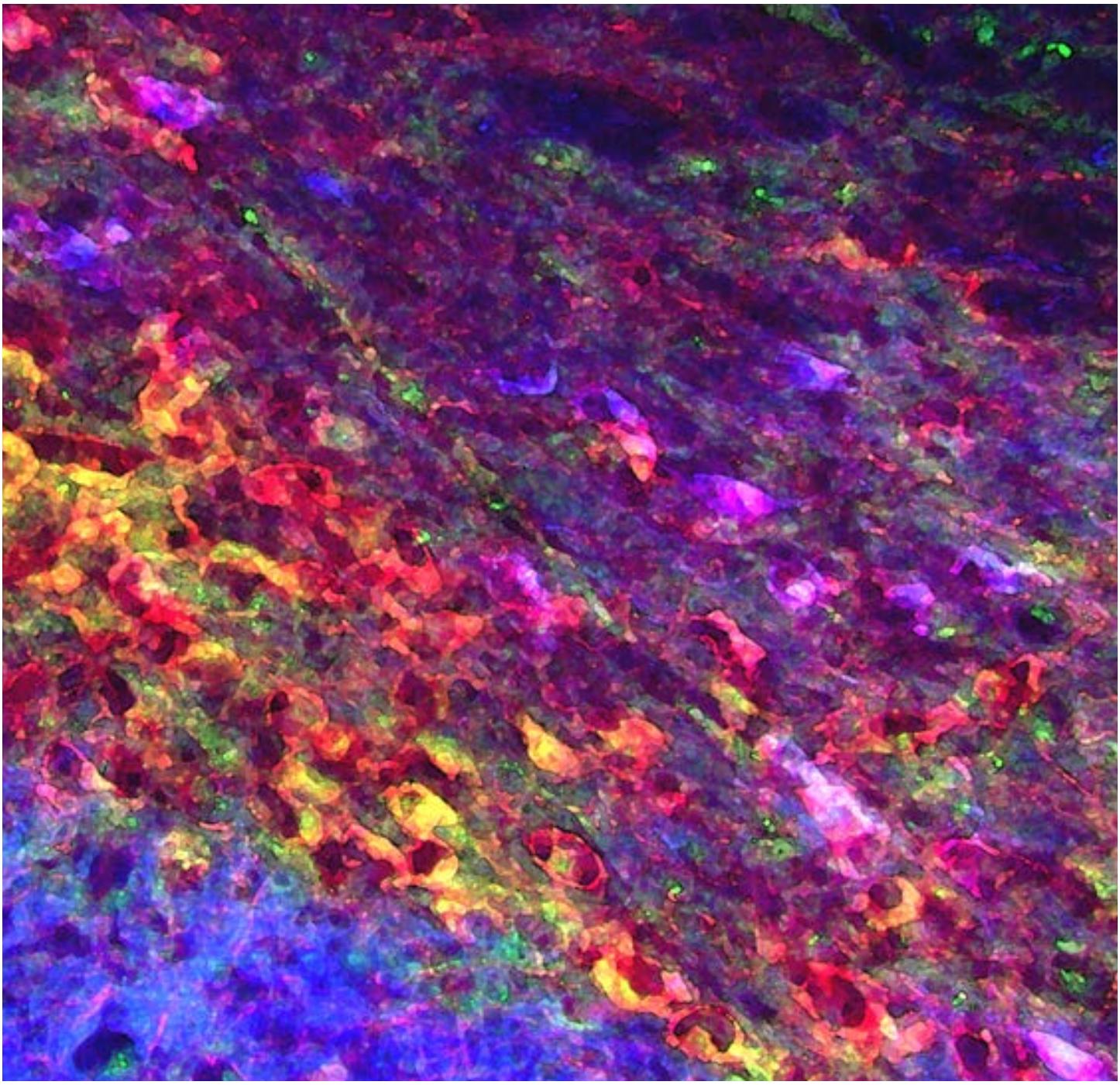

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Neurobiology of Disease Workshop

Stroke Recovery: Connecting Neuroimmunology, Regeneration, and Engineering to Restore Functional Circuits

Organized by Marion Buckwalter, MD, PhD, and Claudia Testa, MD, PhD

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NEUROSCIENCE 2014

Stroke Recovery: Connecting Neuroimmunology, Regeneration, and Engineering to Restore Functional Circuits

Organized by: Marion Buckwalter, MD, PhD and Claudia Testa, MD, PhD
Friday, November 14
8 a.m.–5 p.m.
Location: Walter E. Washington Convention Center | Ballroom C | Washington, DC

TIME	TITLE	SPEAKER
7:30–8 a.m.	CHECK-IN	
8–8:05 a.m.	Opening Remarks	Claudia Testa, MD, PhD *Virginia Commonwealth University*
8:05–8:35 a.m.	Patient Presentation	Amie Hsia, MD *NINDS/MedStar Washington Hospital Center*
PATHOPHYSIOLOGY OF STROKE RECOVERY		
8:35–9:10 a.m.	Acute Immune Responses After Stroke	Costantino Iadecola, MD *Weill Cornell Medical College*
9:10–9:45 a.m.	Late Neuroinflammation After Stroke and Post-Stroke Dementia	Marion Buckwalter, MD, PhD *Stanford School of Medicine*
9:45–10:20 a.m.	Recovery Mechanisms — Rewiring and Recovery	Tom Carmichael, MD, PhD *Geffen School of Medicine at University of California, LA*
10:20–10:35 a.m.	MORNING BREAK	
FRONTIERS OF STROKE RECOVERY THERAPY		
10:35–11:10 a.m.	Stem Cells and Stroke, Adult and Neonatal Models	Cindi Morshead, PhD *University of Toronto*
11:10–11:45 a.m.	MRI Measures of Recovery and Transcranial Magnetic Stimulation	Rick Dijkhuizen, PhD *Utrecht University Medical Center*
11:45 a.m.–12:20 p.m.	Brain–Machine Interfaces and Bioengineering Approaches to Stroke Recovery	Leigh Hochberg, MD, PhD *Brown University*
12:20–1 p.m.	SUMMARY, DISCUSSION, AND Q&A	
1–1:10 p.m.	BREAKOUT GUIDE	
1:10–2 p.m.	LUNCH	

Neurobiology of Disease Workshop (cont.)

TIME	BREAKOUT SESSIONS*		SPEAKERS	ROOM
	* Participants select discussion groups at 2 p.m. and 3:30 p.m.			
2–3:30 p.m.	Group 1	Brain Age as a Factor in Stroke: Pediatric and Neonatal Stroke	Zena Vexler, PhD Cindi Morshead, PhD	146C
	Group 2	Stem Cell Therapies	Stuart Lipton, MD, PhD Lawrence Wechsler, MD	147A
	Group 3	Modeling Recovery in Animals	Theresa Jones, PhD Tom Carmichael, MD, PhD	147B
	Group 4	Helpful and Harmful Motor Plasticity	David Burke, MD, DSc Leigh Hochberg, MD, PhD	150A
	Group 5	Gender Effects on Stroke	Frank Sharp, MD Louise McCullough, MD, PhD	150B
	Group 6	Comorbidities: Hypertension, Diabetes, Cholesterol, and the Neurovascular Unit	Ken Arai, PhD Rick Dijkhuizen, PhD	152A
	Group 7	Adaptive Immunity After Stroke	Kyra Becker, MD, PhD Marion Buckwalter, MD, PhD Costantino Iadecola, MD	152B
3:30–5 p.m.	REPEAT SESSIONS ABOVE. SELECT A SECOND DISCUSSION GROUP.			
5–6 p.m.	RECEPTION			208AB

The Neurobiology of Disease Workshop is being recorded and will be available for access at a later date. Visit SfN.org for more information.

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Introduction

Stroke is a leading cause of death and disability worldwide. Our understanding of the molecular and cellular changes that happen after stroke is rapidly improving; however, effective treatments are not identified for the vast majority of stroke survivors. The aim of this Neurobiology of Disease Workshop is to review stroke research concepts beyond the acute injury setting, exploring efforts to define and promote productive long-term recovery. The course will cover a range of topics, emphasizing research areas that connect basic neuroscience-mechanism work with poststroke therapeutic development.

Multiple disciplines within neuroscience are being utilized to expand our knowledge of the brain's damage and recovery pathways. Poststroke molecular and neural network pathway plasticity is an example of a pathophysiology and recovery concept being approached from different scientific angles. This course also covers neuroinflammation after stroke, encompassing both acute immune responses and the consequences and treatment opportunities in late poststroke neuroinflammation. Neuroinflammation data feed into potential pharmacotherapeutics.

Research bridging disease mechanism with therapeutics development is a theme of multiple course topics. Work in model systems on potential stem cell therapies is driving cell-based therapeutics development. Novel MRI techniques are being used to study brain recovery poststroke, and could also be used to track the efficacy of poststroke therapeutic interventions. Stroke treatment is a key example of bioengineering approaches to neurological disease therapy: poststroke states are an exciting setting for brain–machine interface development.

Course topics and manuscripts will provide the foundation for faculty and attendees to frame and explore intriguing unanswered research questions in the neurobiology of disease.

Plasticity in the Injured Brain: Experience-, Activity-, and Injury-Induced Processes

Justine J. Overman, PhD, and S. Thomas Carmichael, MD, PhD

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Introduction

A major component of the development and maturation of the CNS is an individual's interaction and experience with the environment. During the brain's development, environmental experience shapes synaptic strength, anatomical connections, and brain structure. Recent evidence indicates that, after injury in the adult brain, environmental experience or neuronal activity influences a surprisingly broad amount of brain connections and structural remodeling.

After CNS injury, such as stroke, functional deficits are caused by a loss of axonal and dendritic connections in motor, sensory, and cognitive areas of the CNS; thus, restoration of these preinjury connections or development of new ones will facilitate functional recovery. Although the adult CNS normally restricts the formation of new connections, spontaneous circuit reorganization takes place through axonal sprouting and results from a combination of molecular and experience- or activity-driven physiological changes. By understanding the molecular and environmental substrates and interactions that lead to activity-, experience-, and injury-dependent plasticity, we may be able to devise more effective treatment strategies for repair after CNS injury.

