapy treatments (Zhang et al., 2012b; Gutierrez-Fernandez et al., 2013) with a correlated improvement in post-stroke functional outcome. From our work in neurorestorative therapies for stroke, we have found that all agents and cell types that are effective in promoting recovery of neurological function evoke common responses in cerebral tissue (Chen et al., 2001a; Li et al., 2001a, 2002; Zhang et al., 2001, 2002; Wang et al., 2004).

NEUROVASCULAR NICHE

The neurovascular unit is a conceptual model that describes functional interactions and signaling between neurons, capillaries, and glia in the brain. Since stroke inflicts both neural and vascular damage, therapeutic interventions targeting the vascular neural network warrant investigation as a fundamental component for post-stroke neurorestoration (Zhang et al., 2012a). Angiogenesis and neurogenesis in the neurovascular niches of the CNS play key roles in recovery. Based on *in vivo* and *in vitro* murine models of sublethal hypoxia, it has been suggested that the neurovascular niches of the CNS, in response to hypoxia, trigger HIF-1α-mediated responses (Madri, 2009). HIF-1α is modulated in part by NO, modulates brain-derived neurotrophic factor (BDNF), VEGF, and stromal cell-derived factor 1 (SDF-1), and induces their autocrine and paracrine signaling, which in turn mediates endothelial cell and neural stem cell survival and proliferation (Madri, 2009). Thus, the optimization of the expression levels of hypoxia-induced induction of HIF-1α and its downstream signaling components BDNF, C–X–C chemokine receptor type 4 (CXCR4), Neuropilin-1 (Nrp-1), NO, SDF-1, and VEGF may maximize recovery (Madri, 2009). In a model of focal cortical stroke, migration of newly formed neurons from the SVZ to cortex, neurogenesis from a glial fibrillary acidic protein (GFAP)-expressing progenitor cells in the SVZ, and migration of neuroblasts to a neurovascular niche in peri-infarct cortex can improve behavioral recovery post-stroke (Ohab et al., 2006). Behavioral recovery is thus, attributed to a process linking neurogenesis and angiogenesis by growth factors and chemokines and to the trophic action of SDF-1 and Ang1, which are up-regulated by blood vessels within the neurovascular niche (Ohab et al., 2006).

NEURORESTORATIVE TREATMENT OF STROKE WITH CELL-BASED THERAPY

HUMAN UMBILICAL CORD BLOOD CELLS

Human umbilical cord blood cells hold great promise as therapeutic agents, since they are easy to isolate without serious ethical and technical problems. HUCBCs are a rich source of mesenchymal and hematopoietic progenitor cells (HPCs). The number of

highly proliferative HPCs in bone marrow is equaled or exceeded by those found in HUCBC (Almici et al., 1995). HUCBCs induce strong immunomodulatory properties by the host and yet remain weakly immunogenic themselves (Vendrame et al., 2006; Nikolic et al., 2008). As observed in an animal stroke model, HUCBCs inhibit the pro-inflammatory T helper cell type 1 (Th1) response, while promoting a strong anti-inflammatory T helper 2 (Th2) response (Vendrame et al., 2004; Nikolic et al., 2008). Numerous studies having shown that HUCBC treatment of rodents does not elicit GVHD (Graft Versus Host Disease), a leading cause of death in patients that have received stem cell transplants (Li et al., 2001b; Lu et al., 2002; Henning et al., 2004; Hu et al., 2006). Patients who receive HUCBC transplants from a relative are significantly at a lower risk of GVHD, and are less likely to reject the transplant compared to either bone marrow or peripheral blood stem cells (Takahashi et al., 2007; Morgado et al., 2008). Factors that may be beneficial to the host brain *in vivo* are secreted by HUCB-derived mononuclear cells as they proliferate and differentiate (Neuhoff et al., 2007). Umbilical cord blood can provide a significant number of stem/progenitor cells, for hematopoietic as well as other tissue-specific lineages, including nervous tissue (Li et al., 2001b; Kozlowska et al., 2007). HUCBCs, when intravenously (i.v.) administered, migrate selectively to the ischemic area in the brain, enhancing functional recovery post-stroke (Chen et al., 2001b; Li et al., 2001b; Zhang et al., 2011).

The mechanism of transplanted HUCBC-induced functional benefit after stroke is not clear. The beneficial effects of HUCBC treatment may be due to multiple causes, such as improved cell survival, increased angiogenesis, nerve fiber reorganization, reduced inflammation, and trophic actions, among other restorative events (Vendrame et al., 2006; Arien-Zakay et al., 2011; Liu et al., 2014).

Anti-inflammatory effects

Beneficial effects include reduction in the extent of ischemic damage, and CD8+ T-cell counts in MCAo rat model (Vendrame et al., 2006). HUCBC treatment at 48 h post-stroke significantly decreased infiltration of granulocytes and monocytes and reduced astrocytic and microglial activation in the parenchyma (Newcomb et al., 2006). Functional recovery from permanent MCAo was also seen upon intravenous HUCBC administration in spontaneously hypertensive rats (Miller et al., 2013). While both human $CD34^-$ and $CD34^+$ cells derived from HUCB were found to be equally competent in stroke treatment, easy attainability of $CD34^-$ cells in comparison to purified $CD34^+$ cells, makes it a promising source for cell-based therapies for humans (Miller et al., 2013). HUCBC administration suppresses pro-inflammatory factor expression, including cytokines, CD45/CD11b-, CD45/B220-positive (+) cells, nuclear factor-κB (NF-κB) DNA binding activity (Vendrame et al., 2005), tumor necrosis factor-α (TNF-α) (Chen et al., 2008), and suppression of pro-inflammatory isolectin binding cells (Leonardo et al., 2010), which may lead to functional and anatomical recovery by attenuating neuroinflammation and inducing neuroprotection (Vendrame et al., 2005; Leonardo et al., 2010).

Trophic factor effects

The therapeutic benefits of HUCBC treatment likely derive from enhancement of endogenous brain recovery mechanisms (Chen et al., 2001b). Only a small percentage of HUCBCs employed to treat stroke expresses proteins phenotypic of neural-like cells and functional recovery occurs within days after HUCBC administration (Chen et al., 2001b). Additionally, the number of HUCBCs administered intravenously that enter the brain was small, and the tissue that was replaced would make up no more than a cubic millimeter. HUCBC treatment of stroke also elevated levels of glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and BDNF, thus, trophic factor-mediated mechanisms contribute to improved behavioral outcome, rather than cell replacement (Yasuhara et al., 2010). HUCBCs contain many hematopoietic colony-forming cells (CFS) (Nakahata and Ogawa, 1982), as well as produce IL-11 and thrombopoietin (Suen et al., 1994). CSF-1, a hematopoietic cytokine, is a CNS growth factor (Berezovskaya et al., 1995). As such, it is likely that HUCBCs act as sources of trophic factors (Chen et al., 2008). In spinal cord injury (SCI), treatment with HUCBCs in rats increased serum levels of GDNF, IL-10, and VEGF, which may contribute to the observed beneficial effects (Chen et al., 2008). Cytokines and growth factors in murine NPCs are survival and/or differentiation factors (Mehler et al., 1993) that may play a critical role in neural tissue proliferation or differentiation (Cairns and Finklestein, 2003). When HUCBCs are administered intravenously and migrate to the injured tissue, they may function as a site of trophic factor production, which bypasses the blood–brain barrier (BBB). Therefore, functional benefit of i.v. administration of HUCBC, likely, does not derive from cellular replacement of tissue but from trophic factor expression by the injected cells and/or induced by the injected cells within the parenchymal tissue (Chen et al., 2008; Liu et al., 2014).

Studies also point at OL protection and survival as a means of HUCBC-induced neuroprotection (Rowe et al., 2010, 2012). Umbilical cord blood cell-derived CD34+ cells promote angiogenesis, neurogenesis (Chen et al., 2013), neuronal regeneration resulting in enhanced neovascularization (Taguchi et al., 2004), which amplifies the neurorestorative effects and improves functional recovery after stroke. But without reducing infarct volume and, necessarily, providing neuroprotection (Nystedt et al., 2006).

MULTIPOTENT MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) can be isolated from bone marrow (most common), cord blood cells, placenta, muscle, skin, and even liposuction fat. In this review, we will focus on MSCs derived from bone marrow. BMSCs are constituted by a heterogeneous collection of mesenchymal stem and progenitor cells. Human BMSCs (hBMSCs) can transdifferentiate into neural and mesodermal cell lines (Friedenstein et al., 1968, 1987; Bianco and Gehron Robey, 2000). In animal models, post-stroke hBMSCs transplantation improves sensory–motor function (Chen et al., 2001a; Zhao et al., 2002; Chen and Chopp, 2006; Weng et al., 2008; Huang et al., 2013b), enhances synaptogenesis (Weng et al., 2008), stimulates nerve regeneration (Tohill et al., 2004), decreases tPA-induced brain damage (Liu et al., 2012), and can mediate immunomodulatory effects and reduce inflammation (Yoo et al., 2009).

BMSC transplantation induces neurorestorative effects and ameliorates neurological functional deficits after stroke (Chen et al., 2001a, 2003a; Shen et al., 2007). We have demonstrated

that BMSC therapy can reduce neurological functional deficits when administered intravenously at 1 or 7 days after stroke (Chen et al., 2001a) and even at 1 month after stroke (Shen et al., 2007). Transplanted adult BMSCs migrate to damaged tissue in brain and decrease post-stroke functional deficits (Chen et al., 2001a). Migration may be aided by the disruption of BBB enabling selective entry of BMSCs into ischemic brain compared to normal cerebral tissue. Neurological benefit is derived mainly by triggering the release of growth and trophic factors, as a very small percentage of cells migrate, differentiate, and contribute toward neuroprotection/restoration. BMSCs trigger the release of growth factors in ischemic tissue that can initiate cell repair mechanisms, enhance cell proliferation in SVZ, and reduce apoptosis and neuronal death/damage in ischemic region (Li et al., 2002).

The mechanisms of action of BMSC treatment of stroke significantly differ from those initially targeted for progenitor and stem cells. Progenitor and stem cells are placed in the injured brain, they are designed to replace injured and dead tissue (Riess et al., 2002). It is likely that BMSCs also contain a stem-like subpopulation of cells that can differentiate into brain cells (Zhang et al., 2009; Shichinohe et al., 2010; Ding et al., 2011). However, this is a minor subpopulation of BMSCs where only a minute percentage assumes a parenchymal cell phenotype, and do not contribute to functional recovery (Li et al., 2002).

Transplanted BMSCs, by releasing soluble trophic factors and cytokines, promote endogenous repair of neurologically damaged tissues (Hardy et al., 2008). We and others have shown that exogenous cells produce various factors, and more importantly, stimulate neuroprotective (Chen et al., 2003a) and neurorestorative factor production in parenchymal cells. Intravenously administered BMSCs used to treat stroke or CNS disease produce trophic factors and stimulate parenchymal cells to express trophic factors (Chen et al., 2003b). BMSCs express mRNAs covering a wide range of angiogenic/arteriogenic cytokines that include Ang1, basic fibroblast growth factor-2 (FGF2), insulin-like growth factor (IGF), placental growth factor, and VEGF (Chen et al., 2003b; Zacharek et al., 2007). hBMSCs that had been cultured with extract from ischemic rat brain showed increased levels of BDNF, hepatocyte growth factor (HGF), and VEGF (Chen et al., 2002a,b). Intravenous administration of BMSCs leads to a time dependent release of neurotrophins and angiogenic growth factors like BDNF, VEGF, bFGF, NGF, HGF, and GDNF (Chen et al., 2002a,b, 2003b; Zacharek et al., 2007; Wakabayashi et al., 2010). These cytokines and growth factors have both autocrine and paracrine activities (Matsuda-Hashii et al., 2004), which are the molecular signals of the body uses to regulate differentiation, proliferation, and cell survival. GDNF promotes neurogenesis, endogenous cell repair mechanisms, neuroblast proliferation, and migration from the SVZ and decreases apoptosis (Kobayashi et al., 2006; Yuan et al., 2013). Intravenously injected BMSCs enter the brain and stimulate the local parenchymal cells, mostly astrocytes and endothelial cells, to produce growth factors promoting angiogenesis and vascular stabilization, which are partially mediated by Ang1/Tie2 and VEGF/Flk1 (Zacharek et al., 2007). Elevated IGF-1 mRNA expression was seen in cells subjected to ischemic stress and enhanced IGF-1 mRNA, IGF-1, and IGF-1R immuno reactive cells seen upon treatment with BMSCs, indicating that IGF-1-mediated self repair mechanism may contribute to the gain in neurological function (Wakabayashi et al., 2010).

BMSCs increase angiogenesis and arteriogenesis (Kinnaird et al., 2004; Zhu et al., 2011). BMSC conditioned cell culture media promotes integration and proliferation of endothelial cells and SMCs *in vitro*, and when injected directly into the hind limb of an ischemic mouse, this media enhanced collateral flow recovery and remodeling (Zhu et al., 2011). Treatment with autologous BMSCs significantly increased arteriogenesis and improved blood flow in the chronic limb ischemia model (Zhu et al., 2011). BMSCs selectively migrate to the site of injury, participate in angiogenesis and arteriogenesis, as well as induce a neovascular response that results in a significant increase in blood flow to the ischemic area, aiding the repair of the injured brain (Cui et al., 2009). Trophic and growth factor production is stimulated from angiogenic and arteriogenic vessels by BMSCs, which enhance brain plasticity and recovery of neurological function after stroke (Kinnaird et al., 2004). Therefore, it is possible to think of BMSCs as behaving like small biochemical "factories," busily producing as well as inducing many cytokines and trophic factors in vascular and parenchymal cells that contribute to the improvement of functional outcome after stroke (Liu et al., 2014). Intracarotid BMSC transplantation also promotes white matter remodeling in the cortical IBZ and corpus callosum by increasing axonal sprouting and remyelination (Shen et al., 2006). Other beneficial effects observed upon BMSC treatment include enhanced structural neuroplasticity and increased axonal outgrowth from healthy brain tissue (Andrews et al., 2008).

miRNA on MSC-induced neurorestorative effects

microRNAs are short sequences of non-coding RNA (ca. 22 nucleotides) found in animals and plants, which regulate gene expression both transcriptionally and post-transcriptionally. miRNAs can regulate many genes, pathways, and biological networks, either acting alone or with other miRNAs. It appears that miRNAs act like molecular rheostats, fine-tuning many biological processes regulating tissue repair like angiogenesis, inflammation, hypoxia–response, and stem cell biology by affecting gene regulation (Sen, 2011). Therapies aiding in tissue repair based on miRNAs are beginning to hold promise as we learn how to control and manipulate cell-specific miRNAs (Juranek et al., 2013). However, using miRNAs as therapeutic targets still hold many challenges, due to possible delivery and potential off-target effects. Cell-based therapy may be an effective means to manipulate miRNA expression (Juranek et al., 2013). Cells delivered by intravenous injection release microvesicles that contain enriched miRNA that can in turn stimulate endogenous brain cells to express and release miRNA, ultimately promoting neurorestorative effects after stroke (Juranek et al., 2013).

BMSCs are abundantly present in our body and they secrete extracellular vesicles, which depending on parent phenotype, may carry miRNA, mRNA, proteins, lipids, etc (Katsuda et al., 2013) and transport miRNAs from cells of origin to target cells (Collino et al., 2011). miRNAs play a key role in BMSC neuronal differentiation during which miR-34a is downregulated. miR-34a mediates neuronal precursor motility, that is crucial for homing of stem cells to target tissue (Chang et al., 2011). In addition, miR-96,

miR-124, and miR-199a regulate gene expression critical for differentiation of BMSCs, and were expressed differentially during adipogenic, chondrogenic, and osteogenic induction of hBMSCs (Laine et al., 2012). miRNAs regulate neurogenesis, mediate the trans-differentiation of MSCs into functional neurons and are also useful in restoration of lost or damaged neurons in neurological disorders (Lim et al., 2010). miRNAs, with their varied biological functions and regulatory capabilities, open new avenues and strategies for BMSC therapy and manipulation with potential therapeutic benefits.

Microvesicles provide bidirectional miRNA exchange between injured cells and BMSCs, which in turn facilitates neuronal differentiation and activation of regenerative pathways in injured cells. These extracellular vesicles can mediate intracellular communication and be manipulated to induce therapeutically beneficial effects. While in resting or active states, BMSCs secrete microvesicles, both microparticles and exosomes that can be manipulated to deliver miRNAs to enhance recovery of injured tissues (Wang et al., 2013). A novel treatment strategy for malignant glioma using miRNAs that are known to have anti-tumor properties (e.g., miR-146b), employs exosomes secreted by BMSCs transfected with miR-146b as a delivery vehicle to decrease tumor volume (Katakowski et al., 2013). Exposure to ipsilateral ischemic tissue extracts obtained from MCAo rats elevates miR-133b expression in BMSCs and in exosomes derived from BMSCs (Juranek et al., 2013). miR-133b transfer from BMSCs to neurons and astrocytes results in an increased miR-133b level, and when exposed to post-MCAo brain extracts for 72h, a significant increase in neurite branch number and total neurite length is observed. Neurite outgrowth also can be promoted by delivery of miR-133b to neurons and astrocytes by transfer from BMSCs via exosomes (Juranek et al., 2013). Manipulation the expression of miR-133b in BMSCs and thus, in their exosomes, regulates neurological recovery after stroke (Xin et al., 2013). These data clearly indicate that BMSCs mediate their functional benefit post-stroke, by the transfer of exosomes with active miRNAs to parenchymal cells. The miRNAs, and as shown specifically for the transfer of miR-133b, regulate their downstream targets, and thereby impact brain plasticity, and neurovascular remodeling to promote neurological recovery (Xin et al., 2013).

