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Functional MRI and other non-invasive imaging technologies: Providing visual biomarkers for spinal cord structure and function after injury

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Abstract

Substantial progress has been made towards understanding the molecular basis for limited endogenous central nervous system (CNS) axonal growth after injuries such as spinal cord trauma. Realization of the potential benefit of therapeutic interventions requires methods to assess axonal growth and functional reorganization over time after neurological damage. Here, we discuss the technical challenges of analyzing and interpreting the effects of various interventions on CNS repair, specifically in the context of spinal cord injury. Evolving technologies such as functional magnetic resonance imaging and other non-invasive imaging techniques will be reviewed. These technologies should revolutionize our ability to track changes in both CNS structure and function.

Introduction

Potential treatments for spinal cord injury (SCI) are evaluated in two ways, anatomically and functionally, in an attempt to correlate changes in anatomy with changes in functions such as ambulation. However, several enormous gaps exist in our ability to investigate the effects of various interventions on recovery from SCI: One, we cannot perform histopathological analyses of injured spinal cord tissue in living patients. Two, even in animal models, it is not often feasible to perform serial anatomical studies of the CNS over time in the same animal. Generally, different cohorts of animals are examined at different time points after injury and intervention, inevitably introducing inter-subject variation that may confound results.

Furthermore, despite intense research into tracing and visualization techniques, the interpretation of anatomical changes during and after spinal cord injury and associated interventions is fraught with difficulty. The distinction between true axonal regeneration, sprouting from uninjured fibers, and synaptic plasticity continues to present a challenging technical task, often leading to heated debate over which processes have occurred in any given experiment, which process matters more, or whether the difference really matters in the first place.

As for functional analysis, there is more consensus over standardized motor behavior tests, but tests to assess sensory changes or pain are woefully inadequate in animals. Moreover, all too often, behavioral changes after SCI interventions do not correlate well with anatomical changes (or lack thereof).

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An increasing number of axon growth-promoting drugs are currently in or on the verge of entering human clinical trials (Dergham, et al., 2002, Freund, et al., 2006, Lee, et al., 2003, Rossignol, et al., 2007). Therefore, both to improve the reliability of animal models, and to allow tracking of the effects of interventions on human recovery from SCI, we require technology that enables non-invasive serial imaging over time of both spinal cord anatomy and function. Correlating changes in function with changes in structure in real time will help resolve debates over the contribution of regeneration versus sprouting versus plasticity toward recovery from SCI. In addition, these measures can provide crucial surrogate endpoints in future clinical trials.

Such technology is rapidly evolving, in both animal models and humans. Of several non-invasive methods for assessing spinal cord anatomy and function in the living animal, magnetic resonance imaging (MRI)-based approaches may hold the most potential. A recent publication in *Experimental Neurology* by Endo et al. exemplifies the use of functional MRI (fMRI) to open windows upon spinal cord functions that were previously almost impossible to observe in animal models (Endo, et al., 2008). We will attempt to put these findings into the broader context of human application, and discuss future issues in the field of structural-functional spinal cord imaging.

Imaging neuronal activity through blood oxygenation

Neurons voraciously consume oxygen derived from a rich vascular supply. Stimuli resulting in areas of increased neuronal activity lead to increased extraction of oxygen from local capillaries, followed within seconds by increased local blood flow. In 1990, Ogawa et al. exploited the different magnetic properties of deoxyhemoglobin relative to oxyhemoglobin to demonstrate that deoxyhemoglobin can be utilized as a natural MRI contrast agent (Ogawa, et al., 1990). This blood oxygen level-dependent (BOLD) signal increases as deoxyhemoglobin decreases due to increased local blood flow.

Numerous studies in the next few years showed that areas of increased neuronal activity display increased BOLD-fMRI signal, allowing non-invasive real-time imaging of brain activation patterns in both normal and diseased subjects. For instance, visual stimulation with a red checkerboard pattern stimulates increased BOLD-fMRI signal in the calcarine cortex of normal volunteers (Frahm, et al., 1992). The BOLD-fMRI-positive area of motor cortex associated with a fine motor task expands over weeks of task-specific training in normal volunteers, thus directly demonstrating cortical plasticity in the living human brain (Karni, et al., 1995).