"Activity-dependent plasticity" and "experience-dependent plasticity" refer to molecular or anatomical changes that take place in the CNS that are associated with behavioral activity (e.g., altered movement patterns) or changes in environmental conditions. Activity-dependent plasticity (or altered environmental experience) alters the adult CNS at the level of synaptic plasticity of the involved brain areas and in the formation of new connections. An example of this process would be the connections in immature neurons in the dentate gyrus that form during memory tasks, in which these cells form new circuits that are modulated by experience (Kim et al., 2012). Activity-dependent plasticity is also seen with changes in motor or sensory cortical maps or synapse number that are associated with training or exercise: specific paradigms of behavioral engagement that emphasize the activity pattern over a normal environmental interaction (Adkins et al., 2006; Tennant et al., 2012). In the absence of changes caused by experience or activity, the CNS is also capable of robust injury-induced plasticity, which includes changes in synaptic strength (Carmichael, 2012) and anatomical changes that are observed in response to injury itself (Benowitz and Carmichael, 2010).

Each of these drivers for changes in adult neuronal circuits (activity-dependent plasticity, experience-dependent activity, and injury-induced plasticity) is influenced by different external factors. However, the internal molecular substrates that underlie these persistent anatomical changes can overlap greatly. This chapter focuses on molecules that are differentially regulated in sprouting neurons after stroke and that are known to be involved in experience- or activity-induced plasticity. Identifying the most prominent molecular factors that contribute to successful activity-induced, experience-induced, or injury-induced plasticity may inform future injury-recovery studies and open up new avenues for neural repair strategies.

Functional Recovery After Stroke

Stroke alters movement and sensory functions, and may impose an activity- or experience-dependent plasticity on the brain, apart from any effect that the lesion itself has in inducing activity. However, injury-induced plasticity and the way the brain responds to changes in activity are likely to mutually influence each other. Indeed, the molecular and anatomical changes that confer recovery of function are already occurring when activity-dependent plasticity is superimposed on injury-induced plasticity. Thus, the most effective repair strategies after stroke may turn out to rely on a combination of appropriate molecular manipulation of injury-induced plasticity and patterned behavioral activity.

Constraint-induced movement therapy

In humans, a treatment strategy that consistently produces improvements in behavioral recovery after stroke is the forced use of the affected limb. This technique drives an increase in patterned or task-specific behavioral activity (Wolf et al., 2002, 2006; Grotta et al., 2004; Sawaki et al., 2008). In this paradigm, patients with an upper extremity that is affected by stroke have the unaffected limb restrained by a sling or a mitten in order to keep it from making skilled movements. The "good limb" or "unaffected limb" is then given shaping sessions to facilitate its use in the constraint of the affected limb. After this shaping period, the patient is forced to overuse the affected limb in specific tasks and activities for a period of time, that is, in an activity-dependent manner.

One hypothesis to explain the success of this therapy is that the injured brain undergoes injury-dependent plasticity, after which it undergoes activity-dependent plasticity. This hypothesis is supported by the finding that constraint-induced therapy accelerates or enhances cortical remapping

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in peri-infarct or hemispheric areas ipsilateral to the stroke, which might normally experience remapping during recovery (Wittenberg and Schaechter, 2009). From early postinjury constraint studies in the 1940s (Tower, 1940) to the technique's clinical inception in treating a single stroke patient in 1981 (Wolf et al., 1981) to a recent landmark clinical trial (Wolf et al., 2006), constraint-induced movement appears to be a promising tool in poststroke rehabilitative therapy (Wolf et al., 2006).

It is assumed that permanent or semipermanent structural changes in the brain are responsible for recovery of function and cortical remapping following constraint-induced movement therapy. Such changes likely result from augmented dendritic branching and axonal sprouting in combination with specific gene regulation. This explanation is supported by the finding that skill learning (a specific form of activity-dependent plasticity) promotes dendritic spine morphogenesis and synaptogenesis (Adkins et al., 2006; Xu et al., 2009) and that injury-induced plasticity is also associated with dendritic spine morphogenesis and synaptogenesis (Ito et al., 2006; Brown and Murphy, 2008; Mostany et al., 2010).

The sprouting transcriptome

An important initial subject to address when trying to understand injury-induced plasticity is that injury initiates molecular programs that lead to the formation of new connections. Using a method to selectively label sprouting neurons in peri-infarct cortex after stroke, we applied whole-genome expression analysis in order to identify the distinct transcriptional profile of a neuron that undergoes axonal sprouting after stroke, as compared with a nonsprouting neuron (Li et al., 2010). In sprouting neurons, differentially regulated genes form concerted signaling systems from cell-surface receptors to nuclear transcription control; these systems relate to axonal guidance, calcium signaling, extracellular matrix function, growth factors, transcription factors, cytoskeletal modifying genes, and epigenetic or DNA-modifying genes. Interestingly, sprouting neurons in the condition of stroke in the aged brain show a greater activation of immune-related genes and bone morphogenic protein family genes compared with neurons that engage in axonal sprouting in young brains from young adult stroke.

Paradoxically, sprouting neurons in aged animals activate known inhibitors of axonal outgrowth. Therefore, molecules that could control the expression pattern of larger cassettes of genes, through either direct transcription control or epigenetic control, would be promising targets as "master switches" that might exert a phenotypic switch within an adult cortical neuron: from a static neuronal phenotype to active axonal outgrowth. One of these candidates is the gene product *ATRX* (alpha thalassemia/mental retardation syndrome X-linked), a DNA helicase that controls gene expression through chromatin remodeling. *ATRX* is important in brain development (Seah et al., 2008) and regulates the formation of axonal sprouting and the formation of new connections after stroke (Li et al., 2010).

One surprising finding in the poststroke sprouting transcriptome is the upregulation of growth-inhibitory receptors in sprouting neurons after stroke, particularly in aged animals. Lingo-1, EphA4, and its downstream effector chimaerin-1 are upregulated in aged sprouting neurons after stroke. These gene systems block the formation of new connections after stroke (Li et al., 2010; Overman et al., 2012). These gene systems are also regulated by brain activity (Trifunovski et al. 2004; Inoue et al., 2009) and, along with other molecules in the sprouting transcriptome, are in a position to interact with experience or activity to influence plasticity in the injured CNS. These findings suggest a common molecular substrate for activity-dependent and injury-dependent gene regulation during axonal sprouting. It may be that these molecular systems are regulated in common directions by both activity-dependent and injury-induced stimuli, or that they are differentially affected by the stimulus (injury or activity) or the context (young or aged).

Inhibitors of axonal outgrowth

The adult CNS presents a largely inhibitory milieu to axonal sprouting: After injury in the adult brain, the injured tissue itself becomes a major barrier to axonal outgrowth and axonal sprouting. Stroke (and other types of CNS injury) induces a specific set of glial, myelin-based, and developmentally associated growth-inhibitory molecules during the process of axonal sprouting. There are three major classes of growth inhibitors in the CNS:

- Myelin-associated proteins (NogoA, myelin-associated glycoprotein [MAG], and oligodendrocyte myelin-associated protein [OMgp]);
- Extracellular matix proteins (chondroitin sulfate proteoglycan [CSPGs], lecticans, and NG2); and
- Developmentally associated and canonical axon guidance molecules (ephrins, semaphorins, and netrins) (Silver and Miller, 2004).

Here we focus on the subset of these molecules that plays a role in experience- or activity-induced plasticity and is expressed (or related to molecules that are induced) in sprouting neurons after stroke.

Myelin-associated proteins and activity in the normal CNS

Among the myelin-associated proteins, NogoA, MAG, and Omgp all share a single receptor complex. This complex includes the Nogo receptor (NgR), Lingo, and $P75^{NTR}$, and the paired-immunoglobulin-like receptor B (PirB). Many of these ligands and receptor-complex components have been shown to play a role in activity-dependent plasticity and axonal growth and guidance—either during development or in the adult, and in the normal or injured brain.

PirB. PirB is a major histocompatibility complex class 1 (MHC1) receptor that is expressed by neurons, regulated by experience and activity (Huh et al., 2000), and upregulated in sprouting neurons after stroke (Li et al., 2010). In mice lacking PirB, cortical ocular-dominance plasticity is greater in both the developing and the adult brain. This role for PirB in experience-dependent plasticity may be recapitulated during injury-induced plasticity.

NogoA and NgR1. While NogoA is normally expressed in myelin, areas of the brain that are capable of high levels of experience- or activity-dependent plasticity (including the hippocampus) express high levels of NogoA (Lee et al., 2008) and NgR in their neurons (Josephson et al., 2002). Deletion of NgR1 has been shown to decrease working memory (Budel et al., 2008) and to regulate the formation of lasting memories (Karlén et al., 2009). NgR1 itself is regulated in an activity-dependent manner (Josephson et al., 2003) and has been implicated as a strong regulator of activity-dependent synaptic strength and a regulator of synaptic plasticity. After adult rats are exposed to a running wheel for 7 d, expression of NgR is downregulated in their hippocampus and cortex (Josephson et al., 2003).

The ability of acute structural changes to be transformed into permanent anatomical changes, during experience- or activity-dependent plasticity, relies on the downregulation of NgR1 but not necessarily on myelin-associated ligands. This finding suggests that activity or experience allows neurons in the normal brain to become transiently insensitive to the inhibitory effects of Nogo, MAG, or OmgP and to allow for persistent synaptic reorganization. These data show that Nogo signaling is regulated by activity and experience and plays a role in the development of brain connections and in the normal plasticity that underlies learning and memory in the adult. Further, they show that Nogo-expression changes may allow neurons to activate a period of reduced sensitivity to the adult blockades for synaptic plasticity. These features define a molecular system that has dynamic features that position it to play a role in injury-induced plasticity.

Ephrins and activity-related plasticity during development

Another class of axonal guidance cues that are expressed by or near sprouting neurons after stroke are the developmentally associated ephrins. There are 16 known Eph receptors and 9 ephrin ligands (Pasquale, 1997; Murai et al., 2003). These ligands are separated into classes A and B based on ligand-binding affinity for the extracellular glycosylphoshatidylinositol (GPI)–anchored ephrin-A ligands or transmembrane ephrin-B ligands (Pasquale, 1997). Ephrins bind to their tyrosine kinase receptors (Ephs) and activate RhoA and ROCK to promote destabilization of the axon growth cone (Gale and Yancopoulos, 1997). This interaction is mediated, at least in part, by proteins that stabilize the active state of RhoA, such as chimaerin-1 (Wegmeyer et al., 2007). RhoA is differentially upregulated in the sprouting neurons of young adult rats after stroke (Li et al., 2010), whereas EphA4 and chimerin-1 are upregulated in the sprouting neurons of aged adults after stroke (Li et al., 2010). Ephrin-to-Eph communication is also bidirectional, with intracellular signaling cascades precipitated not just in the Ephrin-to-Eph direction but also in reverse, for the Ephrin B and A classes (Klein, 2009).