Therapeutic modulation of individual miRNAs generated by BMSCs, and either mimicking or antagonizing miRNA actions, may enhance BMSC therapeutic efficacy. For example, miR-126 is expressed in endothelial cells in blood vessels and capillaries, and mediate angiogenesis (Nikolic et al., 2010). Transplantation of BMSCs over-expressing miR-126, increases the release of angiogenic factors, improves resistance against hypoxia, and activates Notch ligand Delta-like-4, thereby enhancing functional angiogenesis in the ischemic myocardium and improves cardiac function (Huang et al., 2013a). Increased angiogenesis and improved cardiac function may be attributed to stimulation of AKT/ERK-related pathway (Chen and Zhou, 2011). Abnormal down-regulation of miR 146a has been implicated in mediating chronic inflammatory responses that interfere with wound healing in diabetic subjects (Xu et al., 2012). BMSC therapy for post-myocardial infarction, increases miR-146a expression and decreases the expression of pro-inflammatory factors. MiRNAs are also involved in nearly every aspect of the presumed repair mechanisms of BMSC-based therapies in myocardial infarction, such as neovascularization and stem cell differentiation (Wen et al., 2012). Hence, the use of miRNAs as novel regulators and therapeutic modulation of individual cardiovascular miRNAs of BMSCs have been proposed to improve therapeutic efficiency (Wen et al., 2012). Treatment of stroke with exosomes derived from BMSCs, i.e., without the parent BMSC, improves functional outcome, as well as enhances angiogenesis, neurogenesis, and neurite remodeling. This approach, of using purely exosomes derived from BMSCs, represents a potentially novel stroke treatment, with possible broad therapeutic applications (Xin et al., 2013).

STROKE CLINICAL TRIAL FOR CELL-BASED THERAPY

Isolating MSCs from bone marrow for transplantation is considered safe, having been widely tested in numerous clinical trials with encouraging results (Bang et al., 2005; Sykova et al., 2006). BMSC therapies are being evaluated via 79 registered clinical trial sites located throughout the world (Malgieri et al., 2010). Clinical trials conducted to study intravenous infusion of autologous BMSCs had promising results, showing that BMSCs appear to be a safe and are a feasible therapy for improving functional outcome in stroke patients (Bang et al., 2005; Suarez-Monteagudo et al., 2009; Lee et al., 2010). Autologous BMSCs were intravenously infused in a series of patients from South Korea suffering from cerebral infarcts in the middle cerebral artery (Bang et al., 2005). Studies, serial evaluations, and comparisons to control group (did not receive MSCs) for 1 year revealed that the treatment is safe and may improve functional recovery. A follow up long-term evaluation report recorded higher survival among treated patients than control group and revealed no significant side effects, indicating that autologous BMSCs delivered i.v. is safe and may improve functional recovery (Lee et al., 2010). A Phase I/II clinical trial has been initiated by researchers at University of California San Diego with other collaborators in which allogenic BMSCs are being evaluated to treat ischemic stroke (NCT01297413). A Phase I/II clinical trial in Spain revealed feasibility, safety, and improved neurological outcome in stroke patients transfused intra-arterially at 5 and 9 days after stroke with autologous bone marrow mononuclear cells (Moniche et al., 2012). During the follow up period of 6 months, no adverse effects, deaths, tumor formation, or stroke recurrence were reported, except for two isolated partial seizures at 3 months post treatment. From a study of a small group of ischemic stroke patients with infarcts in the middle cerebral artery region, it was found that the delivery of umbilical cord MSCs via intra-arterial catheterization is safe and may contribute to functional improvements (Jiang et al., 2012). There are several other ongoing studies to test the safety and efficiency of umbilical cord blood therapy, umbilical cord blood mononuclear cells, to treat stroke subjects (NCT01884155, NCT01673932).

NIASPAN TREATMENT PROMOTES BRAIN PLASTICITY AFTER STROKE

There is a growing body of evidence that strengthens the link between brain high-density lipoprotein (HDL)-C metabolism and factors involved in synaptic plasticity. Scavenger receptor class B1 (SR-B1) binds HDL and facilitates α-tocopherol and cholesteryl

esters transfer into cells from circulating HDL. Mice with knocked out scavenger receptor (SR-B1) exhibited deficient synaptic plasticity, as measured by long-term potentiation of the CA1 hippocampus region, which leads to impaired recognition and spatial memory (Chang et al., 2009). Furthermore, mice that lacked ATP-binding cassette transporter A1 (ABCA1) in the CNS exhibit reduced plasma HDL-C levels and altered synaptic morphology, including reduced synapse and synaptic vesicle numbers (Karasinska et al., 2009). Niacin is one of the most potent HDL-C promoter drugs used in the clinic. Niaspan, an extended release formulation of Niacin, may be effective in reducing neurological deficits post-stroke by promoting axonal remodeling, angiogenesis, and arteriogenesis (Chen et al., 2007, 2009; Cui et al., 2010; Yan et al., 2012).

Successful axonal sprouting and remodeling in the penumbra region is a critical step in nerve regeneration and brain repair. Niaspan, when administered 24 h after MCAo significantly up-regulates neuronal synaptic rewiring in the per-infarct region and restores connections between different cerebral areas after stroke (Cui et al., 2010; Yan et al., 2012). The increase in axonal density and synapse formation translates into long-term functional recovery after experimental stroke (Cui et al., 2010).

Niacin-induced increase in synaptic plasticity and axon growth may be mediated by the up-regulation in the BDNF–TrkB axis (Cui et al., 2010). In the mature nervous system, BDNF/TrkB plays an important role in regulating neuronal migration, differentiation, synaptic remodeling, and survival. Niacin treatment after stroke significantly increases BDNF/TrkB expression both in the ischemic brain and in primary cortical neuron (PCN) cultures. Furthermore, a TrkB inhibitor significantly decreases HDL and niacin-induced neurite outgrowth, which indicates that the BDNF/TrkB axis may mediate, niacin/HDL-induced synaptic plasticity and axon growth (Cui et al., 2010). In addition, the Ang1 molecular pathway also plays a partial role in Niaspan-induced axonal outgrowth (Yan et al., 2012). Niaspan significantly increases Ang1 expression. Ang1, in addition to being a promoter of angiogenesis and vascular maturation, is also a neurotrophic factor and promotes axonal outgrowth (Yan et al., 2012). In type 1 diabetes (T1DM) rats subjected to MCAo, Niaspan treatment attenuated Ang2 and increased Ang1 expression (Yan et al., 2012).

Early stroke recovery is linked to arteriogenesis (Christoforidis et al., 2005; Liebeskind, 2005). Occlusion of intracerebral arteries elevates fluid shear stress and thus primes the brain for arteriogenesis. Cellular interaction of the endothelial cells in the vascular wall with cytokines like monocyte chemoattractant proteins-1 (MCP-1), TNF-α, and cell adhesion molecules facilitates arteriogenesis, typically triggered by the development of elevated shear stress in the vessel (Hoefer et al., 2002). TNF-α is a pivotal modulator of arteriogenesis and the TNF-α-converting enzyme (TACE) is the primary protease responsible for pro-TNF-α activation (Chen et al., 2009). Treating stroke with Niaspan significantly increases CBF in the ischemic brain, as measured by magnetic resonance imaging (MRI), resulting in increased arterial diameter and proliferation of vascular SMC (VSMC). Treatment with Niaspan increases cultural arterial sprouting and VSMC migration *in vitro*. The increase in arterial sprouting and VSMC migration in stroke after Niaspan treatment is partially attributed to the increased expression of TACE in the ischemic brain and cerebral arteries (Chen et al., 2009).

High-density lipoprotein, in addition to promoting arteriogenesis, also up-regulates angiogenesis post-ischemic stroke (Chen et al., 2007). Recent findings in human and *in vitro* cell culture show that niacin impedes apolipoprotein A-1 (APO A-1) hepatic catabolism, thus prolonging HDL half-life (Jin et al., 1997; Kamanna and Kashyap, 2008). HDL promotes endothelial progenitor cell incorporation, endothelial cell migration, and re-endothelialization, all of which are mediated by eNOS and phosphoinositide 3-kinase (PI3k)/Akt kinase activation (Shehadah et al., 2010b). Treatments that combine low doses of Niaspan and tPA administered 4 h after stroke significantly improved functional outcome, reduced lesion volume, decreased expression of TLR4 and TNF-α, and decreased apoptosis in MCAo rats (Shehadah et al., 2011). Combination of Niaspan with Simvastatin helped improve overall functional outcome significantly and decreased axonal damage and density (Shehadah et al., 2010a).

CONCLUSION

Primary physiological mediators of neurorecovery post-stroke, including angiogenesis, arteriogenesis, neurogenesis, and white matter remodeling, have been described in this review. In addition, select cell-based therapies (HUCBCs and BMSCs), and an example of a restorative pharmacological agent, Niaspan, which increases HDL, which amplifies these restorative processes as restorative treatments for stroke have been discussed. Elucidating the underlying mechanisms of cell-based and pharmacological restorative therapies is of primary interest and crucial for translation of treatments to clinical use. miRNAs are major molecular regulators and appear to have pivotal roles in cell-based and possibly pharmacological restorative therapies for stroke. Clarification of their roles in mediating neurorecovery post-stroke, warrant further investigation.

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Induction of neuroplasticity and recovery in post-stroke aphasia by non-invasive brain stimulation

Priyanka P. Shah[1,2], Jerzy P. Szaflarski[3], Jane Allendorfer[3] and Roy H. Hamilton[1,2]*

[1] *Department of Neurology, University of Pennsylvania, Philadelphia, PA, USA*
[2] *Laboratory for Cognition and Neural Stimulation, Center for Cognitive Neuroscience, University of Pennsylvania, Philadelphia, PA, USA*
[3] *Department of Neurology, University of Alabama at Birmingham, Birmingham, AL, USA*

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Victor W. Mark, University of Alabama at Birmingham, USA
Gitendra Uswatte, University of Alabama at Birmingham, USA

Correspondence:
Roy H. Hamilton, Department of Neurology, Center for Cognitive Neuroscience, University of Pennsylvania, 518 Goddard Building, 3710 Hamilton Walk, Philadelphia, PA 19104, USA
e-mail: roy.hamilton@uphs.upenn.edu

Stroke victims tend to prioritize speaking, writing, and walking as the three most important rehabilitation goals. Of note is that two of these goals involve communication. This underscores the significance of developing successful approaches to aphasia treatment for the several hundred thousand new aphasia patients each year and over 1 million stroke survivors with chronic aphasia in the U.S. alone. After several years of growth as a research tool, non-invasive brain stimulation (NBS) is gradually entering the arena of clinical aphasiology. In this review, we first examine the current state of knowledge of post-stroke language recovery including the contributions from the dominant and non-dominant hemispheres. Next, we briefly discuss the methods and the physiologic basis of the use of inhibitory and excitatory repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) as research tools in patients who experience post-stroke aphasia. Finally, we provide a critical review of the most influential evidence behind the potential use of these two brain stimulation methods as clinical rehabilitative tools.

Keywords: TMS, rTMS, fMRI, tDCS, rehabilitation, aphasia

INTRODUCTION

Aphasia, defined as an impaired ability to communicate, is one of the most feared symptoms of stroke. About 21–38% of acute stroke survivors suffer from aphasia (Berthier, 2005), a devastating neurological condition affecting a person's ability to communicate and, thus, reintegrate into the society. It is a consequence of damage in a widely distributed and complex language network involving the fronto-temporal areas in the dominant hemisphere (typically left). Aphasia usually impacts all areas of communication including language formulation and comprehension as well as the ability to read and write. These deficits are attributed to damage in higher cognitive areas involved in language processing rather than to areas involved in motor control of the articulatory structures (Allendorfer et al., 2012a), although aphasia and disorders of speech articulation often coincide.

The first 2 to 3 months after stroke are crucial for spontaneous neuroplasticity, which refers to the natural course of neurophysiological repair and cortical reorganization of language functions (Robertson and Fitzpatrick, 2008). During this period, restoration of some language functions is common and usually fairly rapid (Lazar et al., 2008). However, the slope of spontaneous recovery tends to level off within the first year of stroke (Pedersen et al., 1995; Berthier, 2005), resulting in chronic impairments in language processing in many patients.

Despite availability of pharmacological treatments and professionally-administered speech-language therapy (SLT), new strategies e.g., adjuvant therapies, are required to boost recovery, especially in the chronic stages of stroke. While SLT is the most commonly employed treatment of aphasia, its therapeutic effects are quite variable and are generally modest (Berthier, 2005; Brady and Enderby, 2010). Recently, non-invasive brain stimulation (NBS) techniques, including repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) have shown promise as potential approaches for enhancing aphasia recovery. A number of research studies employing these techniques, especially repetitive rTMS, have reported lasting improvement in specific language functions in patients with chronic post-stroke aphasia. In addition to behavioral improvement, evidence of induced neuroplasticity has further validated the efficacy of these interventions. However, application of therapeutic NBS within few days after stroke i.e., in sub-acute and acute phase, is still in its infancy.

In this article, we will explore the neuroplastic processes that underlie spontaneous recovery in patients with aphasia, and present the methods and discuss the physiologic basis of NBS techniques. Next, we will discuss recently published and influential work in which NBS has been used to enhance recovery from post-stroke aphasia. Lastly, we will review studies that investigate the effect that NBS has on neuroplasticity in patients with aphasia; specifically, we will examine studies that address the functional neuroimaging and electrophysiologic correlates of neuroplastic changes after brain stimulation.

NEUROPLASTICITY IN SPONTANEOUS RECOVERY OF POST-STROKE APHASIA

Converging evidence indicates that recovery in post-stroke aphasia is supported by compensatory changes in the representation of language functions, either involving recruitment of

areas surrounding lesions in the language-dominant left hemisphere, or altered activity of intact homotopic language areas in the non-dominant right hemisphere, or both (Hamilton et al., 2011). Recruitment of previously inactive pathways, appears to apply to motor rather than language recovery and will not be discussed here (Lee and Vandonkelaar, 1995). Recently, modern investigative techniques such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), electroencephalogram-based event related potentials (EEG-ERP), and diffusion tensor imaging (DTI) have been applied to understand neuroplasticity in the context of spontaneous language recovery after stroke [for details of the application of various neuroimaging techniques to the evaluation of post-stroke recovery see recent review by Eliassen and colleagues (2008)]. In this section, we will discuss the proposed models of neuroplasticity in aphasia recovery with supporting evidence from neuroimaging investigations that capture changes in brain function as a measure of neuroplasticity after stroke.

Based on assembled evidence from prior studies, three models of neuroplasticity underlying aphasia recovery in adults were outlined by Hamilton et al. (2011). These are: (1) recruitment of residual perilesional language areas in the language-dominant left hemisphere (Ohyama et al., 1996; Karbe et al., 1998a,b; Warburton et al., 1999; Cornelissen et al., 2003), (2) compensatory recruitment of homotopic language areas in the non-dominant right hemisphere (Musso et al., 1999; Thulborn et al., 1999; Tillema et al., 2008), and (3) inefficient recruitment of sites in the non-dominant right hemisphere, which hinders rather than aids recovery (Turkeltaub et al., 2011). In addition, increased involvement of right homotopic language areas due to release from transcallosal inhibition may also negatively affect spontaneous neuroplasticity. By this account, interhemispheric inhibitory connections that normally modulate and effectively suppress right hemispheric activity are disturbed due to damage in the left hemisphere, enabling areas in the contralesional right hemisphere to become increasingly involved via disinhibition. It has been proposed that increased involvement of right hemispheric regions in post-stroke language production in adults may exert an increased inhibitory influence on perilesional areas in the left hemisphere, interfering with ability of these regions to contribute to language recovery (Belin et al., 1996; Rosen et al., 2000; Naeser et al., 2004). This last model provided the rationale for a number of studies in which suppression of right hemispheric activity or stimulation of the left hemispheric peri-stroke areas with NBS has been employed in order to enhance language performance in patients with aphasia (Naeser et al., 2005; Kang et al., 2011; Szaflarski et al., 2011a,b; Marangolo et al., 2013). As outlined below, most of these studies target specific sites in the right hemisphere.

Evidence regarding the role of the right hemisphere in language recovery is mixed; while some studies suggest that recruitment of right homotopic areas may be beneficial or compensatory in nature (Thulborn et al., 1999; Tillema et al., 2008), other studies show that activation of these right hemisphere regions during language performance may indicate a maladaptive strategy of recovery (Winhuisen et al., 2005; Thiel et al., 2006). For instance, Winhuisen et al. (2005) found that right-hemispheric involvement may only be partially compensatory in sub-acute patients with aphasia. They applied inhibitory rTMS (4 Hz, 10 s trains) to the right and left inferior frontal gyrus (IFG) in 11 patients, where the exact loci of IFG stimulation were based on maximum functional activation on PET during a language task. They showed that individual patients' response to rTMS with left vs. right IFG varied: 8 patients showed increased naming reaction time (RT) with left IFG while 4 patients showed increased RT with right IFG stimulation. Interestingly, the group that showed increased latency after left IFG stimulation performed significantly better on a verbal fluency task than the group that responded to right IFG stimulation. Based on this finding, the authors suggested that patients with residual language function in the left hemisphere, functionally defined by an inhibitory response to rTMS, performed better on the language task than those with right hemispheric involvement. Their findings further suggested that recovery of function in the dominant left hemisphere may be essential for optimal language reacquisition after aphasia, while right hemisphere recruitment may only be partially compensatory or may be maladaptive in some cases.