By the mid-1990s, the power of fMRI to image functioning areas of the CNS was being applied toward the injured CNS, shedding more light on which regions are necessary for proper task performance, and allowing non-invasive tracking of disease course and post-injury recovery. One of the more exciting recent findings using fMRI of the injured human brain showed that cerebral activation areas of patients in a minimally conscious or even a vegetative state resemble that of healthy controls in response to a verbal stimulus (Owen, et al., 2006, Schiff, et al., 2005). Thus, fMRI provides a means of measuring higher cortical activity in subjects who otherwise cannot communicate.

Likewise, fMRI can be used to non-invasively monitor neuronal functions in animals that otherwise could be measured only by observing behavioral responses to various stimuli. This has opened the doorway to studying experimental injury paradigms that are not possible with humans.

fMRI in spinal cord injury

Obtaining high-resolution images of the spinal cord via MRI is complicated by its narrow, elongated geometry as well as by motion artifacts induced by respiration and heartbeat. These technical problems have been steadily reduced by the use of stronger magnetic fields and synchronized scanning protocols timed to occur at the same phase of each respiratory or cardiac cycle (Stroman, 2005). Lars Olson and colleagues have published several papers in *Experimental Neurology* and other journals applying MRI to animal models of SCI, with particular focus on somatosensory function and its relation to pain. Lilja et al. demonstrated that spinal cord fMRI can distinguish nociceptive from tactile stimuli (Lilja, et al., 2006). In unlesioned rats, sub-painful sensory stimuli result in increased BOLD signal in the medulla, whereas stronger stimuli cause increased BOLD signal in the ipsilateral spinal cord dorsal horn. These sites of BOLD activation correlate with the primary relay stations for the posterior columns and spinothalamic tracts, respectively. Thus, by visualizing different distributions of neuronal activation in response to painful versus non-painful stimuli, fMRI provides a more informative readout than behavioral assays such as limb retraction from a hot water bath. But how does this readout change in response to SCI?

Using somatosensory cortical fMRI, Hofstetter et al. found that whereas rats suffering either mild or moderate thoracic spinal contusion injury both recover near-normal motor function, they display divergent recovery of sensory function (Hofstetter, et al., 2003). Only mildly injured, not moderately injured rats recovered sensorimotor cortex activation on BOLD-fMRI in response to hindlimb stimulation. Interestingly, the region of cortical activation in recovered rats was significantly larger than that of control rats, a finding that could correlate with the increased incidence of chronic pain syndromes in human SCI patients.

Olson and colleagues have gone on to examine sensory circuit changes in rats following spinal cord transection rather than contusion. Though cord transection is not as closely correlated with clinical SCI, it provides a more definitive and reproducible experimental paradigm than cord contusion. In January's issue of *Experimental Neurology*, Endo et al. analyzed activation of the nociceptive spinothalamic pathway by measuring BOLD-fMRI signal in the dorsal horn caudal to a thoracic transection injury (Endo, et al., 2008). At three to six months post-injury, hindlimb stimulation with a weak electrical current resulted in significant lumbar dorsal horn (spinothalamic) activation in injured rats. The same weak current applied to control rats usually activates neurons only in the medulla/posterior column pathway. Stronger stimuli resulted in larger dorsal horn BOLD responses in the injured rats than in control rats. These findings suggest possible mechanisms not only for increased pain in SCI patients, but also for another unfortunate sequela of SCI – the phenomenon of allodynia, the transformation of normally innocuous stimuli into painful sensations. Perhaps fMRI could reveal a similarly maladaptive increase in neuronal activity in response to stimuli such as bowel distention, thereby improving our understanding of autonomic dysreflexia following SCI.