EphrinA5 is induced in reactive astrocytes in peri-infarct tissue, and this induction is associated with a substantial increase in EphA activation (Overman et al., 2012). Like its ligand, EphA4, EphrinA5 is induced even further in the aged brain after stroke (Li and Carmichael, 2006). Ephrin activation after stroke is surprisingly widespread—extending millimeters away from the infarct into tissue well removed from the site of damage; this tissue contains reactive astrocytes (Overman et al., 2012). In this tissue, the functional consequences of EphrinA5 are mediated through "forward signaling," from EphrinA5 to EphA receptors (Overman et al., 2012). These data mean that stroke activates multiple elements of the EphrinA signaling system—a ligand, its receptor, and its downstream molecules—and does so in an age-dependent manner. Based on this molecular anatomy, it is tempting to speculate that EphrinA5 may block axonal sprouting and contribute to the reduced recovery seen with age after stroke.

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Future Areas of Study

Future studies are needed to fully elucidate the molecular substrates of injury-, activity-, and experience-induced plasticity and to understand how the complex interaction between molecules and activity affect persistent anatomical change in the CNS. Molecular substrates of plasticity likely overlap across developmental processes, adult tissue homeostasis, various types of CNS injury, and activity paradigms. How these plasticity-associated molecules interact with activity, are influenced by activity, or directly affect activity could have profound effects on the development of strategies for repair.

One critical feature of functional recovery is that activity likely facilitates appropriate rewiring of the CNS after injury and may prevent maladaptive patterns of connections from forming. Activity shapes these connections by inducing both growth-promoting and growth-inhibiting molecules, neutralizing or enhancing either subset to drive structural and anatomical changes. Molecules themselves can limit or enhance the extent to which activity can effect physiological change. The most effective treatment strategy for CNS injury repair will likely combine multifactorial molecular manipulation with behavioral activity to drive the most robust and functionally appropriate plasticity.

Acknowledgments

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Neural Precursors for Stroke Repair

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Introduction

Stroke is the leading cause of long-term disability and the fourth leading cause of death in American adults (Roger et al., 2012). Stroke results in the necrosis of brain tissue, leading to the loss of the intricate arrangement of neurons, glial cells, and vasculature. Currently, the only treatment for stroke is thrombolytic tissue plasminogen activator (t-PA), which has been shown to reduce ischemic damage and permanent disability if administered within hours of onset. A long-term treatment is needed to repair infarct damage and improve functional recovery in patients.

Neural stem cells in the adult brain hold great promise for the development of neural repair strategies. Specifically, neural stem cells and progenitor cells (collectively known as neural precursor cells [NPCs]) possess the ability to generate the neural cell types present in the brain. The use of NPCs in cell-based therapy has focused on two distinct approaches: endogenous and exogenous cell-based strategies. In the endogenous approach, NPCs resident in the brain are induced to proliferate, migrate to the infarct site, differentiate into various neural cells needed for repair, and functionally integrate into the tissue to promote recovery. The exogenous approach involves the propagation of NPCs *in vitro* and their subsequent transplantation to achieve regeneration and recovery. This chapter aims to provide an understanding of NPCs and to present current findings in the two approaches of cell-based therapy for stroke, with a focus on endogenous repair strategies.

Neural Precursor Cells

By definition, a stem cell has the capacity to self-renew indefinitely and the ability to give rise to cells that generate differentiated progeny. A neural stem cell generates multipotent progeny which can differentiate into neurons, astrocytes, and oligodendrocytes. A neural progenitor cell possesses limited self-renewal capacity and differentiation potential (Gage, 2000).

Historically, NPCs were thought to exist only in the developing brain, whereas the adult mammalian brain was presumed postmitotic and devoid of regenerative capacity. However, seminal works by Reynolds and Weiss (1992) showed the presence of NPCs in the adult mammalian CNS. Cells cultured in the presence of growth factors (epidermal growth factor [EGF] and fibroblast growth factor 2 [FGF2]) gave rise to clonally derived free-floating colonies (termed "neurospheres") *in vitro*. Individual neurospheres could be dissociated and replated in growth factors to generate new colonies, thereby revealing the stem-cell property of self-renewal. The cells were also shown to differentiate into the three CNS cell types, confirming their multipotentiality. The discovery of NPCs in the adult brain offered new hope for the possibility of regeneration in the adult brain.

Adult NPCs are found in two discrete regions: the subventricular zone (SVZ) (Morshead et al., 1994) and the dentate gyrus (DG) of the hippocampus (Palmer et al., 1995). Neural stem cells in the SVZ are slowly proliferating cells that express glial fibrillary acidic protein (GFAP) and give rise to rapidly dividing progenitor cells, which subsequently give rise to neuroblasts (Doetsch et al., 1999). In rodents, neuroblast cells migrate through the rostral migratory stream (RMS) to the olfactory bulb, where they differentiate into interneurons and become functionally integrated into the neuronal network (Carleton et al., 2003). In humans, samples from the SVZ of adult brains have shown the presence of $GFAP^+$ cells capable of forming self-renewing, multipotent neurospheres *in vitro*, confirming the presence of NPCs in the adult human brain (Sanai et al, 2004; van den Berge et al 2010). Furthermore, neuroblasts migrating from the SVZ to the olfactory bulb, analogous to the rodent RMS, have been observed in humans, albeit in much smaller numbers than what is observed in rodents (Curtis et al. 2007; Wang et al., 2011).

NPCs that express GFAP are also found in the subgranular zone of the DG, between the hippocampal hilus and granular layer (Palmer et al., 1995; Seri et al., 2001). These NPCs proliferate, migrate into the granular layer, and extend processes that functionally integrate into the overlying molecular layer (Kempermann et al., 2008). In humans, postmortem analysis of hippocampi has revealed the presence of neurons derived from proliferating cells in patients injected with the proliferation marker bromodeoxyuridine (Eriksson et al., 1998). Given the critical role of the hippocampus in learning and memory, the NPCs in this region are considered an important target for treating the stroke injured brain.

Exogenous Approach: Transplanting NPCs Following Stroke

A variety of stem-cell types have been used for decades in cell transplantation studies to treat stroke, and while some have shown promise, the success of this treatment strategy is yet to be realized. Initial pioneering work using fetal brain cells in ischemic

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rat models provided some evidence of synaptic integration and cognitive recovery, although the limited supply, difficulty ensuring the purity of the cell source, and the ethical issues surrounding the cell type have largely impeded the progress of these studies. For examples, clinical trials using human NT2 teratocarcinoma cells and fetal porcine neural cells were met with risks of adverse effects and limited improvement (Konziolka et al, 2005; Savitz et al., 2005). More promising was the use of autologous bone marrow mononuclear cells (BMMCs) and mesenchymal stem cells (MSCs), which were shown to be safe and further demonstrated some neurological improvements (Lee et al., 2010; Burns and Steinberg, 2011). More recently, induced pluripotent stem cells (iPSCs) have gained attention owing to their nonembryonic origin and their potential to be induced to a neural lineage (Yu et al., 2013). Despite the optimistic prospects of these cells, considerable safety concerns remain regarding their potential to form teratomas. For this reason, NPCs are considered advantageous over other cell sources, given their neural restricted lineage potential. Notably, however, difficulties associated with accessing the NPCs in the adult brain have driven preclinical studies to employ NPCs derived from other stem-cell types, such as iPSCs (Yu et al., 2013).

A number of questions remain outside of the cell source to be employed, including the route of administration and time to transplant poststroke. While intracranial injections are commonly studied, there has been increasing interest in intravascular delivery of NPCs. Reports indicate that NPCs can "home" into sites of ischemic injury, reduce infarct volume, and lead to behavioral improvements (Jin et al., 2005; Shen et al., 2010). The NPCs were shown to have immunomodulatory and anti-apoptotic effects (Shen et al., 2010). A meta-analysis examined the effect of intravenous delivery of NPCs in 60 preclinical studies for neurological diseases and summarized the overall outcomes into morphological, behavioral, and molecular effects. This analysis showed that NPCs have a greater effect on behavioral outcomes than MSCs, BMMCs, and other stem cell types (Janowski et al., 2010).

Successful application of exogenous NPCs in the clinic requires the optimization of various parameters, including the time of transplantation and dosage. Indeed, the survival of transplanted cells is dependent on changes in the brain environment after ischemia. There is no current consensus on the most effective time of delivery. The meta-analysis by Janowski et al. (2010) found no significant difference in behavioral, molecular, or morphological outcomes in relation to timing of cell delivery; however, it did find a significant positive correlation between cell dose ($\leq 10^7$ cells) and improved molecular outcomes. Hence, the cell type, dosage, time of delivery, and administration route need to be considered when developing exogenous strategies for human use.

The Endogenous Approach: Activating NPCs to Promote Self-Repair

NPCs in the adult brain demonstrate the fundamental properties *in vivo* that would be necessary for developing strategies to promote their contribution to neural repair: proliferation, migration, and differentiation into neural phenotypes. Demonstration of the recruitment of NPCs in stroke repair was seen using immunohistochemistry. It revealed SVZ-derived NPC proliferation, migration to the site of infarct (Parent et al., 2002), and the generation of neurons in the forebrain that persists for several months after stroke (Thored et al., 2006), with newborn neurons becoming synaptically integrated (Hou et al., 2008). These phenomena have more recently been examined in humans, where postmortem biopsies of stroke patients have shown the presence of proliferating and differentiating cells in the ischemic penumbra as well as the ipsilateral SVZ (Jin et al., 2006; Martí-Fàbregas et al., 2010). However, this endogenous activation is clearly not sufficient for functional recovery, as demonstrated by the persistent and devastating functional impairments observed in patients following stroke.

Developing endogenous activation strategies to promote tissue repair and functional recovery relies on the ability to stimulate endogenous precursors in the brain using a variety of exogenous "activation factors," including growth factors and cytokines. The premise of the endogenous repair strategy is to take advantage of the inherent properties of NPCs to proliferate (and permit an expansion of the NPC pool), migrate to the site of the injury, and differentiate into mature cells in the injured brain. Similar to the challenges faced by exogenous transplant therapies, there are many unanswered questions, such as which types of mature cells (glial and/or neuronal) need to be replaced in the stroke-injured tissue in order to facilitate recovery. Understanding the mechanisms that promote regeneration and recovery will ultimately underlie the choice of NPC activation factors. Hence, the activation factors may mediate their effects by modifying cell-proliferation kinetics, promoting cell survival, enhancing cell migration to

the infarct site and/or promoting neurogenesis, and angiogenesis.