The maladaptive role of at least one specific site in the right homotopic language areas was recently suggested by Turkeltaub et al. (2011). The authors employed Activation Likelihood Estimation (ALE) meta-analysis of functional neuroimaging studies involving language tasks in 105 patients with chronic aphasia and 129 control subjects. While control subjects showed functional activation patterns predominantly in left perisylvian language areas, patients with aphasia consistently involved spared left hemisphere areas as well as right hemispheric homotopic language areas. While recruitment of some right hemispheric homotopic areas appeared to be beneficial with respect to language performance, activation of the right pars triangularis (PTr; Brodmann areas 45 in the IFG) was found to be inefficient, perhaps even deleterious, with respect to language performance. This finding corroborates the observed therapeutic effects of inhibitory rTMS of right PTr on several language functions, which in effect may act due to suppression of "noisy" or maladaptive activation of the right PTr (Naeser et al., 2005; Barwood et al., 2011a, 2013; Weiduschat et al., 2011; Kindler et al., 2012; Medina et al., 2012; Thiel et al., 2013).

Evidence further suggests that the neuroplastic mechanisms that underlie spontaneous recovery vary greatly among patients with aphasia. Prior research suggests that language recovery in adults depends on several clinical factors, such as the extent and location of lesions (Heiss and Thiel, 2006). With small lesions that spare some areas of eloquent cortex, recovery may rely up on recruitment of residual language areas along with increased perilesional activity. By contrast, in the case of large lesions that engulf primary language regions, recovery may rely on recruitment of homotopic non-dominant language areas (Rosen et al., 2000; Heiss and Thiel, 2006).

Data also indicate that at more than 1 year after aphasia-producing stroke the cortical participation in language production remains relatively stable in absence of intervention. For example, a study utilizing three different fMRI tasks (verb generation, semantic decision, and picture-word matching) assessed language recovery mechanisms in a group of chronic aphasic

patients by comparing blood-oxygen-level-dependent (BOLD) signal changes over a period of 10 weeks (Eaton et al., 2008; Szaflarski et al., 2011a). Four chronic patients and an equal number of age-matched healthy controls underwent 5 fMRI sessions (2 runs of each fMRI task per session) over a course of 10 weeks. As expected, patients with aphasia performed worse than the controls on these tasks. In addition, these differences in language performance were associated with differences in cortical activity between the two groups. While control subjects exhibited overall typical bilateral, left greater than right activation in the frontal and temporal language areas as well as symmetric retrosplenial and posterior cingulate areas, the stroke patients exhibited increased and consistent activation in perilesional areas with minimal activation in the contralesional right hemisphere. The authors concluded that among patients with chronic aphasia activation of the left hemispheric language regions and deactivation of the right homologs suggests cortical reorganization after stroke. They further posited that activity in the perilesional areas, rather than in the non-dominant homologs may be the more critical mechanism for language recovery.

More direct evidence of hemispheric changes in brain activity over time is provided in a study by Saur et al. (2006). In a group of 14 patients with post-stroke aphasia, Saur et al. examined the neural correlates of language recovery. They evaluated language task-related fMRI activation patterns in these patients at acute (average of 1.8 days after stroke), sub-acute (average of 12.1 days after stroke), and chronic (average of 321 days after stroke) stroke stages (Saur et al., 2006). In the acute phase, they observed little or no perilesional activation of undamaged areas in the left hemisphere. Whereas in the sub-acute phase, a large increase of activation in a bilateral language network was observed with a peak in the right Broca's homolog (Heiss et al., 1999; Thulborn et al., 1999; Winhuisen et al., 2005) and right supplementary motor area; these increases were strongly associated with improved performance on language tasks. Further, since the authors observed that language improvement in the chronic phase was associated with a redistribution of activation toward the dominant left-hemispheric language areas, recruitment of right hemisphere language homologs may suggest their beneficial role for the early but not the late language recovery. Based on this finding, the authors suggested that the involvement of the right hemispheric areas in recovery may be transient before more favorable perilesional recruitment takes place. However, the exact role of right language homologs during the sub-acute stage of recovery is still unclear. These findings are potentially consistent with the notion that recruitment of right hemispheric areas may only be partially compensatory, and that optimal neuroplastic changes eventually involve recruitment of perilesional areas.

This hypothesis was recently addressed directly by Szaflarski et al. (2013). While Saur et al. (2006) focused on a group of patients who eventually experienced recovery in their language functions at the chronic stage, Szaflarski et al. evaluated the neural correlates of good vs. poor recovery in a group of 27 chronic stroke patients (Saur et al., 2006; Szaflarski et al., 2013). Similar to the findings in Saur et al. (2006), normalization of language functions at least 1 year after stroke was associated with typical fMRI activation patterns i.e., fMRI activity with left hemisphere distribution when compared to healthy controls. However, the reorganization of the language function in the non-recovered group was characterized by activation patterns in the right hemispheric areas. Specifically, increased activity in the left superior frontal and parietal areas and bilateral cerebellum was observed in the recovered vs. the non-recovered group. In addition, a decrease in activation was found in the right superior temporal areas in the recovered vs. non-recovered group. Language performance and the level of hemispheric activation were also associated, in that increase in activation in the right areas was associated with poor trajectory of performance, while increase in activation in the left areas was associated with improved performance. Lesion size also affected language performance consistent with the theory of regional hierarchy (Heiss and Thiel, 2006). Overall, the authors posited that the recruitment of right areas in the poor recovery group may be an indication of a maladaptive or an inefficient pattern of language recovery.

In short, the balance of evidence leads us to conclude that sparing of language areas in the lesioned left hemisphere and/or cortical reorganization of brain activity during recovery to the left hemispheric perilesional areas may be the optimal mechanism of neuroplastic changes with respect to language outcomes. However, the importance of right hemispheric homologs to the process of recovery is not clear. Although the evidence from neuroimaging studies suggests a negative association between language recovery and right hemispheric activation, we posit that involvement of some of the right hemispheric areas may not be deleterious to language recovery and that a specific "noisy" or inefficient site may hinder the downstream recruitment of perilesional and residual language areas, and, therefore, adversely impact recovery (Turkeltaub et al., 2011).

NEUROREHABILITATION WITH NON-INVASIVE BRAIN STIMULATION

The field of neurorehabilitation broadly aims to develop therapies that: (1) derive from an understanding of the mechanisms of healthy brain function and neurological dysfunction after a brain injury, and (2) improve not only behavioral or cognitive performance but also the function of neural systems, which translates into favorable outcomes on everyday quality of life (Robertson and Fitzpatrick, 2008). In light of these goals, NBS techniques provide a unique opportunity for neurorehabilitation after stroke. In the recent years, investigation of NBS techniques to promote stroke recovery has grown immensely and is continuously supported by the advent of new technologies. The application of NBS specifically to post-stroke aphasia rehabilitation leverages current understanding of the models of spontaneous language plasticity discussed above with various neuroimaging techniques such as fMRI (Szaflarski et al., 2011a,b) or EEG-ERP (Barwood et al., 2011b,c) to provide further evidence of stimulation-induced neuroplasticity. Also, insofar as improving function in everyday life is of paramount concern to neurorehabilitation, changes in functional communication outcomes after therapeutic NBS, have been assessed in at least 2 studies (Szaflarski et al., 2011b; Marangolo et al., 2013), where patients with aphasia tended to report improved ability in communication after tDCS and rTMS.

In this section, we will introduce basic principles of TMS and tDCS and summarize the current literature describing the therapeutic effects of these two technologies in stroke patients with aphasia (See **Table 1**). In a subsequent section, we will summarize the accounts of rTMS-induced neuroplasticity.

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION (rTMS)

TMS is a focal NBS method, which employs the principle of electromagnetic induction. A TMS stimulator unit consists of capacitors that store large electrical charges, which is connected to a casing with coil of copper wires. For TMS delivery, this coil is held tangentially to the scalp. When the stored charge is discharged to the coil, a brief and time-varying magnetic field is produced at the scalp. This magnetic field penetrates through the skull, and depending on stimulation intensity, coil shape, and coil orientation, an electrical current is generated in the cortical neurons near the coil. This current is sufficient to depolarize neuronal membranes and generate action potentials. TMS can be delivered either via single pulses or repetitively at a set number of pulses per second (repetitive TMS or rTMS). Typically, low-frequency rTMS (<5 Hz) is characterized by decreased cortical excitability, whereas high-frequency rTMS (≥ 5 Hz) is characterized by enhanced excitability (Pascual-Leone et al., 1998; Fitzgerald et al., 2006). Recently, a new rTMS protocol, theta burst stimulation (TBS), was introduced which can produce longer-lasting and more stable changes in cortical excitability compared to standard rTMS (Huang et al., 2005). While standard rTMS consists of single pulses of stimulation delivered repeatedly over a unit of time, TBS consists of 3 pulses delivered very rapidly (at 50 Hz) every 200 ms, which can either be interrupted every few seconds [intermittent TBS (iTBS)] or can be uninterrupted (cTBS). ITBS typically increases cortical excitability, while cTBS decreases cortical excitability, and such changes in excitability over the motor cortex have shown to last for about an hour with more intense TBS methods (Huang et al., 2005).

A review of recent and influential rTMS studies in post-stroke aphasia revealed that most intervention studies administered low-frequency inhibitory rTMS (1–4 Hz) for 20–40 min a day over 10–15 days, on sites in the right hemisphere that were homotopic to left hemisphere sites in the fronto-temporal language network (Broca's or Wernicke's; **Figure 1**) (Naeser et al., 2005; Barwood et al., 2011a,b,c; Kakuda et al., 2011; Weiduschat et al., 2011; Abo et al., 2012; Kindler et al., 2012; Medina et al., 2012; Waldowski et al., 2012; Barwood et al., 2013; Thiel et al., 2013). Thus, far only 1 group, Szaflarski et al. (2011b), administered iTBS, an excitatory TMS protocol, to increase the cortical excitability in perilesional left-hemispheric language areas (**Figure 2**) (Szaflarski et al., 2011b). In addition, some studies combined therapeutic rTMS with 45–60 min of speech and language therapy (Kakuda et al., 2011; Abo et al., 2012; Thiel et al., 2013).

Outcome measures, methods of finding the appropriate stimulation site, inclusion of patients by disease duration, and number of long-term follow-up evaluations after rTMS vary considerably between studies. Improvement on subtests of clinical aphasia diagnostic or severity scales [Boston Diagnostic Aphasia Examination (BDAE), Aachen Aphasia Test (AAT), or Western Aphasia Battery (WAB)], Boston Naming Test (BNT), and accuracy and RT in picture naming tasks (Snodgrass and Vanderwart, 1980; Bates et al., 2003) are the most commonly used outcome measures. Most of these studies demonstrated improvement after rTMS in one or more outcome measures. For example, improved picture naming accuracy and RT, auditory comprehension, verbal fluency and repetition have all been observed after daily sessions of low-frequency rTMS (**Table 1**). In addition, improvement in global scales of aphasia severity was also reported in some studies. These findings clearly suggest the beneficial role of rTMS in improving some language functions in patients with aphasia.

Although right PTr (BA 45), a site in the IFG, was most frequently stimulated, some studies adopted a site-finding protocol (**Figure 1**) either among several pre-defined right hemispheric sites in individual patients (Martin et al., 2009; Hamilton et al., 2010; Naeser et al., 2011; Medina et al., 2012), or used activation patterns in fMRI (Szaflarski et al., 2011b; Abo et al., 2012; Allendorfer et al., 2012b) to find the most optimal site (**Figure 2**). For example, Medina et al. (2012) adopted a site-finding protocol similar to Hamilton et al. (2010) and Naeser et al. (2011). They carried out 6 separate rTMS sessions [600 pulses of 1 Hz at 90% resting motor threshold (RMT)] before the daily treatment sessions, where 6 different sites in right IFG were stimulated. Sites included the mouth area in the motor cortex, pars opercularis (POp; BA 44), three separate sites on PTr (dorsal posterior, ventral posterior and anterior PTr), and the pars orbitalis (BA 47). Optimal site for stimulation was determined by evaluating improvement in picture naming accuracy after each stimulation session. A site with the greatest increase in naming accuracy was considered optimal, and patients were stimulated for 10 daily rTMS sessions at this site (1200 pulses of 1 Hz at 90% RMT). Right PTr was the optimal site for 9 of 10 patients, while for 1 patient, right pars orbitalis was the optimal site. After the treatment sessions, the patients that received rTMS improved in several measures of fluency, while patients in the sham group did not improve on any language measures; the beneficial effects persisted for at least 2 months after the treatment ended. (Szaflarski et al., 2011b) adopted an fMRI-based activation approach to finding optimal stimulation sites. Perilesional stimulation targets were identified as regions that exhibited increased activation on fMRI during a semantic language task. Subsequently, iTBS was delivered to each patient's target site in 10 daily sessions lasting 200 s (3 pulses at 50 Hz given every 200 ms in 2 s trains for a total of 600 pulses). Each patient underwent fMRI pre- and post-iTBS as he or she performed a semantic decision/tone decision (SDTD) task, which has previously been shown to reliably localize residual language areas in patients with aphasia after stroke (Eaton et al., 2008). The authors reported significant improvement on a semantic verbal fluency task, as well as a trend toward improved functional communication, collected by self-report. Recently, Abo et al. (2012) extended this work by defining stimulation sites not only by fMRI activation patterns but also by the type of aphasia. In patients with non-fluent aphasia they applied inhibitory rTMS to either the right or left IFG and in those with fluent aphasia to either the right or left superior temporal gyrus (STG); stimulation application (STG or IFG) was based on

Table 1 | Summary of non-invasive brain stimulation intervention studies for post-stroke aphasia.

Study	*N*	Stroke onset	Methods	FU	Site	Tests	Findings—significant improvement in:
rTMS: CASE REPORTS/SERIES							
Naeser et al., 2010	1	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day Optimal site finding CPAP	3, 6, 2.4 years	Right PTr (5 sites: Motor mouth area, and 4 subregions within Broca's area)	Picture naming, BDAE, BNT	Phrase length, auditory comprehension and BNT at 3 and 6 months post-TMS
Cotelli et al., 2011	3	Chronic	20 Hz rTMS 90% RMT 10 or 20 days 25 min/day 25 min of SLT	About 1, 3, 6, 11	Left dlPFC	AAT, BADA, picture naming, verbal fluency and reasoning	Picture naming accuracy; persistent benefit present 48 weeks after treatment
Hamilton et al., 2010	1	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day Optimal site finding	2, 6, 10	Right PTr (sites: POp, dpPTr, vpPTr, aPTr, PO, Motor mouth area)	WAB, BDAE-Cookie theft, picture naming	Naming and spontaneous speech; improvement in picture description sustained at 2, 6 and 10 months
Barwood et al., 2012	7	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day	2, 8	Right PTr	BNT, BDAE, picture naming	Naming accuracy and latency, generalized speech output, and auditory speech comprehension; effects sustained up to 8 months
Martin et al., 2009	2	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day Optimal site finding fMRI: changes in activation patterns	2, 6, 16, 43	Right PTr (4 sites: POp, aPTr, pPTr, Motor mouth area)	BNT, BDAE—Cookie theft, picture naming	Picture naming and phrase length in one patient (responder; best response site right pPTr); No improvement in the other patient (non-responder; best response site right aPTR)
rTMS: GROUP STUDIES							
Naeser et al., 2005	4	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day	2, 8	Right PTr	BNT, BDAE, picture naming	Picture naming at both 2 and 8 months in 3 patients
Kakuda et al., 2011	4	Chronic	10 min of 6 Hz followed by 20 min of 1 Hz rTMS 90% RMT 11 days 2 sessions/day (except on 1st and last day) 60 min of SLT	–	Right IFG (F8)*	SLTA, J-WAB	(greatest) repetition and naming; 4 patients showed improvement in different categories including naming, repetition, writing, auditory and visual comprehension and speech; none showed deterioration
Barwood et al., 2011a	6 real 6 sham	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day	2	Right PTr	BNT, BDAE, picture naming	Naming, aspects of expressive language and auditory comprehension

(Continued)