The Olson group has also found that forelimb stimulation after thoracic cord transection results in enlargement of the normal area of sensorimotor cortex activation, from forelimb cortex toward the adjacent hindlimb cortex, which had lost its afferent input due to the transection (Endo, et al., 2007). Additionally, the fMRI findings were compared with in situ staining of brain tissue for expression of myelin-associated growth inhibitors such as Nogo Receptor. Levels of Nogo Receptor and LINGO-1 decreased in precisely the border areas between the forelimb cortex and the deafferented hindlimb cortex, providing another mechanistic clue to cortical plasticity following CNS injury.

These papers from the Olson group, along with several other papers focusing on fMRI in SCI (Stroman, et al., 2004, Stroman, et al., 2002), have established baseline parameters for the serial

non-invasive assessment of neuronal function after SCI in animal models. The stage is set for expanding these studies to assess the effects of various interventions on recovery from SCI. The functional effects of these interventions can now be monitored more directly than by cumbersome behavioral assays subject to numerous confounders. Of course, it would be essential to learn the results of simultaneous monitoring of the entire spinal cord-cerebral axis under these conditions, rather than isolated segments of these circuits, as in the papers described above. How might other technologies compare with and possibly aid BOLD-fMRI in these investigations?

Other methods of visualizing neuronal activity within the spinal cord

Neurophysiological ‘imaging’ techniques

Neuroscientists owe much of the foundations for structural-functional brain correlation to Wilder Penfield’s pioneering studies using direct electrocortical stimulation on awake patients during neurosurgical operative procedures (Penfield and Jasper, 1954). Likewise, techniques using surface electrodes to record endogenous cortical electrical activity (electroencephalography or EEG) or evoked cortical potentials from stimulatory limb electrodes (somatosensory evoked potentials or SSEP) are still a mainstay in the fields of epilepsy and multiple sclerosis, respectively. However, despite the enormous utility of Penfield’s homunculus, and the clinical value of EEG and SSEP, these methods are not actually ‘imaging’ techniques, nor are they a feasible approach for large-scale, longitudinal studies of either human or animal CNS.

Transcranial magnetic stimulation (TMS)

TMS holds the distinction of doubling as both a diagnostic and therapeutic non-invasive modality. Brief external magnetic pulses trigger action potentials in underlying neurons. These magnetic evoked potentials (MEPs) may be triggered over the motor cortex and recorded in peripheral muscle via electromyography. Parameters such as conduction time, stimulus threshold, and regional boundaries of trigger activation can be measured to reveal both cortical and spinal cord functional connections and plasticity (George, et al., 2003). Longer trains of magnetic pulses, known as repetitive TMS (rTMS), can disrupt function of specific brain areas, taking functional mapping a step further by allowing direct demonstration of a region’s necessity for performing a given task (George, et al., 2003). Therapeutically, rTMS can induce long-term synaptic changes (either inhibitory or stimulatory) in specific brain regions, with promising initial results in diseases ranging from depression to pain to dystonia and other diseases (Klein, et al., 1999, Lefaucheur, et al., 2001, Siebner, et al., 1999). However, unlike BOLD-fMRI, TMS cannot visualize endogenously initiated neural activity. Two other drawbacks to TMS are its potential to induce seizures in susceptible patients, and its lower spatial resolution compared with MRI.

Diffusion tensor imaging (DTI)

Like fMRI, DTI-MRI represents a non-invasive imaging technique that can be applied to both healthy and injured humans and animals. DTI exploits the more orderly diffusion of extracellular water parallel to organized fibrous tissue such as white matter tracts. DTI-MRI therefore represents a potential complement to BOLD-fMRI, as it visualizes the anatomy of nerve tracts, whereas fMRI visualizes activity of neuronal cell bodies. DTI has successfully depicted axon tracts in the healthy brain as well as disrupted tracts due to tumors, stroke, or other lesions (Tharin and Golby, 2007). Fujiyoshi et al. recently showed the potential of DTI’s application to SCI by obtaining beautiful images of lesioned descending and ascending tracts after lateral cervical hemisection in marmosets (Fujiyoshi, et al., 2007). DTI also shows promise for tracking axonal degeneration secondary to demyelinating disease (DeBoy, et al., 2007). A significant remaining technical hurdle derives from the voxel-based volume-