Proliferation

Proliferation represents the first step of the adult NPCs' response to ischemia. Indeed, the injury alone is sufficient to enhance the NPC proliferation and thereby increase the size of the NPC pool (Zhang et al., 2004). Given the fact that NPCs make up a very small fraction of cells in the adult (Morshead and van der Kooy, 1992; Morshead et al., 1994), increasing the size of the NPC pool is an important factor in endogenous repair strategies. Intraventricular administration of EGF has been shown to promote NPC proliferation in the SVZ of uninjured and stroke-injured animals (Craig et al., 1996; Teramoto et al., 2003), and NPCs in injured brains migrated to an injury site following stroke (Kolb et al., 2007). Adult NPCs express the EGF receptor (EGFR) and increase their sensitivity to EGF and other EGFR ligands in response to ischemia by upregulating EGFR expression (Alagappan et al., 2009).

A plethora of other growth and trophic factors have been shown to increase NPC proliferation:

- Vascular endothelial growth factor (VEGF) (Jin et al., 2002);
- Glial cell line–derived neurotrophic factor (GDNF) (Kobayashi et al., 2006);
- Granulocyte colony-stimulating factor (G-CSF) (Schneider et al., 2005);
- Fibroblast growth factor 2 (FGF-2) (Leker et al., 2007);
- Insulin-like growth factor (IGF-1) (Dempsey et al., 2003);
- Bone morphogenic protein 7 (BMP-7) (Chou et al., 2006);
- Transforming growth factor–α(TGF-α)(Guerra-Crespo et al., 2009); and
- Brain-derived neurotrophic factor (BDNF) (Watson et al., 1985).

Whereas many of these factors have been shown to act directly through their associated receptors, it is also possible that they mediate their proliferative effects indirectly, via immunomodulation, neuroprotection, and angiogenesis.

From a clinical perspective, enhancement of NPC proliferation increases the risk of aberrant cell growth and tumor formation, especially because some growth factors are well-known mitogens. Using certain hormones, such as erythropoietin (EPO), to stimulate NPCs may be a safer approach, as their physiological levels fluctuate dramatically with minimal tumorigenic effects. Recent studies have shown a decreased number of apoptotic nuclei ipsilateral to the infarct, suggesting an additional role for EPO in extending cell survival (Wang et al., 2012). Hormones such as growth hormone, melatonin, and estradiol have also been shown to enhance neurogenesis (Kilic et al., 2008; Li et al., 2011a; Blackmore et al., 2012; Chern et al., 2012), and the use of hormone therapy in a clinical setting demands further study.

Promoting Survival

Proliferation of NPCs can occur by shortening the cell cycle, and supporting this process may increase the risk of tumorigenesis. An alternative approach to expanding the NPC pool postischemia is to promote cell survival through inhibition of apoptosis. Within the adult NPC population, 60% of the newborn progeny undergo cell death under baseline conditions (Morshead et al., 1998); hence, sparing the NPCs from cell death would effectively increase the size of the NPC pool. The PI3K-Akt pathway has been shown to be important in NPC survival through the upregulation of anti-apoptotic genes (Sinor and Lillien, 2004). Administration of G-CSF in culture led to strong activation of the PI3K-Akt pathway, resulting in decreased caspase 3/7 activity and decreased apoptosis (Schneider et al., 2005). Adult NPCs express G-CSF receptors, and intravenous administration of G-CSF in stroke-injured rats promoted NPC survival *in vivo*, suggesting a possible anti-apoptotic role for G-CSF on NPCs following ischemia (Schneider et al., 2005). Other factors, such as IGF-1, activate the PI3K-Akt pathway and inhibit apoptosis, thereby promoting cell survival (Kalluri et al., 2007).

More recently, we found that cyclosporine A (CsA), a commonly used immunosuppressive drug, acts directly on NPCs to increase their survival without affecting cell-cycle kinetics (Hunt et al., 2010; Sachewsky et al., 2014). Administration of CsA *in vivo* resulted in >2-fold increase in the size of the NPC pool, and mice that received CsA following stroke showed an expansion of the NPC pool, migration of the NPCs to the site of injury, new tissue formation at the site of cortical ischemia, as well as recovery of motor function (Erlandsson et al., 2011). Hence, prosurvival pathways have demonstrated efficacy in models of stroke.

Other possible mechanisms to increase the size of the NPC pool include activation of signaling pathways that promote a change in the mode of division of NPCs. For example, a change in the mode of

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division of stem cells from asymmetric division (producing one stem cell and one progenitor cell) to symmetric (producing two stem-cell progeny) could effectively double the size of the stem-cell pool with each division. One factor recently identified as a regulator of symmetric divisions in adult neural stem cells is Wnt signaling (Piccin and Morshead, 2011). Active Wnt signaling was found to be necessary for regeneration of the SVZ following ablation of the NPCs *in vivo*. Indeed, in the absence of Wnt signaling, no repopulation of the neural stem-cell pool occurs, as the stem cells do not undergo symmetric division. Moreover, Wnt signaling is upregulated in NPCs in the SVZ following stroke, suggesting that this pathway plays a role in the normal expansion of NPCs observed following stroke alone (Piccin and Morshead, 2011).

Thus, various signaling pathways contribute postischemia to the expansion of the NPC pool through the inhibition of apoptosis, change in the cell-cycle kinetics, mode of cell division, and regulation of proliferation.

Migration

In order for endogenous NPCs to contribute to recovery, it is necessary for NPCs to migrate from the SVZ through the brain parenchyma into the peri-infarct region and further, which must occur in sufficient numbers in order to generate sufficient numbers of cells. A number of studies have looked at the receptor–ligand signaling pathways that are involved in the modest NPC migration that occurs poststroke. Some of the factors involved include the following:

- Stromal-cell-derived factor 1 (SDF-1, also called CXCL12) and CXC chemokine receptor 4 (CXCR4) (Parent et al., 2002);
- Angiopoietin-1 (Ang1) and Tie-2 (Ohab et al., 2006); and
- Monocyte chemoattractant protein-1 (MCP-1; also called CCL2) and CC chemokine receptor 2 (CCR2) (Yan et al., 2007).

With the goal of enhancing migration, controlled delivery of ligands has been examined *in vivo*. Intraventricular administration of SDF-1 in ischemic mice increases the number of doublecortin-expressing neuroblasts that migrate to the peri-infarct region 7 d poststroke (Ohab et al., 2006). CXCR4 antagonists suppress the migration of neuroblasts into the peri-infarct region (Parent et al., 2002), causing neuroblasts to migrate nondirectionally and disperse diffusely throughout the cortex following stroke (Ohab et al., 2006). A similar effect on cell migration is observed with Ang1/Tie-2 signaling modulation by administering Ang1 and Tie-2 antagonist intraventricularly (Ohab et al., 2006). Notably, SDF-1/CXCR4 is also involved in the chemotaxis of inflammatory cells (Stumm and Höllt, 2007); hence, these molecules may lead to a more pronounced immune response and may subsequently counteract neurogenic recovery.

Matrix metalloproteinases (MMPs) have also been implicated in neuroblast migration from the SVZ. MMPs are capable of degrading extracellular matrix proteins, which are involved in the cleavage of cell-surface receptors, and are thought to play a role in many cell behaviors, including proliferation, migration, differentiation, and apoptosis (Van Lint and Libert, 2007). In the adult brain, MMP-3 and -9 have been shown to be expressed by NPCs, and inhibition of MMPs in *in vitro* siRNA experiments (as well as MMP inhibitors in a mouse model) have revealed a reduction in NPC migration following middle cerebral artery occlusion (MCAO) ischemia (Lee et al., 2006). The working hypothesis is that MMPs released by the NPCs digest the ECM to clear the path for neuroblast migration. Considering that MMPs are also expressed by endothelial cells and astrocytes (Wang et al., 2006; Kang et al., 2008), and in light of the increased gliosis that occurs following stroke, these cells may also be contributing to the enhanced migration of NPCs following stroke.

Differentiation and Neurogenesis

The importance of generating new neurons in cell-based therapies has not been completely established, and this is partly due to the fact that when NPCs do appear to differentiate into mature neurons, it is challenging to determine the extent to which these newborn neurons have synaptically and functionally integrated into the neural circuitry. Indeed, it is possible that neurogenesis is not required for functional recovery. At least two studies have shown improvements in functional outcomes despite the lack of neurogenesis at the infarct site after activation factor administration. In a study by Erlandsson et al. (2011), CsA administration led to newly formed tissue at the site of the infarct that contained only newborn glial cells. In Chern et al. (2012), intraperitoneal administration of melatonin after MCAO stroke in mice resulted in a reduction in neurological deficit, even though the increase in mature neurons was not significant. These studies support the possibility that NPCs contribute to recovery through other mechanisms, such as providing factors that prevent secondary stroke damage, or by providing trophic support to existing cells in the infarct region (Lindvall and Kokaia, 2011).

Role of Angiogenesis

Angiogenesis plays an integral role during postischemic recovery and is closely tied to neurogenesis (Zhang and Chopp, 2009). Vasculature not only provides blood supply to the infarct region but also stimulates NPCs by the endothelial secretion of various growth and chemotactic factors. VEGF and IGF-1 are released by endothelial cells in response to ischemia and promote NPC proliferation and differentiation (Dempsey et al., 2003; Teng et al., 2008). The receptor–ligand interaction is also thought to act reciprocally; that is, signaling from NPCs promotes neovascularization, leading to the "coupling" of angiogenesis and neurogenesis (Teng et al., 2008).

Vasculature can also provide a scaffold on which NPCs can migrate, observed by the close association of migrating NPCs and neuroblasts with vascular endothelial cells (Ohab et al., 2006; Kojima et al., 2010). Selective inhibition of VEGF receptor 2 (VEGFR2) attenuates postischemic NPC migration, supporting the role of VEGF in coupled angiogenesis and neurogenesis in stroke recovery (Li et al., 2011b). Similarly, IGF-1, SDF-1/CXCR4, and Ang1/Tie-2 signaling from endothelial cells facilitate the migration of NPCs along blood vessels (Ohab et al., 2006; Thored et al., 2007). These studies strongly support the development of strategies that promote angiogenesis, which will ultimately lead to enhanced migration of NPCs and neurogenesis.

Challenges

One of the challenges in the application of NPC activation in the clinic is the delivery of activation factors. Some studies have used peripheral modes of administration (e.g., intravenous, subcutaneous, intraperitoneal), while others have attempted to target the brain directly. Peripheral administration minimizes trauma to the brain but does not target NPCs directly and may lead to systemic effects that confound the activation factor's effect on NPCs. Alternatively, modes of administration that target the brain directly (e.g., intracerebral/intraventricular infusion of factors/viral vectors) may avoid systemic effects, but damage to the brain tissue and the increased risk of infection make them clinically impractical options. Recently, some bioengineering strategies have looked at a less invasive delivery of factors using an epicortically implanted hydrogel that releases factors in a spatially and temporally defined fashion (Cooke et al., 2011; Yu and Morshead, 2011; Wang et al., 2012). The hydrogel technology delivered the factors of interest locally and resulted in greater brain tissue penetration than when the factors were delivered systemically or intraventricularly. This delivery strategy is a promising methodology for clinical application.