Table 1 | Continued

Study	*N*	Stroke onset	Methods	FU	Site	Tests	Findings—significant improvement in:
Medina et al., 2012	5 real 5 sham (crossed-over to real after 2 months)	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day Cross-over Optimal site finding	2	Right IFG (sites: POp, dpPTr, vpPTr, aPTr, PO, Motor mouth area)	BDAE, BNT, narrative speech production	Fluency at 2 months after rTMS, specifically in discourse productivity; no benefit in sentence complexity, grammatical accuracy or lexical selection; for 9/10 patients, the optimal site was right PTr
Kindler et al., 2012	18	Sub-acute and Chronic	cTBS (3 pulses at 30 Hz) 2 days—Sham/real Cross-over 44 s/day	—	Right PTr	Timed picture naming, alertness task	Naming and reaction time after TBS vs. sham; no differences observed in arousal; patients in sub-acute phase were best responders
Waldowski et al., 2012	13 real 13 sham	Sub-acute	1 Hz rTMS 90% RMT 15 days 30 min/day 45 min SLT	3.5	Right PTr and right POp	ASRS, BDAE, picture naming[7]	Aphasia severity (ASRS) in real group compared to the sham 15-weeks after treatment; naming accuracy did not differ between groups but reaction time was slightly faster in the real group after treatment; real subgroup with lesions involving frontal area showed slower reaction times
Barwood et al., 2013	6 real 6 sham		1 Hz rTMS 90% RMT 10 days 20 min/day	2, 8, 12	Right PTr		Naming, expressive language and auditory comprehension up to 12 months in the real group compared to sham
Barwood et al., 2011b	6 real 6 sham	Chronic	1 Hz rTMS 90% RMT 10 days 20 min/day N400 ERP	2	Right PTr	BNT, BDAE, picture naming, SJT	(Differences in) mean and peak amplitudes and area under the curve measures of N400 ERP component between real and sham group at 2 months
Weiduschat et al., 2011	6 real 4 ctrls	Sub-acute	1 Hz rTMS 90% RMT 8–10 days 20 min/day PET SLT	—	Right PTr or Vertex	AAT	AAT; activation shift toward right hemisphere in control group, absent in intervention group; laterality shift and clinical improvement were not related
Szaflarski et al., 2011b	8	Chronic	fMRI-guided iTBS (3 pulses at 50 Hz) 10 days 200 s/day 80% AMT LI	—	Left PTr	BNT, SFT, COWAT, PPVT, CAL, BDAE CompId	SFT; activation shifts to the affected left hemisphere; self-reports of improved communicative ability (tendency)
Allendorfer et al., 2012b	8	Chronic	fMRI-guided iTBS (3 pulses at 50 Hz) 10 days 200 s/day 80% AMT DTI—FA	—	Left PTr	BNT, SFT, COWAT, PPVT, CAL, BDAE CompId	SFT; higher DTI-FA values in the left fronto-temporo-parietal areas

(Continued)

Table 1 | Continued

Study	*N*	Stroke onset	Methods	FU	Site	Tests	Findings—significant improvement in:
Abo et al., 2012	24	Chronic	fMRI (right or left) and aphasia type (STG or IFG)-guided 1 Hz rTMS 90% RMT 10 days 40 min/day 60 min of SLT	–	Fluent: Right STG (CP6*; $n = 5$), Left STG (CP5; $n = 5$); Non-fluent: Right IFG (F8; $n = 11$)Left IFG (F7; $n = 3$)	SLTA, J-WAB	Auditory and reading comprehension, and repetition in non-fluent aphasia patients; improvement in spontaneous speech in fluent aphasia patients
Thiel et al., 2013	13 real 11 sham	Sub-acute	1 Hz rTMS 90% RMT 10 days 20 min/day PET 45 min of SLT	–	Right PTr or Vertex	AAT	Global AAT and naming subtests; larger activation index in the left hemisphere in rTMS group post-treatment compared to the sham group
tDCS: GROUP STUDIES							
Monti et al., 2008	4 a-tDCS 4 c-tDCS	Chronic	a-tDCS and c-tDCS 2 mA 10 min Reference on right shoulder Cross-over	–	Left fronto-temporal (crossing point between T3-Fz and F7-Cz)*	Picture naming task	Picture naming accuracy after c-tDCS whereas a-tDCS and sham induced no changes
Baker et al., 2010	10	Chronic	a-tDCS and sham 1 mA 5 days/condition 20 min/day Reference on right shoulder Cross-over Online anomia treatment[4]	–	fMRI-guided left frontal areas	Picture naming task, WAB-R, ABA-2	Naming accuracy after a-tDCS compared to sham; effects persisted at least 1-week post-treatment
Fiori et al., 2011	3	Chronic	a-tDCS and sham 1 mA 20 min/day 5 days/condition Online language training	–	Left Wernicke's	Picture naming task, BADA	Picture naming accuracy with a-tDCS and sham; shorter naming latencies during a-tDCS compared to sham. Accuracy and RT were better at 1 and 3 weeks after a-tDCS
Fridriksson et al., 2011	8	Chronic with posterior lesions	a-tDCS and sham 1 mA 5 days/condition 20 min/day Reference on right forehead Cross-over Online anomia treatment[4]	–	fMRI-guided (left) perilesional areas	Picture naming task	(Reduction in) reaction times during naming after a-tDCS compared to sham; effects persisted at least 3-weeks

(Continued)

Table 1 | Continued

Study	*N*	Stroke onset	Methods	FU	Site	Tests	Findings—significant improvement in:
Floel et al., 2011	12	Chronic	a-tDCS, c-tDCS, and sham 1 mA 20 min/day 3 days/condition Cross-over Reference in contralateral supraorbital Online anomia training	–	Right temporo-parietal	AAT; picture naming	Picture naming accuracy after all stimulation conditions observed with anomia training; sustained benefits were found 2-weeks after a-tDCS as compared to sham and c-tDCS
You et al., 2011	7 a-tDCS 7 c-tDCS 7 sham	Sub-acute	a-tDCS on left or c-tDCS on right or sham 2 mA 10 days 30 min Reference on contralateral supraorbital Online SLT	–	Left and Right Wernicke's (STG)	K-WAB	Auditory verbal comprehension after right c-tDCS compared to left a-tDCS and sham; overall improvement in AQ and spontaneous speech also observed across groups
Kang et al., 2011	10	Chronic	c-tDCS and sham 2 mA 20 min 5 days/condition Cross-over Reference on left supraorbital Online word-retrieval training	–	Right Broca's homolog	Korean-BNT	Naming accuracy at 1 h after c-tDCS but no changes in sham tDCS
Marangolo et al., 2013	12	Chronic	a-tDCS and sham 1 mA 20 min 10 day/condition Reference on right frontopolar Cross-over Online conversational therapy	1	Left Broca's (F4)* and Wernicke's (CP5)*	BADA, token test, ecological measure, attention and memory tests	Informative speech–increase in content-units, verb and sentence production– after a-tDCS on left Broca's area; effects sustained up to 3 months

*CPAP, Continuous Positive Airway Pressure; AAT, Aachen Aphasia Test; ABA-2, Apraxia Battery for Adults—Second Edition; AMT, Active Motor Threshold; ASRS, Aphasia Severity Rating Scale; a-tDCS, anodal transcranial direct current stimulation; BADA, Battery for the Analysis of Aphasic Disorders; BDAE, Boston Diagnostic Aphasia Examination; BNT, Boston Naming Test; CAL, Communicative Abilities Log; CompId, Complex Ideation subtest; COWAT, Controlled Oral Word Association Test; cTBS, Continuous Theta Burst Stimulation (inhibitory rTMS protocol); c-tDCS, cathodal transcranial direct current stimulation; ctrl, control; dlPFC, Dorsolateral prefrontal cortex; DTI-FA, Diffusion Tensor Imaging—Fractional Anisotropy; EEG, Electroencephalogram; FU, Follow-up in months after treatment; IFG, Inferior frontal gyrus; iTBS, intermittent theta burst stimulation; J-WAB, Japanese-Western Aphasia Battery; K-WAB, Korean-Western Aphasia Battery; LI, Lateralization index; mA, milliamperes (unit of current); PET, Positron emission tomography; PPVT, Peabody Picture Vocabulary Test; PTr, Pars triangularis (Anterior portion of Broca's area); RMT, Resting Motor Threshold; SFT, Semantic Fluency Test; SJT, Word, picture semantic judgment task; SLT, Speech language therapy; SLTA, Standard Language Test of Aphasia; STG, Superior temporal gyrus; WAB-R, Western Aphasia Battery—Revised. *EEG International 10–20 system.*

fMRI activation patterns during a language task. They observed improvement after 10 daily 1 Hz rTMS sessions (40 min/day) in auditory and reading comprehension and repetition in patients with non-fluent aphasia, and in spontaneous speech in patients with fluent aphasia.

Application of optimal site-finding protocols, either rTMS- or neuroimaging-driven, is likely an improvement over blinded application of therapeutic rTMS as it accounts for individual variability in clinical factors such as lesion size and volume that could differentially influence the mechanisms of neuroplasticity

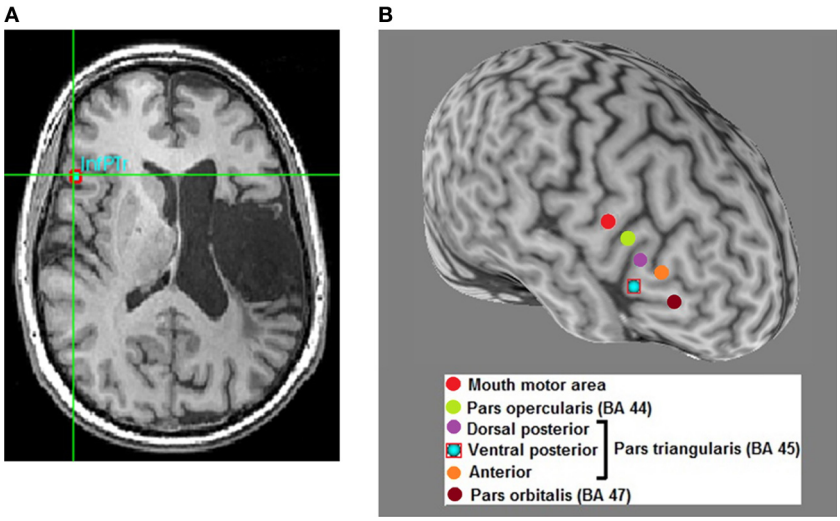

FIGURE 1 | Optimal site-finding among right hemispheric homolog areas and rTMS in a left hemisphere stroke patient with aphasia. (A) Among several right hemispheric sites, an optimal site is identified on the subject's high-resolution anatomical scan (red square); optimal site is the one that exhibits better transient language improvement compared to other sites. Most patients respond optimally to the right inferior pars triangularis (InfPTr) site. **(B)** A 3-dimensional reconstruction of the subject's high resolution anatomical scan with the 6 sites-of-interest highlighted in different colors in the right hemisphere. Optimal site for this patient is the ventral posterior (inferior) pars triangularis (PTr).

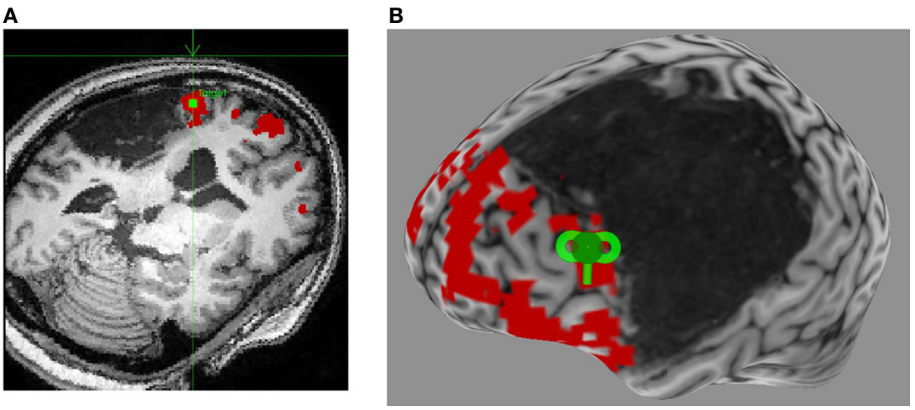

FIGURE 2 | Neuronavigated rTMS in a left hemisphere stroke patient with aphasia. (A) Language fMRI activation in left perilesional frontal area is identified on the subject's high-resolution anatomical scan as the stimulation target (green square), and a trajectory is set for optimal stimulation (green arrow). **(B)** A 3-dimensional reconstruction of the subject's high-resolution anatomical scan allows for visualization of the optimal coil placement (green coil) for iTBS.

in each patient (Heiss and Thiel, 2006). For example, one site that may be optimal in a patient with a small lesion may not be appropriate for another patient with more extensive damage. The extent of transcallosal disinhibition, or propensity of involvement of the perilesional and potentially beneficial contralateral homologs, differs among patients, and therefore, site-finding protocols help to meet individual treatment needs. One limitation in some of the above studies is a lack of sham or a control group. Without a comparison group in which rTMS was not applied, it is difficult to conclude whether the observed beneficial effects were stimulation-specific or whether they were simply a result of increased general arousal or placebo effect. Additionally, the recently conducted fMRI-driven treatment studies have focused on relatively short outcomes. Thus, the long-term therapeutic benefits of this approach are yet to be explored in large, randomized, and sham-controlled clinical trials (e.g., an ongoing NCT01512264).

Kakuda et al. (2011) recently utilized a novel rTMS approach employing two different frequencies of stimulation. They primed 4 chronic patients with motor-dominant aphasia with 6 Hz rTMS for 10 min before applying the standard 1 Hz rTMS for 20 min for 18 sessions in 11 days. An intensive 60-min speech therapy was also provided to all patients after the TMS protocol. Improvement in several language functions was observed; however, the results differed in each patient with most improvements observed in naming and repetition. Based on prior

studies (Iyer et al., 2003; Carey et al., 2010), the authors posited that priming with 6 Hz rTMS would provide "more potent and long-standing suppressive effect" than the more typical 1 Hz rTMS 20 min protocol. However, since the authors did not directly compare effects of a standard approach vs. 6 Hz priming in either this study or a subsequent follow-up study, it is difficult to directly compare their therapeutic effect. Another limitation of this study was lack of long-term follow-up, so persistent effects of this stimulation approach also remain unknown.

While most studies have examined therapeutic effects of rTMS in chronic aphasia, more investigations are beginning to emerge that focus on patients in the sub-acute phase of stroke recovery (Weiduschat et al., 2011; Kindler et al., 2012; Waldowski et al., 2012; Thiel et al., 2013), a period during which spontaneous physiological restoration may still be ongoing. Kindler et al. (2012) assessed the effects of cTBS applied to the right hemisphere Broca's homolog in patients in sub-acute or chronic phases of post-stroke aphasia recovery. They observed that though both patient groups receiving cTBS significantly improved when compared to the sham group, patients in the sub-acute phase were the best responders as tested by timed picture naming accuracy and RT post-cTBS. This finding is crucial as it favorably supports the application of therapeutic rTMS or TBS early on, even sub-acutely. However, yet again, long-term follow-up was not carried out in this study, preventing an assessment of whether this approach had enduring benefits for patients.

Long-lasting effects of inhibitory rTMS have been reported in several studies involving patients with chronic aphasia. For example, Martin et al. (2009) showed that the improvements in picture naming task and phrase length post-rTMS in a chronic patient with non-fluent aphasia lasted for at least 43 months (over 3 and half years). Another study reported the symptomatic benefits post-rTMS lasting up to 12 months (Barwood et al., 2013) as compared to the group that received sham stimulation. Unfortunately, most studies that focused on sub-acute patients have lacked evaluation of long-term benefits. One exception is Waldowski et al. (2012) who reported reduction in aphasia severity 15 weeks post-stimulation in a group of sub-acute patients receiving rTMS as compared to the sham group (Waldowski et al., 2012). However, accuracy in naming improved similarly across both treatment groups, with only a slight benefit in RT in the treatment group. These findings suggest that improvement in some language functions may in fact be non-specific to stimulation. Because there are ongoing physiological neuroplastic changes in the perilesional and homotopic language areas during the acute and subacute phases of post-stroke recovery (Saur et al., 2006), patients are more likely to improve over a course of weeks irrespective of rTMS application. Therefore, more research is necessary to demonstrate long-lasting and stimulation-specific effects of rTMS, especially when applied early after stroke.

Because mechanisms of neuroplasticity may differ as a function of disease duration, it is important that stimulation be delivered in ways that take advantage of and augment the specific neuroplastic changes thought to be at play during the phase of post-stroke recovery in which TMS is being delivered. For example, in some patients who are recruiting the right hemisphere in a compensatory manner in the sub-acute or acute phases of recovery, applying inhibitory rTMS to the right homologs may not be appropriate. In this case, excitatory rTMS of left perilesional language areas (Szaflarski et al., 2011b) may prove more beneficial. Therefore, individual site-finding, driven either by transient rTMS effects or functional neuroimaging, may be the best approach to take while mechanisms of neural recovery remain dynamic. However, this speculation needs to be evaluated in future studies. Future studies of acute and subacute patients should also include multiple long-term follow-ups, in order to better inform the long-term efficacy of these approaches.

TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS)

Recent years have seen a surge of interest in the use of tDCS in order to modulate cognitive function and to improve outcomes in a variety of clinical areas including stroke recovery. Typically tDCS is administered by delivering small electric currents (1–2 mA) to the scalp by a battery-driven device connected to two large (often $5 \times 7\,cm^2$ or $5 \times 5\,cm^2$) saline-soaked surface electrodes (Nitsche and Paulus, 2000). Although current flows through both electrodes, by convention the electrode that is being used to target the brain regions to be stimulated is considered the "active" electrode; the other electrode—termed the "reference" or "return" electrode by convention—is typically placed on the supraorbital region (over the forehead) or at a site off the head. Currents delivered during tDCS are not sufficient to generate action potentials, but are sufficient to incrementally alter neuronal resting membrane potentials. Thus, tDCS is often conceptualized as a neuromodulatory rather than a neurostimulatory technique. Like rTMS, tDCS can alter cortical excitability in predictable ways; anodal tDCS (a-tDCS) is believed to increase cortical excitability and cathodal tDCS (c-tDCS) decreases cortical excitability (Nitsche and Paulus, 2000, 2001).