averaging calculations involved in DTI – though DTI can show integrity of various white matter tracts, it is less specific at defining relative numbers of axons in each tract (Fujiyoshi, et al., 2007). Therapies for SCI may result in regeneration or sprouting of only a few fibers, often in somewhat tortuous rather than parallel routes. DTI needs further improvement to be able to more accurately depict small numbers of regenerating or sprouting fibers. Finally, though DTI can provide tremendous information on CNS structural integrity, it does not visualize the actual function of neuronal processes.

Manganese-enhanced MRI (ME-MRI)

Divalent manganese enters cells through calcium channels, uses axonal transport machinery to travel along neuronal projections, crosses synapses, and is paramagnetic, allowing visualization with T1-weighted MRI (Pautler, 2006). Since neuronal activation consists partly of calcium influx, ME-MRI provides an alternative to hemoglobin/BOLD for tracking activity. However, this requires direct injection into the nervous system of an ion that has been associated with a variety of neurotoxic effects, especially on basal ganglia pathways (Pautler, 2006). Therefore, this minimally-invasive imaging technique will remain limited to animal use.

Positron emission tomography (PET)

In contrast to other radiological methods in which the subject is exposed to an external radioactive or magnetic field, PET relies on detection of positrons emitted from internally-ingested radioactive tracer compounds. For example, when the oxygen-15 isotope is injected intravenously, its distribution correlates with regional blood flow, analogous to BOLD-fMRI. Fluorodeoxyglucose provides an even more direct correlate of neuronal activity. In the context of spinal cord imaging, PET is generally used to define metabolically-active lesions representing tumors or infections (MacVicar, 2006). In animal models, PET is being adapted to the study of SCI by using tracers that label descending tracts such as the serotonergic raphespinal tract (Huang, et al., 2004). Using such tract-specific tracers, animals can be longitudinally monitored for tract regeneration following various investigational treatments. However, PET has several major disadvantages compared with fMRI. Resolution is lower; partially-invasive intravenous access is required; potentially toxic radioactive isotopes are ingested; and expensive, nearby cyclotron facilities are required to synthesize these ultra-short-lived radiotracers (MacVicar, 2006, Tharin and Golby, 2007).

Multiphoton (MP) microscopy

MP imaging exploits the fact that when photons of lower energy (longer wavelength) collide, they produce a local higher-energy (shorter wavelength) photon that can trigger fluorescent emission from tissue in the immediate vicinity, without exposing other planes of the tissue to toxic high-energy photons. This, combined with the greater tissue-penetration of longer-wavelength light, allows imaging of living tissue at depths of 0.5mm or greater. Using this technique, Svoboda and colleagues have illuminated the dynamics of dendritic spine plasticity in response to sensory experience in rodents (Holtmaat, et al., 2005, Trachtenberg, et al., 2002). However, the level of expertise required, combined with the limited depth penetration and requirement for fluorescently-labeled tissue, limit the widespread use of in vivo MP imaging. Furthermore, the invasive windowing techniques limit the application of MP imaging strictly to research animals.

Limitations and questions regarding spinal cord fMRI

Though spinal cord fMRI opens whole new avenues of potential investigation, several technological hurdles remain to be overcome.

Does BOLD signal accurately reflect neuronal activity in the context of injured tissue?