Another barrier to successful activation of endogenous NPCs is the difference in the neurogenic response between young and aged brains. Many studies of activation factors use young adult rodents for stroke models. However, stroke occurs predominantly in the elderly in humans, and consequently, studies need to take this into consideration in order for the results to be translational. While reports have confirmed that postischemic neurogenesis occurs in the aged brain, the response is significantly attenuated (Darsalia et al., 2005; Walter et al., 2010). Furthermore, the response of NPCs to activation factors such as G-CSF and VEGF have been shown to be attenuated or absent in aged mice (Gao et al., 2009; Popa-Wagner et al., 2010; Piccin et al., 2014). Gene expression analysis in the ischemic young and aged brains have shown that the aged brain upregulates the expression of genes related to DNA damage, cell-cycle arrest, apoptosis, and inflammation, while genes related to axon and dendrite growth are downregulated (Buga et al., 2008). These global changes in gene expression may explain the differential response of the aged brain to ischemia.

Successful translation of the endogenous approach to the clinic requires a thorough evaluation of the magnitude of the effect of individual and combinations of factors. Ultimately, a transition to clinical trials will be necessary to assess functional outcomes in humans, as animal models provide limited information about the recovery of functions such as speech, language, higher cognitive function, learning, and memory.

Conclusion

There is much enthusiasm surrounding the potential for NPCs to promote tissue repair and functional recovery following stroke. Both exogenous and endogenous approaches to cell-based therapy using NPCs have shown promising results in preclinical studies. However, it may be that neither alone will be sufficient for promoting full recovery poststroke, especially when considering the need to apply these strategies in old age, when the numbers of NPCs are fewer. Indeed, transition to the clinic will ultimately require rigorous evaluation of not only the activation factors being employed, but also how the exogenous and endogenous approaches can be used together to complement each other, with the end goal of achieving functional and cognitive recovery.

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MRI-Based Measures of Functional Recovery After Stroke

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Introduction

Stroke is a major cause of long-term disability throughout the world, leaving more than half of the patients dependent on daily assistance. Nonetheless, most patients exhibit a certain degree of recovery in the weeks, months, and sometimes even years following stroke, which may be directly related to structural and functional modifications in surviving brain tissue. Several experimental and human stroke studies have reported vicarious function of ipsilesional and contralesional brain regions (Dancause, 2006), which may contribute to restoration of functions, although the exact mechanisms that lead to functional recovery remain largely unclear. Elucidating the critical pathways in poststroke recovery would not only provide important fundamental insights into brain function and plasticity, but could also lead the way toward development of new rehabilitation strategies for recovering stroke patients.

Imaging modalities, such as magnetic resonance imaging (MRI), may significantly contribute to the research on stroke recovery by enabling serial *in vivo* whole-brain measurements of functional and/or anatomical changes in injured brain. Established MRI methods such as T_2-weighted, diffusion-weighted, and perfusion-weighted MRI are frequently applied for assessment of acute stroke pathophysiology in clinical diagnosis and preclinical research (Dijkhuizen and Nicolay, 2003; Weber et al., 2006; Farr and Wegener, 2010). In addition, functional and structural MRI may be used to characterize long-term alterations in brain structure and function after stroke (Dijkhuizen et al., 2012).

Task/Stimulation–Related Functional MRI of Neural Activation

Functional MRI (fMRI) methods are traditionally sensitized to changes in cerebral hemodynamics in response to neuronal activity. The most frequently applied fMRI method is blood oxygenation level–dependent (BOLD) MRI. Alternative techniques include arterial spin labeling (i.e., cerebral blood flow–weighted MRI) and steady-state susceptibility, contrast-enhanced MRI in combination with a (super)paramagnetic blood pool agent (i.e., cerebral blood volume [CBV]–weighted MRI). The principles of these different fMRI methods have been extensively described by Mandeville and Rosen (2002) and Harel et al. (2006).

fMRI enables assessment of human brain activation in relation to sensory, motor, or cognitive tasks or stimuli. Besides, fMRI can be applied in animal experiments, which has provided important insights into the physiological basis of fMRI responses, fundamentals of hemodynamic and metabolic aspects of neuronal activation, and consequences of lesions on cerebral activity (Van der Linden et al., 2007). fMRI studies in stroke patients as well as animal stroke models have revealed diminished activation in the ipsilesional sensorimotor cortex, enhanced contralesional activation, and chronic reinstatement of perilesional activity (Baron et al., 2004; Dijkhuizen et al., 2012).

fMRI-detected activation response to limb stimulation may be transiently absent in the representational somatosensory cortex after stroke, despite normal structural appearance on diffusion- and T_2-weighted MR images, and intact vasoreactivity (Dijkhuizen et al., 2003; Weber et al., 2008). Functional denervation, depression, or deterioration of surviving neurons, as well as derangement of neurovascular coupling, could explain the temporary lack of responsiveness. The preservation or reinstatement of activation within the ipsilesional sensorimotor cortex has been found to be associated with functional recovery after transient middle cerebral artery occlusion in rats (Dijkhuizen et al., 2003; Weber et al., 2008); these findings emphasize the significance of the reorganization of affected cortical representational fields for poststroke functional recovery.

Analogous to findings in human stroke patients, a rise in contralesional fMRI activity has been reported after experimental unilateral cerebral ischemia; this rise was found to correlate with the extent of tissue injury in the ipsilesional sensorimotor cortex (Dijkhuizen et al., 2003). The relevance of enhanced activation in the unaffected hemisphere remains controversial; it may hypothetically contribute to functional recovery, but could also be a direct pathophysiological consequence (e.g., due to broad disinhibition) of stroke.

Resting-State Functional MRI of Functional Brain Connectivity

An alternative fMRI method, known as resting-state fMRI (rs-fMRI), assesses spatial functional correlations within neural networks without the need for a stimulation paradigm (Biswal et al., 1995). During the 2000s, rs-fMRI has been increasingly applied as a tool for studying alterations in the brain's intrinsic functional architecture as potential physiological correlates of neurological and psychiatric disorders (Fox and Raichle, 2007). Spontaneous fluctuations in baseline ("resting-state") neuronal signaling are reflected in low-frequency fluctuations (<0.1 Hz) of the BOLD signal and show temporal coherence between anatomically

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connected brain regions within a particular neuronal network, such as the sensorimotor system (Biswal et al., 1995). Throughout the gray matter, the extent of synchronization between these low-frequency BOLD fluctuations is related to functional connectivity. Correlation of these signals with EEG-measured brain activity has indicated that these slow hemodynamic fluctuations are associated with neuronal function (Laufs et al, 2003).

Functional connectivity measurements with rs-fMRI provide a unique way to depict spatiotemporal characteristics of reorganization in functional neuronal networks after ischemic brain injury (Grefkes and Fink, 2014). Pioneering studies in humans and rats have revealed that strong correlations between rs-fMRI signals in ipsilateral and contralateral cortical sensorimotor regions disappear during acute to subacute phases after unilateral stroke, indicating loss in interhemispheric functional connectivity (Carter et al., 2010; Van Meer et al., 2010). In subsequent weeks, and coinciding with recovery of sensorimotor function, interhemispheric functional connectivity was partially retrieved, as shown in a rat stroke model (Van Meer et al., 2010) (Fig. 1).

Functional connectivity has been most often computed by calculating correlations between the low-frequency BOLD fluctuations in a brain region selected a priori, and low-frequency BOLD fluctuations from other voxels in the brain. Besides region-based functional connectivity analyses, functional connectivity may also be assessed at a whole-network level by means of graph analysis (Reijneveld et al., 2007; Bullmore and Sporns, 2009). Networks can be represented as graphs containing nodes and edges. In rs-fMRI, the image voxels represent the nodes, and the intervoxel correlations between low-frequency BOLD fluctuations represent the edges. The network's structure can be assessed by measuring its clustering coefficient (a measure of segregation that reflects the degree to which nodes are clustered) and the shortest path length (a measure of integration that reflects the minimum number of edges between any pair of nodes). A high clustering coefficient and low average shortest path length indicate a small-world network topology, which is proposed to be an optimal network configuration for global information transfer and local processing (Reijneveld et al., 2007; Bullmore and Sporns, 2009). Graph-based analysis of rs-fMRI data from chronic human stroke subjects has shown a decrease in clustering coefficient and a shift toward a random network configuration in the motor system (Wang et al., 2010). Graph analysis of rs-fMRI data from rats recovering from unilateral stroke has pointed toward increases in clustering coefficient, average shortest path length, and small-world index in the intact bilateral sensorimotor cortices at early stages, which normalized chronically (Van Meer et al., 2012). These findings emphasize that large-scale networks outside the ischemic area rearrange over time. Speculatively, higher values of the calculated network parameters seen subacutely after stroke may

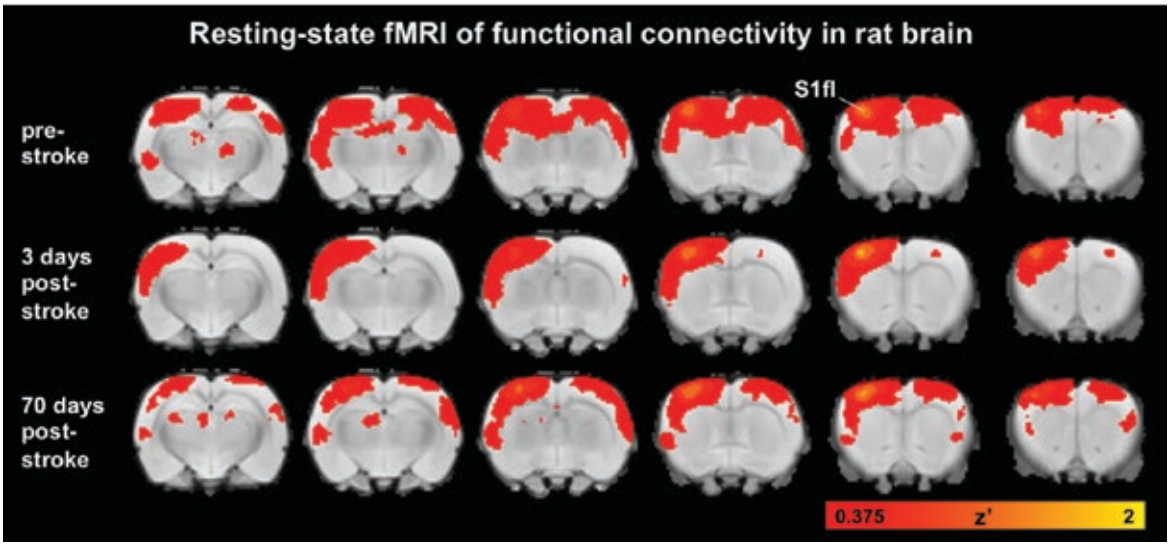

Figure 1. Maps of mean functional brain connectivity of the left (contralesional after stroke) forelimb region of the primary somatosensory cortex (S1fl) in rats before, and at 3 d and 70 d after 90 min occlusion of the right middle cerebral artery ($n = 5$). T_2-weighted multislice anatomical rat brain template. Loss of functional connectivity with the ipsilesional sensorimotor cortex was evident at 3 d, and functional connectivity was restored at 10 weeks after stroke. Adapted with permission from Dijkhuizen et al., 2012, their Fig. 4.

be a sign of diffuse overconnectivity that precedes maturation toward a normally functioning circuitry within the reorganized network.