To date, many studies employing tDCS as a therapy for aphasia have adopted approaches that are broadly consistent with an interhemispheric inhibition model of aphasia recovery (Heiss and Thiel, 2006; Hamilton et al., 2011). That is, most investigations have involved either a-tDCS centered on left hemisphere language areas (Baker et al., 2010; Fiori et al., 2011; Fridriksson et al., 2011; Marangolo et al., 2013) in order to either increase the excitability in the perilesional and residual fronto-temporal language areas, or c-tDCS applied to the right hemisphere homotopic areas (Kang et al., 2011) to inhibit over activation (due to transcollasal disinhibition) in the contralesional right homologs. Most of these studies applied 1–2 mA of current for 10–30 min over 5–10 days in patients with chronic aphasia. Several studies compared the therapeutic effects of a- or c-tDCS to sham treatment in a within-subject and cross-over design and provided concurrent speech and language training.

Outcome measures consisted of aphasia severity scales [WAB, Battery for the Analysis of Aphasic Disorders (BADA)] and language batteries including picture naming tasks such as the BNT. Monti et al. (2008) were first to report transient effects of tDCS in patients with aphasia. In their study 8 patients with chronic aphasia received sham or active tDCS at 2 mA for 10 min over the left frontotemporal area; 4 received a-tDCS and sham, while the other 4 received c-tDCS and sham (Monti et al., 2008). Interestingly, picture naming accuracy improved after 10 min of

c-tDCS, while a-tDCS and sham induced no changes in naming performance. This finding is counterintuitive as it suggests that inhibiting the damaged left hemispheric language network may improve language functions. The authors argued that the mechanism of improvement may be the stimulation-specific inhibition of overactive interneurons in the damaged left hemisphere. However, these findings have not yet been replicated in an intervention study with multiple tDCS sessions and in blinded patients/evaluators.

Naming accuracy also improved in 10 chronic aphasic patients, who received 1 mA a-tDCS on intact left frontal areas for 20 min per day for 5 days (Baker et al., 2010); they also received sham in the same manner, where application of active and sham tDCS was randomized. The active site of stimulation was determined individually by examining fMRI activation patterns during a naming task. In a follow-up study, Fridriksson et al. (2011) applied a similar stimulation and site-finding protocol in 8 chronic patients with fluent aphasia and reported improvement in naming reaction times after a-tDCS as compared to sham. In addition, they reported that these RT benefits persisted at least 3 weeks after stimulation. Both these findings are in line with the notion that perilesional recruitment is necessary for post-stroke aphasia recovery; the beneficial effects of tDCS are presumed to be mediated by enhanced activity of residual left hemisphere language areas as well as compensatory functional changes in left hemisphere perilesional areas.

As noted above, the interhemispheric inhibition model of post-stroke language plasticity posits that activity of right hemisphere structures may interfere with the compensatory recovery of left hemisphere perilesional area. Although this model has underpinned the approach of numerous investigators, Kang et al. (2011) is the only group to date to apply inhibitory right c-tDCS to patients with chronic aphasia with the aim of inhibiting the potentially deleterious right hemisphere activity (Kang et al., 2011). In a cross-over design, Kang et al. demonstrated that picture naming accuracy improved after c-tDCS on right frontal areas compared to sham in 10 chronic patients. In another study, Floel et al. (2011) applied excitatory a-tDCS as well as inhibitory c-tDCS and sham over the right temporo-parietal cortex during anomia training (Floel et al., 2011). The aim was to demonstrate enhancing therapeutic effects of anomia training in context of inhibitory vs. excitatory tDCS of the areas in the right hemisphere. Naming ability improved across all three groups with anomia training, and the effects lasted for at least 2 weeks. Interestingly, a-tDCS, and not c-tDCS, of the right hemispheric exhibited greater and longer-lasting improvement in the naming ability, as compared to sham. This finding suggests that stimulating right homotopic areas may be more reliable in enhancing effects of anomia training than inhibiting them. Beneficial role of right hemispheric areas in language improvement with tDCS is highlighted in this study.

Recently, Marangolo et al. (2013) took a different approach to language training and outcome measures where they assessed whether enhancing activity in the left language areas by a-tDCS can improve informative or pragmatic speech, rather than focusing on improvement on neuropsychological assessments alone (Marangolo et al., 2013). They used different video-clips describing "everyday life situations" for training and testing 12 chronic patients with aphasia who underwent a-tDCS and sham centered on left Broca's and left Wernicke's areas in a cross-over design. The authors reported improved language performance in terms of increased use of content-units and increased verb and sentence production after a-tDCS on Broca's area as compared to Wernicke's and sham tDCS. Additionally, the effects sustained for at least a month after the treatment ended. Sustained improvement in an ecologically valid measure in this study is promising, in that tDCS paired with language training may be able to improve the overall ability of patients with aphasia to communicate in everyday life.

While almost all tDCS intervention studies focused on chronic patients, You et al. (2011) studied effects of tDCS on subacute patients with global aphasia (You et al., 2011). Rather than a cross-over design commonly adopted by other investigators, You et al. included a separate sham control group. Of 21 patients, 7 patients received a-tDCS centered on left STG (Wernicke's), 7 received c-tDCS on right STG and 7 received sham stimulation, for 30 min a day for 10 days. During stimulation (sham and active), patients underwent speech and language therapy. As predicted with stimulation of temporal language areas (regions broadly involved in language comprehension), the authors reported that auditory verbal comprehension improved significantly more in patients receiving right c-tDCS (inhibitory) compared to those receiving left a-tDCS (excitatory) or sham stimulation. In addition, across active and sham groups, improvement was observed in the aphasia severity scale and in spontaneous speech. This finding is similar to (Waldowski et al., 2012) who applied rTMS in subacute patients and observed across group (sham and active) improvements. It appears that these non-stimulation specific improvements may be typical in subacute patients undergoing spontaneous restitution of language functions. Therefore, carefully chosen neuropsychological assessments and inclusion of a sham group are required to establish therapeutic benefits of tDCS in patients who have dynamic rather than static aphasia. Of importance is the finding that inhibition of right temporal language areas, rather than excitatory left stimulation common among other studies involving chronic patients, resulted in improved language functions. These findings suggest that during sub-acute recovery stages, inhibition of right hemisphere activity may be an effective therapeutic approach which is consistent with the trajectory of neuroimaging changes associated with acute, sub-acute, and chronic post-stroke recovery as demonstrated by Saur et al. (2006).

Overall, a review of tDCS intervention studies in post-stroke aphasia reveals that while some studies have made efforts to increase their sample sizes (up to 14 patients) and use ecologically valid outcome measure, several parameters in study design and stimulation methodology could be improved. First, except one study (Marangolo et al., 2013), most current intervention studies did not include a long-term follow-up to address the long-lasting benefits of tDCS in improving language functions in post-stroke aphasia. Secondly, the effects of tDCS in the sub-acute phase of recovery need more consideration. For example, studies comparing the therapeutic effects of a particular electrode montage (c-tDCS vs. a-tDCS on the right or left language areas) in

sub-acute vs. chronic patients are desirable. Based on the effects of disease duration and lesion size and location on the models of neuroplasticity, a mechanistic approach to tDCS electrode montage may be more appropriate. This can be achieved by a multimodal neuroimaging-driven tDCS intervention (Hunter et al., 2013), where the placement of active electrodes in the left or right hemispheres can be based on the fMRI activation patterns. Additionally, a dual-hemispheric tDCS approach i.e., simultaneous right inhibitory and left excitatory tDCS, may also prove beneficial. Evidence of changes in the underlying brain activity as a function of therapeutic tDCS or a particular montage of tDCS has not yet been carried out in patients with aphasia. Improvement in language functions may be directly related to induced neuroplasticity; however, as the current literature stands, there is no evidence of this association in post-stroke aphasia.

As far as tDCS methodologies are concerned, new evidence suggests that c-tDCS does not reliably decrease the underlying cortical excitability. Depending on the duration (>15 min) and intensity of stimulation (>1 mA), c-tDCS may behave more like a-tDCS, in that it increases, rather than decreasing, the cortical excitability (Batsikadze et al., 2013). Specifically, Batsikadze et al. showed that 2 mA c-tDCS, when applied for 20 min on the motor cortex, induced cortical excitation, rather than inhibition, in healthy individuals. While 1 mA c-tDCS for the same duration and site induced cortical inhibition. Interestingly, at least one study (Monti et al., 2008) reported transient beneficial effects of 2 mA 10 min c-tDCS on the lesioned left hemisphere. Speculatively, these effects may have resulted from cortical excitation by c-tDCS of the perilesional or residual language areas, and not cortical inhibition as previously believed. However, effects of c-tDCS in stroke patients, particularly when applied to the lesioned hemisphere, may be different than in healthy individuals. Because of the loss of typical cortical structure, the flow of current in the lesioned hemisphere may be quite diverse. In contrast to Monti et al. (2008), Kang et al. (2011), and You et al. (2011) applied c-tDCS to the unaffected right homologs of the STG and the Broca's area, respectively. Interestingly, this approach also led to improved language functions; c-tDCS applied to the right STG was more beneficial than a-tDCS applied to the left STG and more than sham (You et al., 2011). However, since these studies did not provide a model of current flow (Datta et al., 2009, 2010), nor followed-up with a measure of changes in cortical excitability, it is difficult to infer the mechanism that underlies the observed improvement. It is clear, however, that a multimodal approach to tDCS is required for understanding how different tDCS parameters, especially c-tDCS, remodel the bilateral language networks to enable recovery. Future studies should describe a relationship between tDCS "dose and response," to translate its application as an effective treatment for post-stroke aphasia.

NEUROPLASTICITY INDUCED BY NON-INVASIVE BRAIN STIMULATION

In this section, we will discuss the observed changes in the brain activity induced by NBS. We reviewed only those studies that paired direct measures of neuroplasticity (PET, fMRI, EEG-ERP, etc.) after therapeutic rTMS as no such studies have yet been carried out with tDCS (summary in **Table 1**). Based on neuroimaging accounts of neuroplasticity in spontaneous recovery described earlier, we expect that sparing of key language areas in the left hemisphere and an increased contribution of the left hemispheric residual and perilesional areas after therapeutic rTMS would be associated with improved language functions. However, just as we discussed in the case of spontaneous neuroplasticity, the contributions of right hemisphere homologs to stimulation-driven neuroplasticity is not clear.

In one study, Martin et al. (2009) suppressed right PTr in a chronic non-fluent aphasic patient over 10 daily 1 Hz rTMS sessions to induce long-lasting improvement in naming and propositional speech (Martin et al., 2009). Importantly, at 16 and 43 months after rTMS, fMRI activation patterns revealed a greater recruitment of perilesional (left)/perisylvian areas, specifically the left supplementary motor area and the left and right sensorimotor mouth areas, and areas along the fronto-temporal language network. Activation of the right IFG, observed pre-TMS persisted at 16 months after stimulation. Overall, the authors argued that the increased activation in the bilateral motor and perilesional language areas after repetitive suppression of right PTr revealed a leftward activation shift supported by improvement in language functions. However, the contribution from changes in the right IFG to improved performance after rTMS is unclear. Perhaps, suppressing an inefficient node i.e., the right PTr (Turkeltaub et al., 2011) enabled reorganization of the bilateral functional networks by increasing left hemispheric recruitment as well as refining the beneficial role of right homologs. In contrast, fMRI activation patterns in a second patient, also suffering from chronic non-fluent aphasia, did not show an increased left hemispheric recruitment post-rTMS at 3 or 6 months, and language performance on naming and propositional speech did not improve in this patient (right IFG activation was consistently observed in this patient). In this patient, a larger frontal and temporal lesion and subcortical white matter damage may have been associated with more severe non-fluent aphasia and lack of improvement post-rTMS. The authors argued that since key language regions in the left hemisphere were not spared in this patient, suppression of right PTr by rTMS could not promote recovery. We posit that lesion extent and location may have impeded induced reorganization in the bilateral functional network with rTMS (Heiss and Thiel, 2006).

A different case study by Turkeltaub et al. (2012) shed some light on the role of right hemispheric homotopic areas (Turkeltaub et al., 2012). A right hemispheric stroke in a patient with chronic non-fluent aphasia, who underwent therapeutic rTMS (10 daily 1 Hz rTMS sessions) after an initial left hemispheric stroke, worsened her aphasia symptoms. After her left stroke, repetitive inhibition of right PTr induced improvement in her language functions for up to 2 months. FMRI activation patterns obtained on the first day of rTMS, before and after treatment, indicated that right PTr activity was in fact suppressed but the expected increase in left hemispheric activity was not yet present. However, within 3 months of therapeutic rTMS, she suffered from a second stroke, this time on the right side of the brain, which worsened her language functions. At 3 months after her second stroke, language functions were decreased more than other cognitive functions. This case

provided authors with a unique opportunity to examine the contribution of right homolog damage to the overall language functions after initial dominant hemispheric stroke. If one assumes that recruitment of all homotopic right areas is deleterious to recovery, then in this case the language functions should not have been affected or could have improved. Therefore, consistent with the notion that the "right hemisphere can speak" (Code, 1997) the authors suggest that right hemisphere homotopic areas may support functional recovery.

In contrast to the above studies, Weiduschat et al. (2011) demonstrated that PET activation patterns during a verb-generation task were suppressed in the right hemisphere post-rTMS (compared to pre-TMS), while they were significantly more prominent in the right hemisphere in the sham treatment group (Weiduschat et al., 2011). In this study, 1 Hz rTMS was applied either to Broca's homolog in the right hemisphere (right PTr) followed by speech and language therapy or to control area (vertex); significant improvements in language functions were observed in 6 patients receiving rTMS compared to 4 control patients. Since enrolled patients were in the sub-acute phase of recovery there was a tendency in the control patients to recruit right homologs consistent with the result of the study by Saur et al. (2006). In patients receiving rTMS, right hemispheric involvement was suppressed, presumably by functional inhibition of right PTr, which may have contributed to the observed improvements in this group. Thus, the increased right hemispheric involvement in the absence of right PTr suppression in the control group may represent an inefficient mode of recovery. We posit that inefficient activation of specific sites in the right hemisphere (right PTr) during spontaneous course of recovery may be detrimental to the recruitment of perilesional and residual language areas in favor of recovery. Therefore, suppressing activity in the right PTr may induce neuroplastic changes characterized by release in activation of an inefficient node and thus, promoting recruitment of left hemispheric areas. However, an important caveat in this study is a lack of association between changes in PET activation patterns and language improvements; this may be, in part, related to a small number of subjects enrolled in this study and/or high drop-out rate (4/14). This last shortcoming was recently addressed by Thiel et al. (2013) in a larger group of 24 sub-acute stroke patients with different aphasia types (non-fluent, fluent, global, and amnestic). After 10 sessions of 1 Hz rTMS combined with 45 min of speech and language therapy, 13 patients in the rTMS group showed improvement on the AAT, a comprehensive aphasia severity scale, with largest improvement in naming subtest as compared to the 11 patients in the sham group (Thiel et al., 2013). Relevant to prior findings, a change in bilateral functional activity was observed in this study. Activation volume index, a measure of change in the volume of activation (significant voxels) between left and right hemispheres, revealed increased PET activation in the left hemisphere in the rTMS compared to the sham group; similar right hemispheric activation was observed pre-rTMS between sham and rTMS groups. Importantly, the level of change in activation followed a linear relationship with the change in AAT scores i.e., greater activation shift toward left hemisphere was associated with greater improvement on AAT scores after rTMS. The findings of this study suggest that induced cortical reorganization of language functions to the left hemisphere relate to the improvements after therapeutic rTMS.

Direct measurement of activation changes in the language areas before and after rTMS was also carried out by Szaflarski et al. (2011b) in chronic patients (Szaflarski et al., 2011b). This study differed from other rTMS aphasia intervention studies in 2 important ways: (1) rather than suppressing areas in the right hemisphere, residual left perisylvian areas were stimulated in an excitatory stimulation protocol, and (2) instead of standard continuous delivery of rTMS, iTBS was applied (for more information refer to **Table 1**). Repeated iTBS showed significant improvement in 6 out of 8 patients in a semantic fluency task. Importantly, fMRI activation patterns post-iTBS revealed increased recruitment of perilesional fronto-temporo-parietal areas, as well as a shift in activation toward left frontal and temporal language areas. Recruitment of some right hemispheric subcortical and motor areas was also observed. The findings in this study corroborate with those in other studies using inhibitory rTMS, and suggest that recruitment of perilesional areas as well as reduction in inefficient activation of right homotopic sites may subserve improved language functions.

In addition to functional changes in activation patterns after rTMS, white matter structural integrity may also improve. In a follow-up study, (Allendorfer et al., 2012b) examined whether iTBS administered in the earlier study could also potentially improve structural white matter integrity, specifically in the areas that showed greater fMRI activation with iTBS (Szaflarski et al., 2011b; Allendorfer et al., 2012b). They used DTI, and compared fractional anisotropy (FA) changes pre- vs. post-iTBS in the same group of chronic patients as in (Szaflarski et al., 2011b) DTI-FA provides a measure of white matter integrity and directionality by indexing restricted diffusion of water molecules in different tissue types (Basser and Pierpaoli, 1996; Pierpaoli et al., 1996; Bennett et al., 2010; Allendorfer et al., 2012b); higher FA values correspond to greater white matter integrity. Increases in FA values were observed post-iTBS compared to pre-iTBS in left hemispheric areas close to the stimulation site and also near the regions that showed greater fMRI activation in the earlier study. Specifically, measurable increases in FA were observed in the left IFG, anterior cingulate, insula and right temporal and parietal areas, along with bilateral increases in the posterior cingulate. However, improvement on language performance (semantic fluency task) did not correlate with changes in FA probably because of the relatively low sample size ($N = 8$). Nonetheless, the observed changes in the white matter integrity in the left perilesional areas as well as in some right hemisphere areas present a similar pattern to changes in functional activation. Future studies with a sham-controlled arm in a larger group of patients will validate or disprove these findings (NCT01512264).