CNS trauma leads to both acute and chronic disruptions of vascular supply and inflammation that likely alter local blood flow independently of neuronal activity, potentially resulting in spurious or misinterpreted changes in BOLD signal. This could confound studies in both animal models and human patients. For example, in the context of brain injury, Murata et al. demonstrated that due to increased neuronal oxygen extraction, both oxyhemoglobin and deoxyhemoglobin increase in response to neuronal activation near infarcted cortical areas three to 12 months after ischemic stroke (Murata, et al., 2002). This results in silent or even decreased BOLD signal precisely where neurons are working hardest. In addition, it remains unclear whether increased numbers of reactive astrocytes and other cell types in the vicinity of injured tissue may further distort local blood flow and oxygenation. In fact, even normal brain can show varied correlations of neuronal activation, local blood flow, and local ratios of oxyhemoglobin to deoxyhemoglobin, depending on age, cortical region, and stimulus intensity (Harel, et al., 2002, Hillary and Biswal, 2007, Murata, et al., 2002, Seiyama, et al., 2003, Seiyama, et al., 2004). Functional MRI acquisition and analysis techniques need to be further developed to account for these potential confounders in both normal and injured brain and spinal cord. Until then, ME-MRI provides an alternative correlate of neuronal activity, based on manganese (calcium) influx rather than local blood flow. But as discussed above, this invasive technique is restricted to animal use only.

How many of the demonstrated changes in fMRI are due to *new* connections, versus unmasking of previously *latent* connections?

As mentioned in the introduction, the relative contribution of newly regenerated or sprouted fibers versus plasticity of pre-existing synapses represents a key and controversial question in the field. Altered activity of neuronal cell bodies may not clearly differentiate between regenerated versus plastic synaptic changes. Furthermore, BOLD signal changes are generally detectable only in gray matter. This neglects the functional integrity of spinal cord white matter tracts. Therefore, it is essential that the structural tract anatomy revealed by DTI-MRI be further improved, and that the data obtained from BOLD and diffusion tensor imaging be better integrated to address this question (Fujiyoshi, et al., 2007, Tharin and Golby, 2007).

Animal fMRI requires sedative and paralytic drugs

Anesthetics reduce the magnitude of cerebral activation signals on BOLD-fMRI (Lahti, et al., 1999). How do they affect simultaneous spinal cord activation patterns? Sedative and anesthetic drugs and dosages continue to be refined to allow reliable data to be obtained without unacceptable distress to the animal. However, most protocols still involve the use of muscle paralytics such as pancuronium. Thus, it is difficult to envision a simple method of stimulating and recording motor activity while keeping the small animal immobilized enough to acquire accurate images.

Quantitative fMRI results usually reflect averaging from a population of subjects – how will fMRI provide useful prognostic information for the individual patient?

Many studies comparing groups of subjects project and normalize raw BOLD data onto a standardized anatomical template. The statistical analysis of the raw data and these projections can be quite complicated, but sophisticated techniques have emerged (Skudlarski, et al., 1999). The question remains, however, whether injuries that disrupt the anatomical integrity of the spinal cord will require alternative methods of normalizing raw BOLD data, both for comparison among different subjects, and eventually for deriving useful information that will allow individual doctors to give individualized advice to individual patients.

Future Applications

Current and future advances in structural-functional imaging technology will allow scientists to address numerous questions that have been begging for answers via non-invasive, longitudinal studies, in both human patients and animal models. What are the mechanisms for development of post-SCI neuropathic or ‘phantom’ pain? How do central and peripheral circuits interact to produce problems such as autonomic dysreflexia, bladder dyssynergia, and limb spasms in SCI patients? What are the anatomical correlates of functional recovery and adaptation toward SCI over time? How do various interventions affect these processes? These questions will be addressed not only in the context of traumatic SCI, but also in other diseases affecting the spinal cord, such as amyotrophic lateral sclerosis and multiple sclerosis (not to mention numerous applications in normal and diseased brain).

Different imaging techniques will be (and already are being) combined to exponentially increase the knowledge gained from these types of studies. Combination BOLD-fMRI/TMS will allow simultaneous manipulation and observation of different neural circuits and regions (Bohning, et al., 1999). Combination BOLD-fMRI/DTI-MRI should be able to track the regrowth of damaged tracts connecting functional neurons. BOLD-fMRI/PET studies could demonstrate the location and timing of various radiolabeled drugs’ effects on the nervous system. The information gleaned from these technologies will not just expand our understanding of nervous system function and repair, but will provide crucial new biomarkers for tracking the effects of various interventions in future clinical trials, as well as for more accurately predicting the prognosis for individual SCI patients.

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