Diffusion Tensor Imaging of White Matter Structure

Changes in functional brain organization are often closely associated with structural modification of neuronal elements in the brain. Diffusion tensor imaging (DTI) offers an MRI-based means for assessing neuroanatomical changes associated with brain injury and repair. DTI informs on the three-dimensional displacement of tissue water, mathematically characterized by an effective diffusion tensor consisting of nine matrix elements, which can be exploited to assess the microstructure of gray and white matter tissue (Basser and Jones, 2002; Mori and Zhang, 2006). Because the diffusion of tissue water is restricted by the presence and orientation of biological barriers, such as cell membranes and myelinated fibers, stroke-induced structural modifications therein can significantly alter the characteristics of tissue water diffusion, such as the DTI-derived axial, radial, and mean diffusivity (MD), as well as fractional anisotropy (FA). These changes may involve different processes at different poststroke stages, ranging from acute cell swelling (causing MD decrease) and subacute cell lysis and demyelination (causing MD increase and FA decrease) to chronic axonal regeneration or remyelination and gliosis (causing FA increase) (Sotak, 2002; Jiang et al., 2010; Dijkhuizen et al., 2012).

Because white matter tracts are composed of highly oriented fibers, which cause relatively high anisotropy of diffusing tissue water, DTI is well suited for measuring effects on white matter integrity. For example, loss of FA in ipsilesional white matter after (ischemic) brain injury has been linked to demyelination or axonal loss (Assaf and Pasternak, 2008). Serial DTI studies in experimental stroke models have demonstrated that this initial decrease of FA may be chronically followed by normalization or elevation in the ischemic lesion borderzone (Van der Zijden et al., 2008; Jiang et al., 2010; Van Meer et al., 2012). This area revealed a high density of axons and myelin on postmortem histological sections (Jiang et al., 2010; Van Meer et al., 2012).

Even so, the relationship between tissue diffusion characteristics and histo(patho)logical features is highly complex (Rudrapatna et al., 2014). In the ipsilesional internal capsule with elevated FA in poststroke rat brains, significant manganese enhancement on T_1-weighted MRI has been observed after injecting the paramagnetic neuronal tracer into the perilesional sensorimotor cortex (Van der Zijden et al., 2008). This suggests that rearrangement of white matter in the ischemic boundary is accompanied by preservation or restoration of neuronal connectivity. Figure 2 shows postmortem high-resolution FA maps that were collected at 11 weeks poststroke, which clearly illustrate the FA rise in perilesional white matter at chronic stages after stroke (Dijkhuizen et al., 2012). In accordance with these findings in animal

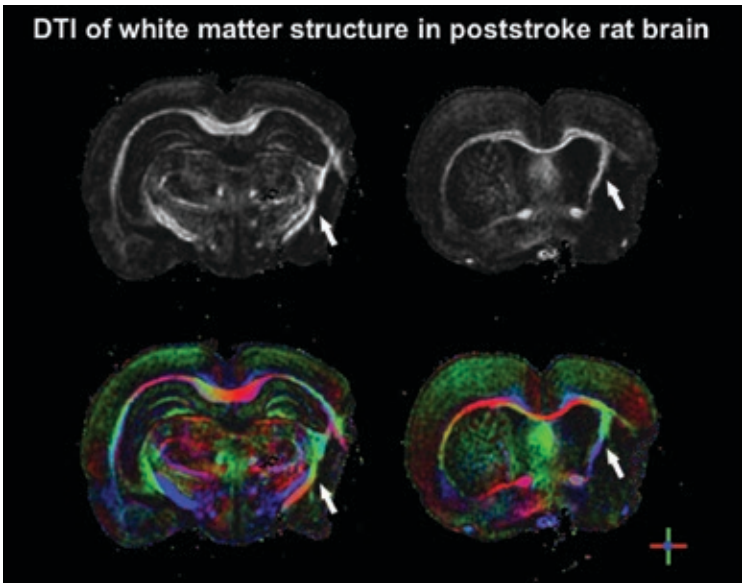

Figure 2. Postmortem FA maps (top, gray-scale values; bottom, color-coded orientations) of consecutive coronal rat brain slices at 11 weeks after 90 min occlusion of the right middle cerebral artery, calculated from DTI with high spatial (200 × 200 × 200 μm^3 voxel size) and angular resolution (120 diffusion-weighted directions). Arrows indicate the increased FA in white matter around the lesion (with low FA). Adapted with permission sfrom Dijkhuizen et al., 2012, their Fig. 4.

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stroke models, elevated FA in ipsilesional corticospinal tracts has also been found in chronic stroke patients, and has been associated with improved motor function (Schaechter et al., 2009). Evidently, structural integrity of the corticospinal pathway appears critical for a favorable outcome in sensorimotor performance after stroke (Van Meer et al., 2012).

Conclusions

MRI offers a powerful means to assess functional activity and structural integrity of the brain that can be exploited to evaluate the spatiotemporal pattern of changes after stroke in both clinical and preclinical settings. Especially the combination of *in vivo* functional MRI and DTI techniques provides a unique complementary approach for investigating the interaction of reorganization of neuronal networks in relation to function. MRI may therefore significantly contribute to (1) elucidating cerebral rearrangements that underlie functional recovery, (2) predicting outcomes, and (3) monitoring therapeutic strategies that promote brain repair. In the coming years, multiparametric MRI studies aimed at mapping the complex process of brain reorganization after ischemic injury, conducted in parallel in human patients and animal models, may help to unravel the mechanisms that underlie loss and restoration of function after stroke. Ultimately, this field of inquiry could lead to the development of more effective diagnosis and treatment strategies for recovering stroke patients.

Acknowledgments

This chapter was modified and excerpted from Dijkhuizen et al., 2012, which gives an overview of the application of fMRI and DTI methods for assessing poststroke brain reorganization.

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Intracranial Brain–Computer Interfaces for Communication and Control

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Introduction

Hundreds of thousands of people worldwide have a CNS injury or disorder that leaves them cognitively intact but unable to move their limbs, and in some cases, unable to speak. For people with brainstem or subcortical stroke, one could identify the core injury leading to paralysis as being a "disconnection" between motor cortical areas and the brainstem or spinal nuclei responsible for effecting speech and movement. Conventional assistive devices for people with severe motor disabilities are inherently limited, relying on spared movements as controllers. Building on decades of publicly funded, fundamental neuroscience research (which, notably, has largely been presented at annual Society for Neuroscience meetings and NIH Neural Interfaces workshops) (Pancrazio, 2009), several laboratories are working to develop a more powerful method for restoring communication and function to people with paralysis: brain–computer interfaces (BCIs). It is hoped that BCIs will allow people to control devices such as computer cursors, robotic limbs, and perhaps their own limbs once again directly with their neural activity.

Signal Sources, Electrode Types, and Recording from Inside the Brain

BCIs consist of electrodes to record neural activity from the brain, a computer algorithm ("decoder") that interprets this activity and turns it into a control signal, and an effector (often a computer cursor or robotic arm) that is directly controlled by this decoded signal. The brain area chosen for BCI electrode placement depends on the desired type of control signal and application (see discussion later in this section). Based on where the electrodes are placed, BCIs can be broadly categorized as "scalp surface" if they do not require surgery (e.g., standard EEG electrodes) or "intracranial" if they require surgery (e.g., depth electrodes). Although there are risks inherent in surgical electrode implantation, the demonstrated safety of implanted neurotechnologies such as cochlear implants and deep-brain stimulators has set a precedent for the anticipated eventual clinical viability of intracranial BCIs.

Intracranial BCIs can be further subdivided into those using electrodes placed under the cranium but above the dura (epidural), placed below the dura (subdural), or placed so that they penetrate into the brain (intraparenchymal), usually the cortex (intracortical). The use of epidural and subdural electrocorticography (ECoG) signals for BCI control has been demonstrated with some success in able-bodied epilepsy patients undergoing seizure focus localization prior to resective surgery (Leuthardt et al., 2004, 2006; Hinterberger et al., 2008; Schalk et al., 2008; Blakely et al., 2009; Miller et al., 2010; Vansteensel et al., 2010; Milekovic et al., 2012). This was also attempted in a person with complete locked-in syndrome who normally communicated using imagery that would change her measured salivary pH, though her attempts at communication via an ECoG hemispheric grid were not successful (Birbaumer et al., 2006).

Although the word "invasive" is sometimes applied to intracranial BCIs and meant to imply "more risky because it's implanted more deeply," depth of implantation is not necessarily linked to risk. For example, more than 90,000 people have received deep-brain stimulators (DBSs) for symptomatic management of movement disorders such as Parkinson's disease. DBS electrodes are approximately 40 times the length of a typical intracortical recording electrode used for BCI control, and yet they have become the standard of care for medically refractory Parkinson's disease. In contrast, the neurotrophic "cone" electrode used in some intracortical BCI studies (Kennedy, 1989; Kennedy et al., 1992) is a thin, 1–2 mm long glass cone containing several conductive wires and nerve growth factor, which encourages neurites to grow through it, allowing spikes to be recorded from several neurons; and the "Utah" microelectrode array (Blackrock Microsystems, Salt Lake City, UT) used in other BCI studies and clinical trials (Hochberg et al., 2006, 2012; Collinger et al., 2013) is a 4 × 4 mm silicon array of 100 electrodes, each 1–1.5 mm long. This array displaces approximately 2 orders of magnitude less volume than a DBS electrode, and can record spikes from dozens of individual neurons, sometimes even after several years of implantation (Suner et al., 2005; Chestek et al., 2011; Simeral et al., 2011; Hochberg et al., 2012). These small platforms also introduce much less total foreign matter into the body than an epidural or subdural ECoG grid, whose surface area is often several centimeters in each dimension.