Additionally, electrophysiologic changes after therapeutic rTMS have also been reported. Event-related potentials (ERP) derived from EEG can also characterize induced neuroplasticity in patients with aphasia as demonstrated by changes related to speech-language therapies (Pulvermuller et al., 2005; Laganaro et al., 2008; Barwood et al., 2011b) and pharmacological treatments (Szelies et al., 2001). Recently, Barwood et al. (2011a,b) examined the effects of 1 Hz rTMS on right PTr in 12 patients with

chronic non-fluent or global aphasia (Barwood et al., 2011a,b,c). At 2 months after stimulation, 6 patients who received rTMS for 20 min/day for 10 days improved significantly more than 6 patients who received sham treatment of same duration; specifically naming, expressive language, and auditory comprehension improved. Further, in a follow-up study, Barwood et al. (2011b) reported stimulation-specific effects on N400, an ERP component time-locked to semantic language processing. Specifically, at 2 months the overall mean amplitude, peak amplitude, and areas under the curve of the N400 component were significantly higher in the treated group than in the sham group; higher amplitudes reflect improvement in the language function, in this case on a semantic-lexical task. Interestingly, in this group of patients, transient changes (increases) on N400 parameters were not found, meaning ERP amplitudes at baseline vs. 1 week after rTMS were not different. The authors speculated that rTMS-specific modulation in the bilateral language network supported by increase in N400 component post-rTMS may be crucial for the improvements observed over time. Additionally, the findings suggest that rTMS-induced neuroplasticity may be time-dependent such that reorganization in the bilateral language network may require protracted time to materialize.

This observation of time-dependency of induced neuroplasticity after therapeutic rTMS reveals one recurring limitation of studies reviewed in this section. Most of these studies lacked a long-term follow-up i.e., these studies reported changes in activation patterns acutely after rTMS, but unlike Martin et al. (2009) and Barwood et al. (2011b), failed to report sustained changes in activation patterns a few months or years after treatment. This information is critical as it will inform us about the direct effects of rTMS on long-term neuroplasticity.

SAFETY OF NON-INVASIVE BRAIN STIMULATION

The most serious, albeit unlikely, health risk associated with TMS is induction of a seizure (Homberg and Netz, 1989; Kandler, 1990). In the years since induced seizures were first observed in association with TMS, rigorous safety guidelines have been developed which specify the number of pulses that may safely be given as a function of stimulus intensity (% of Motor Evoked Potential), frequency, and inter-train interval (Wasserman, 1998; Bolognini et al., 2009; Rossi et al., 2009). Numerous subsequent studies of rTMS have demonstrated that stimulation within these parameters is safe in normal persons, patients with stroke (Hao et al., 2013), and even epilepsy patients (Bae et al., 2007). Administration of TBS within published parameters has been well tolerated in healthy adult studies; only one study reported a seizure in a healthy subject caused by TBS used with intensity set at 100% of the RMT (Oberman and Pascual-Leone, 2009), greater than the 80% of active threshold typically used in recent studies (Huang et al., 2005; Szaflarski et al., 2011b). Thus, there has been no convincing evidence that rTMS performed within established guidelines or TBS performed using published parameters can cause short- or long-term seizures/epilepsy or other ill effects.

To date, there have been no reports of seizures or other short- or long-term severe adverse events related to the use of tDCS. Several recent studies have reported mild side effects of tDCS in both healthy individuals (Brunoni et al., 2011; Kessler et al., 2012) and patient populations (Poreisz et al., 2007) including itching, tingling, burning, pain, and headaches, which were not long-lasting.

FUTURE DIRECTIONS

Neurorehabilitation of post-stroke aphasia with the use NBS shows a lot of promise. Throughout this review, we have highlighted several advantages as well as limitations of current NBS methodologies and study design in an attempt to advance its use as an effective tool for the treatment of post-stroke aphasia. One overarching goal of future studies should be to capture therapeutic benefits of NBS not only on neuropsychological language batteries but also on everyday communication abilities.

Underlying recovery mechanisms and neuroplasticity with NBS in post-stroke aphasia still remain an open question. Recovering language networks are dynamic depending on multiple factors including the location of the lesion and its size, time since injury, intensity and type of provided intervention, age at the time of injury and handedness. There is an agreement in the field about the beneficial role of left hemispheric perilesional and residual language areas in both spontaneous as well induced with NBS recovery. However, the debate on the role of right hemispheric homotopic areas continues. Several investigators concur that recruitment of areas in the right hemisphere is an inefficient mode of recovery in patients with aphasia while some argue that rather than all areas, recruitment of some specific site(s) in the right hemisphere may be inefficient or deleterious to recovery. In future studies, we recommend parsing out specific functions of right hemispheric homotopic areas during spontaneous recovery, and persisting activity in some right hemispheric areas in recovery induced with NBS. Future studies should closely address the individual determinants of patterns of neuroplastic changes both to guide NBS treatment and to assess functional recovery as well as the role of neuronavigation with TMS, fMRI, PET or other techniques. In particular, future multimodal approaches pairing neuroimaging and electrophysiological measures with therapeutic NBS will more clearly define its potential in aiding rehabilitation after an aphasia-producing stroke.

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Finding an optimal rehabilitation paradigm after stroke: enhancing fiber growth and training of the brain at the right moment

Anna-Sophia Wahl [1,2] **** and Martin E. Schwab*** [1,2]

[1] *Brain Research Institute, University of Zurich, Zurich, Switzerland*
[2] *Department of Health, Sciences and Technology, ETH Zurich, Zurich, Switzerland*

Edited by:
Edward Taub, University of Alabama at Birmingham, USA

Reviewed by:
Sara L. Gonzalez Andino, Hôpitaux Universitaires de Genève (HUG), Switzerland
Victor W. Mark, University of Alabama at Birmingham, USA

****Correspondence:***
Anna-Sophia Wahl, Brain Research Institute, University of Zurich; Department of Health, Sciences and Technology, ETH Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
e-mail: wahl@hifo.uzh.ch

After stroke the central nervous system reveals a spectrum of intrinsic capacities to react as a highly dynamic system which can change the properties of its circuits, form new contacts, erase others, and remap related cortical and spinal cord regions. This plasticity can lead to a surprising degree of spontaneous recovery. It includes the activation of neuronal molecular mechanisms of growth and of extrinsic growth promoting factors and guidance signals in the tissue. Rehabilitative training and pharmacological interventions may modify and boost these neuronal processes, but almost nothing is known on the optimal timing of the different processes and therapeutic interventions and on their detailed interactions. Finding optimal rehabilitation paradigms requires an optimal orchestration of the internal processes of re-organization and the therapeutic interventions in accordance with defined plastic time windows. In this review we summarize the mechanisms of spontaneous plasticity after stroke and experimental interventions to enhance growth and plasticity, with an emphasis on anti-Nogo-A immunotherapy. We highlight critical time windows of growth and of rehabilitative training and consider different approaches of combinatorial rehabilitative schedules. Finally, we discuss potential future strategies for designing repair and rehabilitation paradigms by introducing a "3 step model": determination of the metabolic and plastic status of the brain, pharmacological enhancement of its plastic mechanisms, and stabilization of newly formed functional connections by rehabilitative training.

Keywords: stroke, rehabilitation, Nogo-A, critical time window, plasticity, training

INTRODUCTION

The human brain works wonders to fulfill the requirements of every-day life. These unique capacities are then fully esteemed when all of a sudden even simple activities fail or become a problem: cerebral strokes leave the victims with often large psychical and physical impairments—from vision problems to aphasia and motor deficits—leading to the number one cause of adult disability worldwide with great impact on public health. In the acute phase, "time is brain"—ruptured blood vessels (hemorrhagic stroke) or aggregates of platelets and blood cells that clog cerebral blood vessels (ischemic stroke) cause acute shortage of glucose and oxygen resulting in metabolic distress and long-term neuronal cell loss. The destruction process is complex and can only be dampened in the case of the ischemic stroke by very early intervention (within 4–6 h) with thrombolysis, (Hacke et al., 2008). Currently, only about 10% of all stroke patients reach a hospital early enough or fulfill the criteria for being able to receive thrombolysis in the therapeutic time window. Prognosis and recovery then depend on the location and extent of the stroke lesion. Clinically, the most successful therapy to further enhance this recovery of function is rehabilitative training. Rehabilitation as a term "to reach and maintain optimal functioning in physical, intellectual, psychological and/or social domains" (WHO. International classification of functioning disability Health ICF. Geneva: WHO; 2001) is evidence based medicine and does not exclude a specific subgroup of patients.

Nevertheless, for many rehabilitative interventions, in particular those for long-term or chronic rehabilitation, robust data or adequately controlled studies are lacking (Quinn et al., 2009): e.g., comparisons between different training methods in current use could not show that any particular physiotherapy or stroke rehabilitation strategy is superior to another (Johansson, 2000).

Consequently optimal rehabilitation strategies can only be defined if we understand the way in which training and the rehabilitation protocol influences the neurobiology of the central nervous system with priority on the aspects of timing, kind and intensity of rehabilitative training. Measurable endpoint criteria for rehabilitative outcome are required in order to achieve two purposes: the adjustment of the ideal rehabilitative strategy to the individual patient, and the choice of the optimal therapy protocol.

In this review we focus on mechanisms of spontaneous recovery after stroke, on rehabilitative designs to enhance plasticity, on growth promoting mechanisms with an emphasis on anti-Nogo-A immunotherapy, and on the time windows of rehabilitative training and pharmacological interventions and the combination of both.

MECHANISMS OF SPONTANEOUS RECOVERY AFTER STROKE—FROM HUMAN PATIENTS TO ANIMAL MODELS

For many years people have thought that the hardware of the brain is that "hard", that once an incident such as stroke happens, brain areas and functions are lost forever. The old paradigm of the adult CNS as a stable and static structure, consisting of billions of nerve cells and circuits, has now been replaced by a much more dynamic view of the CNS which includes processes of growth, connectivity changes and areal remodeling that can occur after CNS injury or stroke and plays an important role in recovery and functional repair.

Spontaneous recovery is seen in stroke patients weeks to months after the incident. However, due to variability across subjects and across neurological domains efforts of summarizing this process with precision have been frustrating. Among the most obvious factors that contribute to the extent of spontaneous recovery are infarct size, infarct location, age and pre-stroke disability (Cramer, 2008). Most spontaneous recovery tends to occur within the first 3 months. While patients with milder deficits achieve spontaneous recovery more quickly than patients with more severe deficits, the pattern of spontaneous recovery can also differ within the same patient for different functions (Cramer, 2008).

SPONTANEOUS RECOVERY OF SENSORIMOTOR FUNCTION IN HUMANS

Motor recovery has been among the most often examined because motor impairments belong to the symptoms that are most frequently and precisely diagnosed after stroke (Gresham et al., 1998; Rathore et al., 2002; Langhorne et al., 2009). Motor impairment can be regarded as a loss or limitation of function in muscle control or movement or a limitation in mobility. It is a focus of physiotherapy or occupational therapy in terms of stroke rehabilitation (Langhorne et al., 2009). The natural history of motor recovery is considerably heterogeneous: the first voluntary movements can be seen anywhere from 6 to 33 days after a hemiplegic stroke (Twitchell, 1951). The largest improvement occurs in the first 30 days after stroke, though significant progress is still found in patients with more severe deficits up to 90 days after stroke (Wade, 1983; Duncan et al., 1992, 1994, 2005). Studies on arm disability revealed that a maximum of function is reached by 80% of the patients within 3 weeks and by 95% of patients within 9 weeks (Nakayama et al., 1994). Still significant long-term improvement is found if arm function starts to ameliorate 16 weeks after stroke onset (Broeks et al., 1999).

Insights into the underlying remodeling and re-organization processes for functional recovery in the brain after stroke can be obtained in human patients via functional neuroimaging methods and brain mapping. These data suggest that recovery of motor function after stroke leads to brain-wide modifications in neuronal activity patterns and connectivity (Rehme and Grefkes, 2013). While initially tissue function and neurophysiological responses are diminished within the injured primary neocortex, cortical function increases over time (Marshall et al., 2000; Calautti et al., 2001; Feydy et al., 2002; Grefkes and Fink, 2011). In terms of good functional outcome one of the major correlates is the degree of recovery of neurophysiological activity in the affected primary cortical areas (Cramer, 2008). In other terms: the best behavioral outcomes are associated with the greatest restoration/remodeling of brain function towards the normal state of organization (Ward et al., 2003; Zemke et al., 2003; Ward, 2004; Murphy and Corbett, 2009). This is true even if the post-stroke behavior is far from being identical to the pre-stroke motor kinematics. In particular the extent of corticospinal tract integrity is positively correlated to functional recovery as revealed by transcranial stimulation of the motor cortex (M1) and its efferents after stroke (Talelli et al., 2006). In general, if an ischemic event occurs, those areas are recruited for structural and functional modification which are either close or functionally related and connected or both. Therefore, after a small stroke, peri-infarct tissue is mainly involved that has similar function. By contrast, after a large stroke, tissue that has similar functions might be only found at more distant sites or in unaffected regions of the contralateral hemisphere, where still enough capacity for structural remodeling remains (Murphy and Corbett, 2009).

THE ROLE OF THE PREMOTOR AND CONTRALESIONAL MOTOR CORTEX

Which areas are activated and what they contribute in terms of beneficial re-organization for functional recovery is still under debate: a meta-analysis revealed that activation of premotor areas and the contralesional primary M1 are consistent findings (Rehme et al., 2012; Rehme and Grefkes, 2013). Interactions between premotor areas and the lesioned primary M1 are directly related to recovery and functional outcome. For example, Johansen-Berg et al. (2002) showed that disruption of dorsal premotor cortex activity by transcranial magnetic stimulation (TMS) over both the ipsi- and contralateral hemisphere lead to a deterioration of performance in stroke patients, but not in healthy controls (Johansen-Berg et al., 2002). The exact role of the activation of contralesional M1 is a subject to controversy: longitudinal functional MRI studies revealed enhanced neuronal activity in motor-related areas in both hemispheres after a large stroke. But then during the first 12 months post-stroke this activity returns to unilateral levels similar to those of healthy controls for those patients with good motor recovery (Ward et al., 2003). Remaining increased activity in the contralesional M1 was often associated with poor outcome. Further studies have demonstrated that inhibition of contralesional M1 activity using repetitive TMS may lead to ameliorated motor performance of the stroke-affected hand in the subacute and chronic phase (Nowak et al., 2008; Takeuchi et al., 2012). In contrast, Rehme et al. (2011) found that increases in contralesional M1 activity over the first 10 days after stroke correlate with the amount of spontaneous motor improvement in initially more impaired patients. These data suggest a supportive role for functional recovery in the early phase after stroke for the contralesional M1. In addition, disrupting contralesional M1 activity with TMS resulted in a deterioration of motor-performance of

the stroke-affected hand of stroke patients with capsula interna infarcts (Lotze et al., 2006). A clear time-, size or lesion-location- dependent influence of the contralesional M1, be it either beneficial or harmful for functional recovery, remains to be demonstrated.

CHANGES IN CORTICAL EXCITABILITY, LATERALIZED ACTIVATION AND SOMATOTOPIC RE-MAPPING

For the above described remodeling and recruitment of areas three main forms of reorganization have been described: (1) increased cortical excitability in cortical regions distant from, but connected to the stroke core; (2) reduced lateralized activation; and (3) somatotopic modifications within intact cortical regions.

Increased activity, as a first form of reaction to stroke in areas which before stroke formed a distributed network, has been described many times (Brion et al., 1989; Chollet et al., 1991). This phenomenon occurs in several cortical areas which include motor, language, attention and visual functions (Cramer, 2008). Widespread areas of cortical hyperactivity appear days after stroke and diminish within months post incident (Ward, 2004). This form of modification in cortical excitability is thought to be a result of the down-regulation of the $\alpha 1$ γ-amino butyric acid receptor subunit and a decrease in γ-amino butyric acidergic inhibition (Neumann-Haefelin et al., 1998).

The second form of reaction to stroke—reduced lateralized activation—reflects the increased activity in the contralesional hemisphere, which reduces the extent of interhemispheric balance as demonstrated in many stroke studies (Weiller et al., 1993; Seitz et al., 1998). Reduced lateralized activation is a common brain response not only seen in stroke but also in other neurological contexts such as epilepsy, traumatic brain injury and multiple sclerosis (Cramer, 2008). The exact function of this reduced laterality remains to be elucidated: it may be just a subtype of the described increased activity as described in the first form or a passive event reflecting a reduced interhemispheric inhibition resulting from the stroke. Another interpretation is that the contralesional hemisphere has to take over functions that were previously based in the ipsilesional hemisphere.

Both phenomena, increased cortical excitability and reduced laterality, are related to spontaneous functional recovery (Cramer, 2008). Both are time dependent, increasing in the early weeks after stroke and decreasing over months thereafter. This decrease is greater among stroke patients with stronger functional recovery while the persistent increased activity over both hemispheres is greatest in those patients with the poorest outcome (Ward et al., 2003; Cramer and Crafton, 2006). A relation to increased susceptibility for seizures and phantom pain is possible.

The third response to ischemic injury—somatotopic reorganization—implies that intact cortical regions—in particular within the perinfarct area—reassign their functions which they subserved before stroke and take over function, which have been affected or lost by the ischemic event. Some studies suggest that the largest degree of somatotopic reorganization is associated with very large stroke injuries (Cramer and Crafton, 2006). Such map shifts occur in primary and secondary cortical areas (Byrnes et al., 2001).

ANIMAL MODELS TO STUDY STROKE INDUCED CORTICAL RE-ORGANIZATION ON THE ANATOMICAL AND MOLECULAR LEVEL

As studies in stroke patients have limitations, animal models of stroke have been used to describe remodeling and reorganization processes on the macro and molecular level. Although spontaneous recovery in animals tends to occur earlier (depending on stroke size), imaging and mapping data show a number of analogues between recovery in animals and in humans: connectivity changes between sensorimotor cortex and deep grey matter structures after middle cerebral artery occlusion (MCAO) in rats were comparable to results in human stroke patients (van der Zijden et al., 2007). fMRI studies concentrating on the affected upper limb in rats have described a shift in laterality of activation after stroke such that early after stroke, brain activation during affected paw stimulation is mainly in the contralesional cortex, later after stroke activity shifts toward the normal pattern, that is the ipsilesional cortex (Dijkhuizen et al., 2001, 2003). Hsu and Jones (2006) found that the larger the ischemic insult the stronger the activity in the contralesional M1. In accordance with human studies van Meer et al. (2012) could show that functional recovery after MCAO in rats was correlated with the extent of preservation or restoration of the ipsilesional corticospinal tract in combination with reinstatement of interhemispheric neuronal signal synchronization and normalization of focal network organization.

New mapping methods allow describing somatotopic map shifts in animals in greater detail: a recent study using light based motor mapping in transgenic mice expressing light-sensitive channelrhodopsin-2 before and after focal ischemic lesions of the forelimb sensorimotor areas revealed decreased motor output in the infarcted area and spatial displacement of sensory and motor maps (Harrison et al., 2013). While strokes in sensory cortex caused the sensory map to move into the M1, a stoke in the M1 lead to a compensatory increase in peri-infarct cortical motor output, but did not affect the position or excitability of the sensory maps. *In vivo* 2-photon calcium or voltage sensitive dye imaging furthermore opens up new possibilities to study the reorganization of complex neuronal networks and their functional relevance for stroke recovery (Winship and Murphy, 2008; Stetter et al., 2013). Anatomically, different studies have demonstrated that map-shifts and re-mapping can be accompanied by axonal sprouting (Carmichael, 2003), and dendritic spine turnover (Brown et al., 2008, 2009, 2010). Using different tracing techniques, Starkey et al. (2012b) could show which neurons take over when functional map shifts occur: if the forelimb M1 in rats was destroyed, neurons in the hindlimb area took over to enable functional recovery of the forelimbs. This functional shift was based on sprouting of new axon branches from hindlimb corticospinal fibers into the cervical spinal cord, followed by retraction of the original lumbar projecting axon and thus a conversion of a hindlimb into a forelimb projecting neuron.

Animal studies have also provided first insights on underlying molecular changes. A unilateral infarct is associated with a number of growth related processes, in some cases bilaterally. These events include the induction of inflammatory markers, grow-promoting and inhibiting genes, cell-cycle regulatory genes and genes involved in synaptogenesis, dendritic branching and

neuronal sprouting as reviewed elsewhere (Li and Carmichael, 2006; Popa-Wagner et al., 2007).

Three major phases of stroke reaction and repair are often distinguished (Cramer and Crafton, 2006): the first epoch is the acute reaction to the injury and takes place in the initial hours when modifications become apparent in blood flow, edema, metabolism and inflammation. A second epoch is related to repair, starts in the first days post stroke and is on-going for several weeks. During this epoch spontaneous recovery is seen and endogenous repair related events reach their peak levels. The third epoch begins weeks to months after stroke when spontaneous recovery has reached a plateau and represents a stable but still modifiable chronic phase.

On the molecular level stroke induces neuronal growth-promoting genes in sequential waves post insult to initiate axonal sprouting in the peri-infarct cortex, as initially shown in a rat somato-sensory cortex (barrel field) infarct model (Carmichael et al., 2005): in the early phase immediate early genes and growth related mRNAs such as SPRR1 are induced 3–7 days after stroke. Typical growth cone constituents such as GAP43, CAP23 and MARCKS as well as the transcription factor c-Jun are expressed from day 3 onward. Subsequently, the cell adhesion molecule L1, cyclin-dependent kinase inhibitor p21 and embryonic tubulin isoform alpha1 tubulin are induced, followed by the expression of cytoskeletal reorganization genes such as SCG10 and SCLIP. This pattern of growth gene expression described is unique for axonal sprouting as a stroke response compared to expression profiles in neuronal development, peripheral or other CNS injuries (Li et al., 2010). Furthermore, in an early response to stroke (Mattson, 2008; Carmichael, 2012), several neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin 3 (NT-3) as well as fibroblast growth factor (FGF)-2 and insulin-like growth factor (IGF-1), epidermal growth factor (EGF) and glial cell line-derived neurotrophic factor (GDNF) are up-regulated. Each neurotrophic factor species shows a different temporal and cellular distribution pattern (Abe, 2000): while GDNF is mainly expressed by neurons, CNTF induction was predominantly observed in astroglia of the marginal region and VEDF gene expression was found in both non-neuronal and neuronal cell types after stroke.

Axonal sprouting not only requires the induction of growth-promoting programs within perinfarct neurons, but also a reduction in the growth inhibitory environment (Carmichael, 2006): axonal growth inhibition in the adult CNS is mediated through three general classes of proteins: myelin associated proteins (Nogo-A, myelin-associated glycoprotein, oligodendrocyte myelin glycoprotein), extracellular matrix proteins (e.g., chondroitin sulfate proteoglycans) and repulsive cues for growth cones known mainly from development (e.g., ephrins, semaphorins). Interestingly, messenger RNAs for the chondroitine sulfate proteoglycans aggrecan, phosphacan and versican were found to be induced later after stroke than the early and middle phase of the growth-promoting gene expression. A small number of growth inhibitory proteins including Nogo-A (Jiang et al., 2009), ephrin A5, semaphoring IIIa and neuropilin 1 are induced in the early phase, however, but down-regulation of Nogo receptor components were also seen (Li et al., 2010).

Not only a temporal expression pattern of growth promoting and inhibiting genes can be detected, but also the spatial distribution plays a role to induce the brain's self-repair processes at the right location: axonal sprouting e.g., in the peri-infarct cortex takes place in a distinct environment close to but larger than the glial scar. Thus, within the glial scar representing the wall that separates the stroke core from the surviving per-infarct tissue both, growth-promoting and growth inhibiting factors are induced while the growth-permissive and peri-infact cortex shows a reduction of the levels of growth inhibiting molecules such as chondroitin sulfate proteoglycans. In contrast, neurotrophins such as BDNF are highly up-regulated in the growth-permissive penumbra und repressed in the stroke core (Lanfranconi et al., 2011).

Taken together, the data on the time and space dependent processes of intrinsic repair mechanisms after stroke suggest a critical period or time window, in which the CNS recruits factors for plasticity that enhance functional recovery. One of the most crucial questions that has to be addressed from a clinical perspective is whether this period characterized by map shifts, fiber growth and major functional and structural changes is also the time window in which rehabilitative interventions should be initiated. We now give an overview on rehabilitative and repair strategies with an emphasis on timing, kind and intensity.

STRATEGIES TO ENHANCE PLASTICITY AFTER STROKE

GROWTH AND PLASTICITY ENHANCING TREATMENTS

Since the discovery of nerve growth factors and factors that prevent neuronal outgrowth and survival, it became a goal in experimental animal studies to apply or induce growth-promoting factors and inhibit the inhibiting ones. Several preclinical studies have examined various growth factors, hormones and cytokines with the aim to enhance motor rehabilitation—including prominent candidates such as NGF, glia (GDNF) and BDNF, IGF, erythropoietin and the granulocyte colony-stimulating factor. All have met with variable levels of success in animal models; some initial clinical studies have started (The BDNF study group (Phase III), 1999; Nagahara and Tuszynski, 2011).

In adult rats with large strokes, the administration of BDNF resulted in improved recovery rates (Schäbitz et al., 2004), while the beneficial effect of rehabilitation on the improvement of forelimb function was prevented in animals treated with a BDNF antisense oligonucleotide (Ploughman et al., 2009). The translation of these results into clinical trials remains challenging and is a matter of safety concerns: in the case of BDNF applied as a neuro-protective agent after stroke, the administration of very large quantities would be necessary as well as repeated dosing to overcome the limited amount of protein that reaches the CNS, even with transient disruption of the blood-brain barrier after stroke. The adverse effects of these high dosages have not been extensively studied in animal models (Nagahara and Tuszynski, 2011). Furthermore, the largest clinical trial of erythropoietin therapy revealed that, compared with placebo, erythropoietin administration was associated with an increased risk of mortality in patients with acute stroke (Ehrenreich et al., 2009).

Other experimental approaches to enhance the intrinsic regeneration ability of CNS axons include injecting cAMP analogs to

influence intracellular signaling pathways (Hannila and Filbin, 2008), knock down of the protein synthesis inhibitor PTEN (Liu et al., 2010) or blocking the small GTPase RhoA (Ellezam et al., 2002).

Promising results have also been gained if inhibition of neuronal plasticity and outgrowth was decreased either by: (1) digesting growth restricting ECM proteoglycans with enzymes such as chondroitinase ABC; (2) by blocking the growth inhibitory protein Nogo-A; or (3) by grafting growth permissive cells.

The bacterial enzyme chondroitinase ABC digests the glycosaminoglycan chains of the chondroitin sulfate proteoglycans (CSPGs) which are part of the extracellular matrix and usually up-regulated in astrocyctes and oligodendrocytes after CNS injury (García-Alías and Fawcett, 2012). Chondroitinase ABC treatment reduces scar formation and enhances axonal regeneration and sprouting as first shown in several studies after experimental spinal cord injury (Moon et al., 2001; Bradbury et al., 2002; Huang et al., 2006). After stroke, chondroitinase ABC administration promoted functional recovery (Hill et al., 2012; Starkey et al., 2012a). Furthermore, Soleman et al. (2012) could demonstrate that delayed chondroitinase ABC microinjections into the cervical spinal cord induce localized plasticity of the forelimb sensorimotor spinal circuitry without effects on the cortical peri-infarct region.

Inhibition of Nogo-A signaling in animal models of stroke

The well-studied protein Nogo-A, a transmembrane protein of about 1200 amino acids including a C-terminal 200 amino acid reticulon (RTN) domain, is involved in several cellular and molecular events contributing to the failure of CNS axons to sprout and reconnect after CNS injury. Function-blocking antibodies against Nogo-A, Nogo receptor (NgR1)-blocking peptides, antibodies against the Nogo receptor subunit Lingo-1, or pharmacological blockade of the signal transducer RhoA and ROCK have been administered in various laboratories in different stroke and spinal cord injury models in rodents and primates (Pernet and Schwab, 2012 for review). Enhancement of behavioral recovery in a variety of sensory-motor tasks as well as anatomical evidence of fiber growth, increased plasticity and re-organization within the cortex, brain stem and spinal cord have been reported (Zörner and Schwab, 2010 for review). Despite different approaches to interrupt Nogo-A signaling, a high degree of similarity in terms of functional recovery and hardware changes in the CNS was found among research groups and injury models. Acute intrathecal anti-Nogo-A antibody infusion over 2 weeks after stroke, with an application starting early after incident (Wiessner et al., 2003; Tsai et al., 2007), or delayed application starting 9 weeks after stroke in adult rats (Tsai et al., 2011) significantly improved forelimb function and was correlated with a significant increase of midline crossing corticospinal fibers originating in the unlesioned sensorimotor cortex. Robust sprouting of new projections from contralesional brain regions into subcortical structures as well as functional reorganization of contralateral sensorimotor areas were reported after anti-Nogo-A immunotherapy in rats (Markus et al., 2005; Cheatwood et al., 2008). Those newly sprouting cortico-efferent axons terminated in the red nucleus, pontine nuclei and spinal cord. A similar effect was found by down-regulation of the Nogo receptor NgR using adenovirus-mediated RNA interference (Wang et al., 2010) or NgR or Nogo-A/B knockout mice (Lee et al., 2004). Anti-Nogo-A immunotherapy was also associated with increases in dendritic length, complexity, and spine density, both in the lesioned and contralesional hemisphere (Papadopoulos et al., 2006). Functional MR-imaging 8 weeks after unilateral MCAO revealed adaptations in the somatosensory system of rats in the anti-Nogo-A antibody treatment group (Markus et al., 2005). Nevertheless anti-Nogo-A immunotherapy is not neuroprotective in the sense that it would reduce stroke lesion size as reported for anti-MAG immunotherapy (Irving et al., 2005). This opens the therapeutic window for anti-Nogo-A immunotherapy in the subacute and even chronic phase.

The described *in vivo* experiments represent essential preclinical tests to validate the efficiency and safety of intrathecal Nogo-A antibody administration. Three different anti-Nogo-A antibodies (IN-1, 11C7, 7B12) have proved efficient in enhancing axonal regeneration and outgrowth both *in vitro* and *in vivo*. In collaboration with Novartis Pharma, a human anti-human Nogo-A antibody has been developed and tested in extensive toxicological studies with intrathecal antibody application in rodents and primates. In a Phase I clinical trial[1] with 52 acutely injured para- and tetraplegic patients in Europe (European Multicenter Study about Spinal Cord Injury, EMSCI[2]) and Canada pharmacokinetics, safety, tolerance and dosing of intrathecal delivery of the antibody were investigated. The tolerance has been excellent without any adverse effects ascribed to the anti-Nogo-A antibody (Abel et al., 2011). A placebo-controlled Phase II clinical trial is currently in preparation. Anti-Nogo antibodies are also in clinical trials or in preparation for clinical trials for other neurological indications such as multiple sclerosis and amytrophic lateral sclerosis (ALS). For ALS GlaxoSmithKline (GSK) has also developed a humanized anti-Nogo-A antibody (GSK1223249). In a Phase I clinical trial, the intravenous injections of GSK1223249 were well tolerated by the 76 patients enrolled in the study (Pradat et al., 2011).

Several additional molecules restricting axonal growth *in vitro* have been identified including ephrins, netrins, semaphorins and oligodendrocyte myelin glycoprotein (OMgp; Schwab, 1990, 2010; Schwab et al., 1993). Their role *in vivo* after stroke has to be evaluated. How much growth and plasticity of the adult, stroke-injured CNS can be enhanced by single or combined manipulations of growth promoting or inhibitory mechanisms, and if there is a danger of chaotic growth and formation of wrong connections is currently unknown.

Finally, grafting growth permissive cells, such as bone-marrow mesenchymal cells, cord blood cells, fetal cells and embryonic cells as a form of restorative therapy have been studied in animals (Chopp and Li, 2002). E.g., cultivated bone-marrow stromal cells from donor rats were stereotactically implanted into the peri-infarct area in rats resulting in significant recovery of somatosensory behavior. In a first small study, 5/30 stroke patients who

[1]http://clinicaltrials.gov/ct2/show/NCT00406016
[2]www.emsci.org

received autologous bone-marrow mesenchymal cell transplantation showed beneficial effects in clinical stroke scores (Bang et al., 2005). Such cell-based therapies could influence endogenous neurogenesis, axonal sprouting and synaptogenesis in ischemic brain tissue (Zhang and Chopp, 2009), although their effects may be primarily immune-modulatory or neurotrophic. More detailed and systematic studies are certainly needed.

REHABILITATIVE TRAINING IN CLINICAL AND EXPERIMENTAL STUDIES

The brain, including the motor system, learns by repetition and training. Many basic mechanisms, however, are still poorly understood, and rehabilitative training is largely evidence-based medicine (European Stroke Organisation (ESO) Executive Committee; ESO Writing Committee, 2008). Nevertheless there are no generally accepted guidelines and no definite recommendations concerning the timing, kind and intensity of rehabilitative training. Clear end point data and randomized controlled clinical trials are often lacking. Furthermore, stroke recovery is a complex process that probably occurs through a combination of restoration, substitution and compensation of functions. For this reason it has been also difficult to translate results from rehabilitative studies in animals to recommendations for rehabilitative schedules in human stroke patients. A majority of clinical studies has been conducted in chronic stroke patients (> 6 months after the stroke) as recruitment of these patients was easier and baseline performance had stabilized (Krakauer et al., 2012). These circumstances lead to functional outcome measurements probably gained largely from compensatory techniques to improve skills for daily living. In contrast, animal studies had a stronger focus on enhancing impairment with more or less detailed analysis how much of the functional recovery was restoration of baseline (motor) function or compensation. Furthermore, the time courses of motor recovery differ among animal and human studies: While recovery in rodent models reaches its maximum around 4 weeks after stroke, human stroke survivors complete most of their recovery within 3 months (Dimyan and Cohen, 2011; Krakauer et al., 2012).