While surgery must always be considered carefully, "invasiveness" is rarely the relevant metric, and the concept of implanting devices in the body, including in or around the CNS, is now commonplace. In the future, we anticipate that decisions regarding BCI choice will be no different than any other medical decision; they will be made after careful assessment and discussion of relative risk versus benefit, both of which can be understood only after extensive clinical research has been performed.

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Although individual users ultimately will choose which type of BCI to use, some relevant factors to consider include the following:

(1) Compared with "less invasive" methods, intracortical electrodes are smaller and implanted closer to the neurons they record. Hence, the neural signals they record have higher spatial resolution, are less subject to attenuation and distortion by intervening biological tissues, and are less contaminated by artifacts from scalp and ocular muscle activity, thus providing a higher signal-to-noise ratio.

(2) The closer proximity and higher spatial resolution of intracortical recording electrodes allow more information to be obtained about the person's intended movements natively from the recorded neural activity, without requiring extensive training on the part of the user. For example, neurons in many motor cortical areas have "salt-and-pepper" tuning properties; i.e., neighboring neurons are not necessarily tuned to similar movement intentions. Thus, large electrodes such as those used in ECoG arrays (>500 μm of conductive surface) do not record individual neurons' spikes; instead, they record field potentials reflecting the postsynaptic activity summed across thousands of neurons, blurring the specificity of the signals that might have been apparent if they had been sampled at higher spatial resolutions. In contrast, microelectrodes are small enough (~5–10 μm of conductive surface) to record spiking activity from individual neurons in addition to local field potentials. Microelectrode arrays record the activity of many individual neurons within a single motor cortical area, allowing the user to deploy natural, intuitive motor imagery to control the BCI. In other words, using a BCI controlled with signals from intracortical microelectrode arrays, the user might simply imagine moving his or her hand in a particular direction through 3D space to move a robotic hand in that same direction. This approach has been used to allow several participants with long-standing tetraplegia to neurally control computer cursors or robotic arms (Figs. 1, 2) by simply imagining moving their own arm or hand (Hochberg et al., 2006, 2012; Kim et al., 2008; Simeral et al., 2011; Collinger et al., 2013).

(3) The daily setup of intracranial BCIs is quick and easy compared with noninvasive methods, and such setup may entirely disappear once the fully implanted wireless systems under development are deployed (Kim et al., 2009; Nurmikko et al., 2010; Borton et al., 2013). This is an important advantage because only a permanently attached (which usually means fully implanted) device can be ready to function 24 h per day, 7 d per week, and has the potential to be used without assistance from an able-bodied caregiver.

On the other hand, a common reason for using large (EEG or ECoG) electrodes for BCI control is that the recorded signals are believed to be more stable over both short (minutes to hours) and long (days to months) timescales than the spiking activity recorded using microelectrodes (Donoghue et al., 2004; Dickey et al., 2009; Chao et al., 2010). Small physical shifts (on the order of several microns) between the brain and the electrodes, as might happen with, for example, sudden head accelerations (Santhanam et al., 2007) or spurious changes in local intracranial pressure, make less of a difference when the signal combines the averaged activity of thousands of neurons than when the signal is spiking activity from individual neurons. In the latter case, relative movements of several microns can change the amplitude of the recorded neurons' spikes or cause different sets of neurons to appear on those electrodes (Santhanam et al., 2007; Chestek et al., 2011). However, methods are being developed that allow microelectrode-based BCIs to yield stable neural control on both short and long timescales despite these nonstationarities. For example, on short timescales, adaptive decoders can compensate for changes in response properties during real-time neural control by tracking changes in individual neurons' firing rates and/or by changing the model that maps neural activity to desired motor output (Wessberg et al., 2000; Taylor et al., 2002; Wahnoun et al., 2006; Jarosiewicz et al., 2008; Velliste et al., 2008; Shpigelman et al., 2009; Li et al., 2011; Gilja et al., 2012; Hochberg et al., 2012; Homer et al., 2012; Orsborn et al., 2012; Sussillo et al., 2012). Treating all spikes that cross a certain threshold on a given channel as one signal (Carmena et al., 2003; Fraser et al., 2009) instead of attempting to isolate individual neurons' spikes based on their shape or amplitude further increases the short-timescale stability of microelectrode recordings and allows for stable neural control for years (Chestek et al., 2011; Gilja et al., 2012; Hochberg et al., 2012), even if spiking signals are no longer clearly separable from background noise (Polikov et al., 2005; Chestek et al., 2011). And finally, if any biases persist in the decoded output despite the corrections performed on the neural data, they can be ameliorated by estimating and directly subtracting them out from the decoded trajectory command (Hochberg et al., 2012).

Implanted neural interface systems still undoubtedly require more research and development before they

become clinically useful (see Future Engineering Efforts, below), but given the neural control that has already been demonstrated with current technology in persons with tetraplegia (Hochberg et al., 2006, 2012; Collinger et al., 2013), we are optimistic about the future of intracortical BCIs for helping to restore independence to people with paralysis and other neurological and motor disabilities. The rest of this chapter will focus on the current state of research and development of intracortical BCIs.

Direct Speech BCIs

A relatively new line of BCI research is the development of "direct speech" BCIs, whose goal is to decode desired speech sounds in real time, motivated by a desire to improve upon slow, letter-selection-based assistive communication technologies. For direct speech BCIs, electrodes have been placed in the left ventral premotor cortex (Guenther et al., 2009), which is thought to encode the formant frequencies of desired speech acoustics (Guenther et al., 2006). Trajectories through as low as three-dimensional (3D) formant frequency space can be perceived by human listeners as the sentences from which they were derived, despite the absence of harmonics, consonants, and other traditional acoustic cues (Remez et al., 1981); thus, a low-dimensional formant frequency space should theoretically provide a manifold for decoding desired speech sound trajectories from neural activity, using methods analogous to the decoding of trajectories through 2D or 3D physical space from motor cortex (see Motor BCIs, below). Although this method has not yet been demonstrated to allow real-time speech synthesis, preliminary results are promising, demonstrating that a participant who is locked in because of a brainstem stroke improved with practice at neurally mimicking two-vowel sequences in a 2D formant frequency space (Guenther et al., 2009). Direct speech BCIs will undoubtedly be further informed by ongoing research to understand the neural activity underlying speech production (Bouchard et al., 2013).

Motor BCIs

Motor BCIs convert intended movement activities into the motion of an external device. Such devices could be used either for rehabilitation or neurorestoration, and include the continuous point-and-click control of a cursor on a computer screen (as is provided by a standard computer mouse), a robotic assistive device, a prosthetic arm and hand, or even a functional electrical stimulation system that reanimates the movement of one's own paralyzed arm or hand. The rest of this chapter will focus on details of motor BCI research, although many of these concepts will also generalize to direct speech BCIs.

Continuous decoders

For BCIs designed to restore movement, electrodes have often been placed in the arm/hand area (Yousry, 1997) of primary motor cortex (MI), where neurons modulate their spiking activity with specific intended movements of the arm and hand. To a first approximation, each neuron has a baseline firing rate at rest; it increases its firing rate with intended hand movements in a particular direction, called the neuron's "preferred direction" (PD), and decreases its firing rate for intended movements in the opposite direction, with roughly cosine-shaped tuning in between (Georgopoulos et al., 1982; Schwartz et al., 1988; Ashe and Georgopoulos, 1994; Fu et al., 1995; Moran and Schwartz, 1999; Paninski et al., 2004). Modulation of MI neurons with movement intention or motor imagery occurs even in the absence of movement (Taylor et al., 2002; Hochberg et al., 2006; Fetz, 2007), making this area a suitable signal source for BCI control. Intuitively, if spiking activity is recorded from a population of individual neurons with known PDs, it is possible to decode the direction of the desired movement at each moment by comparing the firing rate of each neuron at that moment to its baseline firing rate. This decoded movement intention can then be used to drive the moment-to-moment movement of a cursor on a computer screen, a robotic limb or other prosthetic or assistive device, or (eventually) the person's own limb through functional electrical stimulation (Chadwick et al., 2011; Ethier et al., 2012).

The assumption of cosine tuning with intended movement direction (or velocity) helps to simplify the decoding of intended movements from MI neural activity; thus, it is commonly used as the "encoding" model (i.e., the assumed neural tuning model) in BCI decoding algorithms. The simplest decoding algorithm, called the "population vector algorithm" (PVA), obtains an estimate of the desired movement direction by simply summing the "votes" of all recorded neurons, where each neuron's vote is its PD scaled by its normalized firing rate (Georgopoulos et al., 1983, 1986, 1988; Moran and Schwartz, 1999). The PVA has been shown to yield high-performance 2D and 3D closed-loop neural control over cursors and robotic arms in nonhuman primates (Talyor et al., 2002; Jarosiewicz et al., 2008; Velliste et al., 2008). A major shortcoming of the PVA, however, is that it gives rise to biases in the estimate of both the desired movement direction and speed when the PDs of the population are not distributed uniformly

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(Zhang et al., 1998; Chase et al., 2009; Koyama et al., 2010). The optimal linear estimator (OLE) is a modified version of the PVA that corrects for these biases, providing more accurate decoding of intended movements by essentially shifting its model of each cell's PD (Salinas and Abbott, 1994; Zhang et al., 1998; Chase et al., 2009); the OLE has been shown to provide good multidimensional control over a robotic arm by a person with tetraplegia (Collinger et al., 2013).

Because firing rates are extremely noisy within the small time bins (~20–200 ms) typically used in real-time decoding, smoothing is required to keep the decoded trajectories from also being extremely noisy. In the OLE and PVA, smoothing is often accomplished by averaging the spike rates over the last several time bins using a sliding window (Taylor et al., 2002; Jarosiewicz et al., 2008; Velliste et al., 2008; Chase et al., 2009). An alternative method is to smooth the trajectories themselves. One way to do this is by using state-space methods (Brown et al., 1998; Brockwell et al., 2004) such as the Kalman filter (Wu et al., 2006; Yu et al., 2007; Kim et al., 2008; Malik et al., 2011), which allow a "state model" to be specified that describes the decoded variables (e.g., position, direction, and velocity) as likely to evolve smoothly from one time step to the next. The state model can be calibrated using the statistical properties of natural arm movements (e.g., in able-bodied primate labs) or of a set of idealized trajectories of, say, a cursor moving to targets on a computer screen. The Kalman filter (Wu et al., 2006; Kim et al., 2008; Malik et al., 2011) has been used for real-time decoding in people with tetraplegia in the BrainGate2 pilot clinical trials, allowing for good 2D cursor control and 3D control of robotic arms (Hochberg et al., 2006, 2012; Kim et al,. 2008; Simeral et al., 2011).