Early vs. delayed training

A consensus exists that the effects of early training, whereby "early" should be starting at 1–2 weeks in animals, not earlier (see below), exceed effects of delayed training in terms of functional recovery in both, animals and humans (Nudo, 2006; Murphy and Corbett, 2009; Langhorne et al., 2010; Krakauer et al., 2012). In animal studies, behavioral training after ischemic injury is most effective for restoring behavioral performance, peri-infarct neurophysiological maps and enhanced neuroanatomical changes in the ipsi- and contralesional hemisphere when introduced within the first week of injury (Nudo, 2006). In a rat MCAO stroke model it was demonstrated that functional outcome and dendritic branching patterns in the contralesional hemisphere were restricted when rehabilitative training was initiated 14 and 30 days post insult (Biernaskie et al., 2004). In another study by Hsu and Jones (2005), rats were trained in a skilled forelimb reaching tasking starting 4 or 25 days post stroke. Reaching performance was significantly enhanced in the early trained group. In a small ischemic insult in M1 in squirrel monkeys delayed training resulted in a large decrease in spared hand representation during the spontaneous recovery period that persisted following the delayed training (Barbay et al., 2006).

Concerns about initiating therapy too early following stroke arose from studies where lesion size and cell death rate were seen to be exaggerated after early excessive use of the impaired forelimb in rats while the unimpaired forelimb was casted (Kozlowski et al., 1996). One cause for increased lesion size following early excessive limb training might be NMDA-mediated excitotoxicity in the already hyperexcitable peri-infarct region (Humm et al., 1999). In closer resemblance to clinical practice were animal studies, where training or enriched rehabilitation was initiated a few days after stroke. In these cases early intervention (1–3 days post stroke) again was associated with increased cell-death but also with much improved motor performance on the long-term (Risedal et al., 1999; Farrell et al., 2001). Here, neuronal cell death may be part of a pruning effect in which non- or dysfunctional neurons are eliminated early due to a use-dependent selection. In summary, the overall consensus from animal data is that initiating rehabilitative training 5 or more days after stroke is mostly beneficial and has no adverse effects (Krakauer et al., 2012).

Constraint-induced movement therapy (CIMT), robot assisted training and electrical devices to stimulate the rehabilitation process

For human stroke patients two advanced rehabilitative approaches have proven beneficial for functional outcome: constraint-induced movement therapy (CIMT) and robot-assisted training for upper limb function (Langhorne et al., 2009; Liao et al., 2012; Mehrholz et al., 2012). Extensive preclinical studies in rodents and primates have preceded both rehabilitative strategies (Taub et al., 2002). When somatic sensation is surgically abolished from a single forelimb in a monkey, the animal avoids the usage of this forelimb in the free situation, but monkeys can be induced to use the de-afferented extremity by restricting movement of the intact limb continuously for a period of days. This concept was successfully brought into the clinics when chronic stroke patients wore a sling or cast on their less affected arm during 90% of their waking hours for 14 days (Taub et al., 1993). These patients showed a significant increase in the skill and quality of movement as measured by two laboratory tests and a much larger increase in real-world arm use over the period of these 2 weeks than the unrestricted control group. Two studies addressed the question of intensity and timing for CIMT: In the VECTORS study (Dromerick et al., 2009), 52 stroke patients were randomized at about 10 days post stroke to two levels of intensity of CIMT or standard upper extremity therapy. Intense meant 3 h of CIMT vs. 2 h of shaping therapy. After 90 days the motor outcome was worse for the more intensive CIMT group, although there had been no difference at 30 days. This result reflects the fact that too intensive CIMT can turn into an adverse situation for both the patient and the therapist. In the much larger EXCITE study (Wolf et al., 2006) patients started CIMT therapy 3–9 months post stroke and showed greater motor recovery than the usual care group. In addition Lang et al. (2013) revealed that improvements

in existing motor abilities were possible with both early (3–9 months post stroke) and delayed (15–21 months post stroke) application of CIMT. However, significant reacquisition of the ability to complete tasks was only detected with early CIMT treatment.

A number of arm and also hand training robots have been developed recently with the aim to allow very intense training without continuous, costly physiotherapy assistance. In the most modern set-ups, training devices are combined with interactive video games that can boost the motivation of the patient for the training and facility e.g., precision movements (e.g., grasping eggs and putting them into a basket). The number of well controlled and standardized outcome studies is still very limited. However, differences are discriminated between recovery of specific movements under "laboratory conditions" and functional gains for daily life activities (Mehrholz et al., 2012). Such studies are needed to exactly know the specific advantages (and potential drawbacks) of robot assisted rehabilitation in stroke (Aisen et al., 1997; Balasubramanian et al., 2010; Mehrholz et al., 2012).

Therapeutic approaches which directly stimulate the PNS or CNS electrically or by magnetic pulses may enhance neuroplasticity during poststroke rehabilitation (Dimyan and Cohen, 2011). Numerous research groups have examined the stimulation of the CNS, specifically the primary M1, by noninvasive approaches such as TMS and direct current stimulation as well as experimentally in animals by the implantation of electrodes. Several studies showed that an increase of the excitability in the stroke-affected ipsilesional M1 by electrical devices resulted in improved motor outcome (Hummel et al., 2005; Malcolm et al., 2007; Ameli et al., 2009; Koganemaru et al., 2010). The mechanisms of action of these techniques are under investigation but might involve changes in synaptic activity, gene expression and increases in neurotransmitter, receptor and neurotrophin levels (Dimyan and Cohen, 2011) or even enhanced fiber sprouting (Martin, 2012). Understanding these mechanisms may provide the basis for novel approaches using closed-loop brain machine interfaces (BMIs) that define optimal stimulation parameters from a priori developed experimental models and correctly modulate ionic currents and extracellular electric fields to provoke and guide plastic changes of the CNS (Gonzalez Andino et al., 2011).

COMBINATION OF DIFFERENT REPAIR AND REHABILITATION STRATEGIES

To maximize the effectiveness of rehabilitative therapies after stroke, it is critical to define when the brain is most responsive to sensorimotor input or extrinsic application of plasticity promoting reagents. This becomes particularly important if different rehabilitative approaches are combined.

In one of the first proof of concept studies for a critical period of heightened neuroplasticity, stroke rats were exposed to an enriched environment in combination with daily sessions of grasping training. The most significant gains in the recovery of forelimb reaching ability were achieved when rehabilitation was initiated early, i.e., 5 days after stroke as compared to 14 and 30 days after stroke. Recovery was associated with increased dendritic branching of layer V M1 neurons in the unlesioned hemisphere—a response that was not detected when rehabilitation was delayed by 30 days (Biernaskie et al., 2004).

A few recent studies in which regenerative therapies and rehabilitation have been combined have been conducted since then. These experiments suggest that designing the combination and their temporal pattern of administration are not going to be trivial (García-Alías and Fawcett, 2012; Starkey and Schwab, 2012). The different experiments have revealed a beneficial combinatorial effect, a detrimental effect, no effect at all, or an effect that depends on the relative timing of plasticity treatment and rehabilitation.

Beneficial effects were described in spinal cord injury rat models when agents against inhibitory molecules in the CNS were combined with growth promoting reagents: García-Alías et al. (2011) reported that the combination of Chondroitinase ABC with neurotrophin NT-3 and an increased expression of the NR2D subunit of the NMDA receptor resulted in better body stability and interlimb coordination compared with the single treatment groups. The behavioral data were correlated with the highest number of sprouting axons in the spinal cord and multisynaptic responses in the motor-neurons. Similar results could be found if anti-Nogo-A antibodies were combined with NT-3 and the NMDA-NR2D subunit (Schnell et al., 2011). Furthermore, the combinatorial treatment of acutely applied anti-Nogo-A antibody followed by delayed Chondroitinase ABC treatment starting 3 weeks after spinal cord injury, and forelimb grasp training starting at 4 weeks was much more effective in terms of functional recovery, sprouting and axonal regeneration than the single treatments (Rehme et al., 2011). In rats with large cortical strokes, inosine, a substance which was shown to improve fine motor control after stroke (Zai et al., 2009), augmented the effects of the Nogo receptor blocker NEP1-40 in the restoration of skilled reaching abilities in rats. Similar functional improvements were seen when inosine was combined with environmental enrichment (Zai et al., 2011).

Several recent experiments—mainly in spinal cord injury—have combined growth-promoting agents with rehabilitative training with somewhat different results: García-Alías et al. (2009) investigated whether chondroitinase-induced plasticity combined with physical rehabilitation promotes recovery of manual dexterity in rats with cervical spinal cord injury. While CSPG digestion combined with forelimb-specific rehabilitation lead to improved manual dexterity, animals treated with chondroitinase ABC in combination with environmental enrichment improved in ladder walking but performed much worse in skilled forelimb tasks than untreated control animals. In a second investigation by Maier et al. (2009) adult rats with large but incomplete cervical spinal cord injury received anti-Nogo A antibodies and simultaneous daily forced treadmill training. The simultaneous rehabilitative therapy clearly worsened the functional outcome compared with either treatment alone. When the forced treadmill training was delayed, however, for 2 weeks after the end of the antibody treatment a very good functional outcome was obtained (Marsh et al., 2011). In contrast to these results in spinal cord injured rats, combination of Nogo receptor blockade with skilled forelimb training in stroke lead to a greater degree of recovery than when either of the treatments were applied alone (Fang et al., 2010).

No additive or adverse effects were reported by Boyce et al. (2007) when neurotrophins were combined with rehabilitative training in spinal cord injured cats. Administration of pharmacological neuromodulators such as amphetamine and cholinergic agonists in combination with rehabilitative training are a matter of debate: early animal research had suggested a beneficial effect of amphetamine in recovery of motor function after stroke which could not be sufficiently reproduced in recent human and animal studies (Krakauer et al., 2012). Only for the anti-depressant fluoxetine, a serotonin-selective reuptake inhibitor, which was applied from 9 days post stroke to 3 months in a human stroke study, an impressive degree of increased motor recovery was found when combined with rehabilitative training (Chollet et al., 2011). For all these studies and their quite diverse outcomes, better knowledge of the neurobiological phenomena and mechanisms triggered by the injury, the spontaneous reaction of the nervous tissue to it, and by the different pharmacological and behavioral interventions is urgently required.

FUTURE DIRECTIONS FOR DESIGNING OPTIMAL REHABILITATION SCHEDULES

How can we better understand the neurobiology of rehabilitation? What can we learn from the above mentioned animal and clinical studies to improve current rehabilitation schedules for the best possible recovery after stroke? The presence of critical time windows for the application of growth and plasticity promoting agents and of training-dependent plasticity suggests that careful consideration of rehabilitation onset times, tailored training to the type and extent of stroke and the patient's history are required. Potential future rehabilitation schedules after stroke may therefore include the following "3 step model" (**Figure 1**):

1. Determination of the metabolic and plastic status of the brain by using state-of-the-art imaging technologies and metabolic markers
2. Enhancement of the plastic status of the brain by the application of growth and plasticity-promoting factors
3. Selection and stabilization of newly formed functional connections by rehabilitative training

One obstacle of the implementation of the optimal restorative therapies is the heterogeneity of stroke as injury location and size differ widely from one patient to another. The ability to assign the right therapy to the right patient would maximize treatment effects. Although clinical scores and a number of imaging methods exist for evaluating the state of the central nervous system and its function after stroke as reviewed elsewhere (Burke and Cramer, 2013), these approaches are often insensitive, cost intensive and have logistical difficulties. Nevertheless, neuroimaging is not only essential for the establishment of acute stroke diagnosis but can also serve as a powerful tool for the characterization of disease

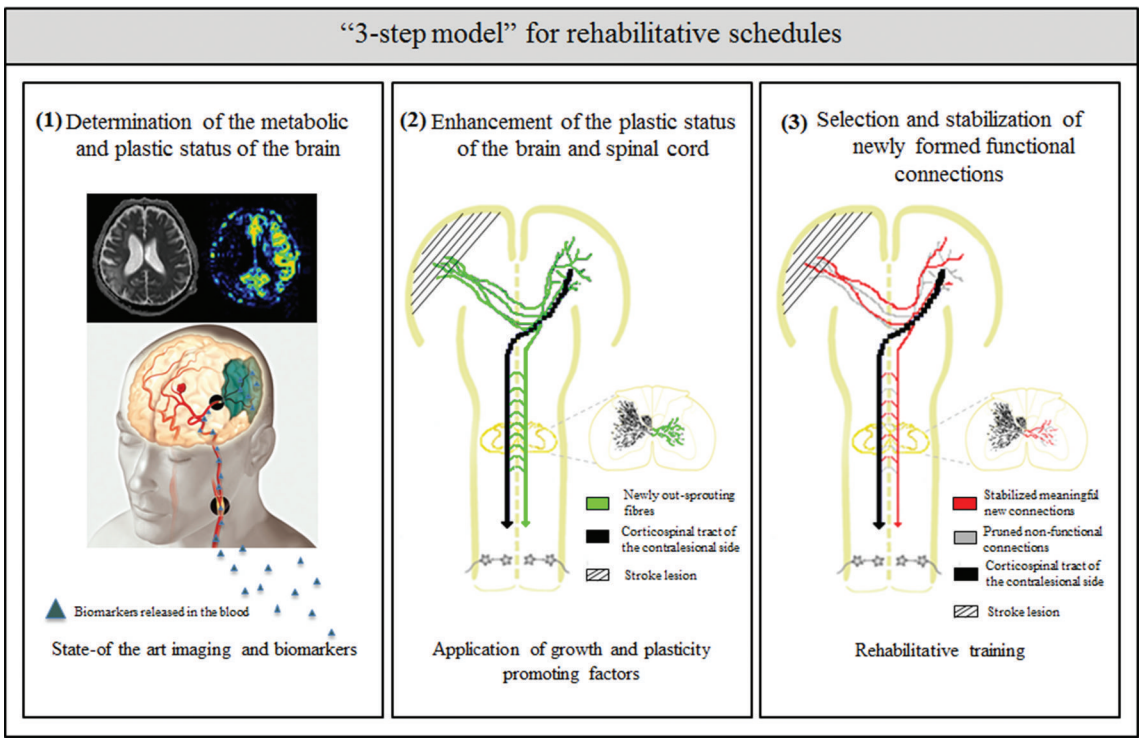

FIGURE 1 | Schematic overview of the "3 step model"—as a possible roadmap for designing future rehabilitation schedules: **(1)** determination of the metabolic and plastic status of the brain by using state- of the art imaging technologies (image taken by the Akashi Municipal Hospital, Japan) and biomarker profiles in the blood and CSF; **(2)** enhancement of intrinsic repair and plasticity mechanisms in the ispi- and contralesional hemisphere as well as the spinal cord by application of growth and plasticity-promoting factors such as anti-Nogo-A antibody or Chondroitinase ABC; and **(3)** selection and stabilization of newly formed functional connections and pruning of non-functional ones by rehabilitative training.

progression and monitoring of the response to rehabilitative interventions. Diffusion-weighted imaging (DWI) and perfusion-weighted MRI (PWI) are widely available MRI modalities that provide valuable information about the tissue characteristics of the ischemic core but also of the tissue at risk in the penumbra (Merino and Warach, 2010; Fisher and Bastan, 2012). Further work is needed to optimize the characterization of penumbra imaging for patient triage into adjusted treatment groups. In the near future we expect to learn if penumbra imaging or other early imaging features provide predictive value of critical time windows in which therapeutic interventions should be initiated or maintained and allow stratification of patients into groups for specific types of therapies.

Biomarker profiles in blood and cerebrospinal fluid (CSF) samples could bring a tremendous advance and are currently a focus of genomic and proteomic profiling studies and of systems biology in several laboratories (Stuart et al., 2010; Hemphill et al., 2011; Whiteley et al., 2012). In this regard, a biomarker or a specific combination and profile of biomarkers may not only speed up diagnosis and initiation of acute stroke treatment but may also help to classify and categorize patient groups for prediction of outcome and target the right rehabilitative approach to those stroke patients who would benefit the most.

Why do we suggest a temporal sequence of first enhancing the plastic state by growth promoting agents followed by a phase of rehabilitative training in our "3 step model"?

The current data suggest that the CNS reacts to the injury by an activation of growth and plasticity mechanisms which, however, seem to also represent a vulnerable phase in which forced activity can be harmful: this phase includes a period of GABA-mediated tonic inhibition, which may also be necessary in the first days after the stroke to limit an expansion of the infarct size (Clarkson et al., 2010), as well as homeostatic plasticity mechanisms, which ensure that neurons receive an balanced amount of synaptic input (Murphy and Corbett, 2009). Intrinsic growth and plasticity as well as exogenous enhancement of growth will lead to the formation of a large number of new connections within and between different areas of the injured CNS. In analogy to the situation in early postnatal development, many of these connections may be weak and imprecise. The functionally meaningful ones will now have to be selected and stabilized, while the malfunctional ones should be pruned, in the next, activity-dependent phase of the recovery process.

In the last step of recovery that is based mainly on rehabilitative training the spared and the new circuitry of the CNS is shaped by selection and stabilization of functional connections and pruning of the non-functional ones. Hebbian learning rules might play a crucial role in this step in the sense that Hebbian plasticity mechanisms redistribute synaptic strength to favor the wiring of pathways that are coincidently active (Murphy and Corbett, 2009). Motor learning in development is a very protracted process, requiring huge numbers of repetitions over a period of many weeks and months. Much too less is known today on the optimal time and intensity requirements for rehabilitation learning. To distinguish optimal rehabilitation schedules from less beneficial ones, strict criteria for functional outcome have to be defined that discriminate compensation and substitution from real restoration of previously impaired function. Much remains to be learned and applied in this fascinating and medically most important field of stroke rehabilitation at the interface between basic neuroscience and clinical neurology.

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