Neural tuning in motor cortex is in reality far more complex than cosine tuning to desired end-point direction or velocity in extrinsic space; neural responses can also be influenced by several other factors, including, for example, the following:

- Position, speed, and acceleration (Georgopoulos et al., 1984; Kettner et al., 1988; Ashe and Georgopoulos, 1994; Fu et al., 1995; Moran and Schwartz, 1999; Paninski et al., 2004; Churchland and Shenoy, 2007);
- Time along the trajectory and reach distance (Fu et al., 1995; Churchland and Shenoy, 2007);
- Posture, arm position, joint angles, muscle activation, and force (Caminiti et al., 1990; Taira et al., 1996; Scott and Kalaska, 1997; Sergio and Kalaska, 1998; Kakei et al., 1999; Ajemian et al., 2000; Wu and Hatsopoulos, 2006; Oby et al., 2013); and
- Motor or visuomotor perturbations (Wise et al., 1998; Gandolfo et al., 2000; Li et al., 2001; Paz et al., 2003, 2004).

Indeed, some studies suggest that motor cortical neurons are not actually "tuned" to any particular (sets of) parameters; instead, their activity can be more succinctly described as part of the neural population's trajectory within a dynamical system state space, and the apparent tuning of individual neurons is an incidental by-product of the spurious correlations between their complex mechanistic roles and specific components of the task (Todorov and Jordan, 2002; Wu and Hatsopoulos, 2006; Churchland and Shenoy, 2007; Churachland et al., 2010).

Although more complex tuning models and more sophisticated decoders that try to capture some of the complexity in the neural signals might give better offline results, evidence is accumulating that they do not necessarily improve real-time, closed-loop neural control because visually guided error correction can readily compensate for inaccuracies in decoding (Chase et al., 2009; Koyama et al., 2010; Cunningham et al., 2011). Furthermore, the longer computation time required by more complex decoders translates into a longer delay between the issued movement command and visual feedback, and real-time neural control is better when the feedback loop is shorter because it allows for faster error correction (Cunningham et al., 2011). Additionally, motor cortical neurons appear to be plastic enough to adapt their tuning to the specific ways in which their activity is decoded by a BCI: If the decoder assumes cosine tuning, the neurons become more cosine-tuned; and if the decoder models the preferred directions of the neurons differently than their native preferred directions, the neurons' actual preferred directions shift toward the modeled ones (Taylor et al., 2002; Carmena et al., 2003; Jarosiewicz et al., 2008; Ganguly and Carmena, 2009). Thus, given the tradeoff between computational speed and the sophistication of the decoding methods, the fact that closed-loop neural control is best when the feedback loop is as short as possible (Cunningham et al., 2011) and that neural plasticity can compensate for slight inaccuracies in the particulars of the tuning model (Taylor et al., 2002; Carmena et al., 2003; Jarosiewicz et al., 2008; Ganguly and Carmena, 2009) suggests that it might make sense for now to err on the side of simplicity and computational speed. However, as computer processors become faster, computational simplicity might become less of a priority, so more

sophisticated decoding models and algorithms might exceed the capabilities of simple models during real-time neural control.

Discrete decoders (classifiers)

In addition to decoding continuous quantities such as direction or velocity for BCI control, it is possible to use a classifier to decode two or more discrete "states" from neural activity. Selecting among a finite, predefined set of states (e.g., targets on a computer screen, discrete hand positions) is computationally simpler than decoding a continuous quantity such as the entire path to the target, and it can be useful in BCI applications when there is a finite set of possible targets. Intuitively, classifiers work by modeling the relationship between "training" sets of data (e.g., neural activity) and their corresponding "labels" (desired states, e.g., discrete screen targets, hand positions) and then using this model to predict the desired states from new, incoming patterns of neural data (Duda et al., 2001; Singer and Kreiman, 2012). For example, one type of classifier, known as linear discriminant analysis (LDA), attempts to maximally separate two classes of data by projecting the data from both classes onto a line that maximizes the variance between groups while minimizing the variance within them, and then choosing a boundary between the two classes (Fisher, 1936). This method has been used in real-time BCIs in people with paralysis to control the open/closed state of a neurally controlled robotic hand (Hochberg et al., 2012) or to "click" in a neurally controlled point-and-click computer application (Kim et al., 2011; Simeral et al., 2011). Other approaches include the use of Bayesian classification methods to decode a desired target among an array of possibilities and immediately move the cursor there (Musallam et al., 2004; Santhanam et al., 2006). These studies often use neural signals recorded from posterior parietal and/or dorsal premotor cortex, which had previously been found to contain neurons that are active during reach planning and that encode high-order, goal-related information (Snyder et al., 1997, 1998; Messier and Kalaska, 2000; Andersen and Buneo, 2002; Shenoy et al., 2003; Hatsopoulos et al., 2004). Such classification methods could eventually be extended, for example, to the fast sequential selection of desired letters in a neurally controlled typing interface.

Figure 1. BrainGate2 clinical trial participant neurally controlling a robotic arm to give herself a drink of coffee, more than 14 years after becoming tetraplegic and anarthric from a pontine infarction (Hochberg et al., 2012). Caution: Investigational device. Limited by federal law to investigational use.

Figure 2. University of Pittsburgh clinical trial participant who has tetraplegia, resulting from spinocerebellar degeneration, neurally controlling a robotic arm to feed herself a bar of chocolate (Collinger et al., 2013). Photo courtesy of UPMC. Caution: Investigational device. Limited by federal law to investigational use.

Although discrete decoders have the potential to allow for faster sequences of selections when there are a finite number of targets, continuous decoding of the entire desired path has the important advantage of generalizability. For example, continuous 2D plus click control over a BCI incorporating a point-and-click communication interface would allow people with severe motor disabilities to use any computer application that an able-bodied person can use, without any modifications to the computer hardware or software other than changing the source of the input signal from a mouse to a BCI (Bacher et al., 2011). Furthermore, a BCI that allowed the user to specify the entire path, and possibly the articulation of each joint, in a 3D robotic arm or functional electrical stimulation (FES) system would allow the user to avoid any physical obstacles using visual feedback, obviating the need for solutions to the difficult 3D computer vision and inverse kinematics problems that would be necessary for automated obstacle avoidance.

Future Engineering Efforts

Several engineering obstacles remain before the current, investigational implanted BCI technologies

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can become clinically useful to a large number of people. First, the quality of neural control obtained with BCIs in people with paralysis is still far from able-bodied motor performance. As mentioned above, efforts are under way in many laboratories to improve neural control by exploring more sophisticated decoding models and algorithms. Furthermore, implanting multiple electrode arrays will increase the amount of signal obtained about movement intentions, increasing the quality of decoding and making the system more robust to signal nonstationarities. Multiple arrays can be placed to span different areas of the motor homunculus to allow the decoding of movement intentions of multiple parts of the body. They can also be placed bilaterally to allow, for example, the independent control of effectors on both sides of the body (e.g., both arms through FES, or two separate robotic or prosthetic arms).

Second, in the current state of intracortical motor BCI development, an able-bodied person must be present for various stages of BCI use. For example, someone must physically attach a cable to a percutaneous connector mounted on the participant's head. Developing a fully implantable, wireless recording system (an ongoing effort in several laboratories [Kim et al., 2009; Nurmikko et al., 2010]) would help to reduce this dependency on caregivers. In addition, it would reduce the risk of infection and render the system more aesthetically acceptable (indeed, it would become "invisible" to someone looking at its user, as is a fully implanted cardiac pacemaker or deep-brain stimulator). Also, intracortical BCIs currently require the intervention of a trained technician to select the task or application to be run, decide when to recalibrate the neural decoder, choose the neural signals and time periods to include in each calibration, etc. If BCIs are to help restore independence to people with paralysis, each of these stages must become automated or user controlled.

One key step, currently under development (Jarosiewicz et al., 2012), will be the automated calibration of the decoder during ongoing, practical use of the BCI. Decoder calibration has traditionally been performed by recording neural activity while the user imagines moving a preprogrammed cursor or robot arm (Hochberg et al., 2006; Simeral et al., 2011) or while he or she actually moves it under neural control toward prespecified targets (Taylor et al., 2002; Helms et al., 2003; Wahnoun et al. 2006; Jarosiewicz et al., 2008; Velliste et al., 2008; Fraser et al., 2009; Shpigelman et al., 2009; Li et al., 2011; Gilja et al., 2012; Hochberg et al., 2012; Orsborn et al., 2012; Sussillo et al., 2012; Collinger et al., 2013). However, during practical use of the BCI, the targets will not be prespecified; instead, the user will select items from a (possibly infinite) array of possibilities. Recent preliminary work has shown that it is possible to continuously maintain decoder calibration during practical use of a communication interface by mapping ongoing neural activity to desired movements that are retrospectively inferred based on the user's subsequent selections (Jarosiewicz et al., 2012). In theory, this method could maintain effective neural control for an indefinite amount of time in any neurally controlled point-and-click application in which the user's intended movements can be inferred retrospectively, without the need to pause the system for periodic calibration.

Third, the state-of-the-art electrode arrays currently used for human BCI research have not yet demonstrated the desired longevity. Although useful neural signals can still be recorded as long as five years after the implant date (Hochberg et al., 2012), the individual neuronal activities are sometimes not as robustly recorded after years of sensor implant as they were in the first months after implantation, likely owing to a combination of engineering, mechanical, and biological factors (Polikov et al., 2005; Chestek et al., 2011). Efforts are under way to improve the design and functional lifetime of currently used electrode array technology, and in parallel, to develop novel electrode technologies that might be able to provide new solutions in the future (Kennedy, 1989; Neves and Ruther, 2007; Kipke et al., 2008; Kozai et al., 2008; Grill et al., 2009; Harris et al., 2011).

Fourth, the decoding hardware is currently bulky, consisting of a neural signal processing unit and several rack-mounted computers. Efforts are also under way to make the system portable by compiling the neural signal processing and decoding software to run on a field-programmable gate array.

The field of clinical BCI research and development is still in its infancy, but there is a recent expansion in the number of clinical trial sites that are translating the efforts of the nonhuman primate BCI laboratories to people with tetraplegia. Given the progress that has already been made and the ever-growing enthusiasm of talented neuroscientists, engineers, and clinicians for intracortical BCI research, we are optimistic about the prospect of intracortical BCIs soon helping to restore independence to people with tetraplegia and other neurological and motor disabilities.

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