Session Title: Functional neuroimaging within hours and days after stroke or brain injury
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Questions for Discussion:
What limitations and opportunities are posed by neural, metabolic, and blood flow/volume factors?
Can fMRI, TMS, NIRS, etc complement the assessment of diffusion-perfusion MR imaging, DTI or spectroscopy for viable tissue that may be available to subserve later recovery?
What would be a set of standard activation paradigms to test for this possibility?

Limitations & opportunities re: blood flow, etc.

Hemodynamic Changes in Subacute and Acute Poststroke Stages
It is important to consider issues related to hemodynamic changes in the hours and days after stroke or brain injury. Acute stroke is an evolving event in which there may be opportunities to intervene over several days following the insult. Dysfunctional regions within the ischemic penumbra may include viable tissue, but how long it will survive without intervention is unknown.

Hypoperfusion and Reperfusion
Restoration of blood flow is an important mechanism supporting recovery of function in the acute stage. This mechanism can be studied pre- and post-intervention using MR perfusion weighted imaging (PWI) to assess severity of hypoperfusion, diffusion weighted imaging (DWI) to assess extent of lesion, and language or other functional testing. Reperfusion of hypoperfused regions that correlate with improvements in language performance in acute aphasia patients can also provide evidence of regions that are essential to linguistic functions (Hillis et al., 2006). For example, Hillis et al. (2001) performed detailed single subject studies on six patients who had small infarcts surrounded by larger regions of hypoperfusion including BA 22 and BA 37. Patients were tested daily on oral naming and spoken comprehension and scanned pre- and post-intervention to pharmacologically improve blood pressure. Time-to-peak (TTP) was measured and compared with TTP in the contralateral (right) hemisphere in these two regions. Improvement in naming in the first 2-3 days poststroke was correlated with improved blood flow (to less than 2.5s delay relative to normal hemisphere) in all six patients. Use of serial PWI to assess temporarily dysfunctional tissue in acute stroke also provides converging evidence of brain/behavior relationships prior to functional reorganization, in this case demonstrating that BA 22 is essential to oral naming and comprehension.

More recently, in a study of 50 patients, Kannan, Kleinman, et al. (2007) demonstrated that deficits in word comprehension were most strongly associated with abnormal diffusion weighted imaging (DWI) or perfusion weighted imaging (PWI) in posterior superior temporal (BA 22) and parietal cortex (BA40), where blood flow was at least 4s delayed compared to homologous regions.

In a DWI/PWI study of 170 acute patients within 48 hours poststroke, Hillis et al. (2006) provided evidence that semantic errors in oral naming and/or comprehension can arise from damage or dysfunction of different brain regions. Specifically, damage or dysfunction in BA 22 or 21 predicted errors in both naming and comprehension, while hypoperfusion in BA 37 (sparing BA 21) predicted semantic errors in naming alone.

Studies of motor recovery have also demonstrated restoration of function following reperfusion of hypoperfused penumbral areas. For example, Kleiser et al. (2005) demonstrated a “PWI-DWI mismatch” (an area of impaired perfusion at risk of infarction) in two acute stroke patients. After Internal Carotid Artery (ICA) recanalization, language (for patient with LH infarct) and motor task-specific (for patient with RH infarct) activation could be seen utilizing BOLD fMRI in peri-infarct

regions that were previously acutely hypoperfused. This study provides evidence that at-risk tissue can survive, regain functionality, and support poststroke recovery.

The (ICA) Balloon Occlusion technique was utilized to test neurobehavioral effects associated with induced changes in cerebral hemodynamics in 44 patients[1] (Marshall et al., 2001). Variability in patients' performance was monitored during a sustained attention task in which patients were required to estimate 10-13s time intervals with a button press. Three groups emerged after carotid occlusion: one group demonstrated normal absolute cerebral blood flow (CBF) while maintaining consistent performance; a second group experienced a drop in CBF along with deterioration in task performance that spontaneously returned to normal; a third group demonstrated the largest effect in lowered CBF and was unable to recover task performance until the carotid occlusion was reversed. These results suggest that some compensatory mechanisms related to functional reorganization may be occurring in the hyperacute state in the face of persistent hypoperfusion.

Patients without infarct, but with unilateral cerebral hypoperfusion in the absence of stroke, may also demonstrate functional reorganization[2] (Marshall et al., 2006). Fourteen such patients were studied with fMRI BOLD during a motor task. Activations in nine of the 14 with abnormal vasomotor reactivity significantly differed from those of control subjects, showing ipsilateral motor activation in homologous regions in the non-hypoperfused hemisphere. These findings are significant in that they suggest that hypoperfusion alone, in the absence of demonstrable brain injury on anatomical imaing may be sufficient to induce brain reorganization.

Some patients can have normal function in spite of chronic hypoperfusion. Mast et al. (1995)[3] studied a patient with arteriovenous malformation (AVM) who was administered a superselective anesthetic injection (WADA) during language testing. This patient demonstrated very low blood flow (25 mm Hg). In spite of ischemia, tissue was evidently normal, because the patient temporarily exhibited symptoms of Wernicke's aphasia after the injection. In fact, many chronic aphasia patients demonstrate hypoperfusion that doesn't move into infarct. With restored blood flow some do recover function, but it depends on the level of ischemia, area of brain, and timing.

Recommendations:
It is critical to consider the ongoing dynamic state of the vasculature in this equation. Use of multimodal imaging techniques can provide critical diagnostic information that may identify salvageable penumbral tissue in the early hours and days poststroke. These methods can also provide converging evidence regarding necessary and sufficient structure/function relationships in the brain.

1) Can fMRI, TMS, NIRS, etc complement the assessment of diffusion-perfusion MR imaging, DTI or spectroscopy for viable tissue that may be available to subserve later recovery?

It is possible that ischemia and the PWI-DWI mismatch may be part of a neuroprotective mechanism, but studying task-related activation in patients in the hyperacute phase is fraught with difficulties. Not many studies have attempted to examine patients in the early hours poststroke. Kidwell examined 18 patients within the first six hours, but in 16 of the patients massive head movement rendered the results uninterpretable.

Questions also remain concerning the implications of chronic low blood flow with regard to imaging methods. It is unknown, for example, whether the BOLD signal is normal in global hypoperfusion. It has been demonstrated to be undistinguishable from that of control subjects (Krakauer et al Ann Neurol 2004, Chmayssani et al Neurology 2007)[4, 5].However, other studies (e.g., Roc et al., 2006)[6] suggest that BOLD activation in patients with altered CBF may be quite different from healthy controls in spite of equivalent task performance on a simple visuomotor task. Roc and her colleagues demonstrated characteristically different hemodynamic response functions (HRF) in primary motor cortex in patients with hemodynamically significant stenoses,

compared with neurologically healthy controls, in spite of no differences in the motor performance between groups.

The question remains whether or not blood flow is a good marker of neural activity in these patients. In rats, most show a BOLD response but with delayed peak, e.g., 6-8 s. In addition, in the contralateral hemisphere, the signal may be accelerated when one carotid is tied off. Since the BOLD response is associated with recruitment of additional blood flow, and blood flow may be compromised, it is advisable to examine individual patients' HRF, rather than to rely on the canonical HRF during statistical analysis. Even normal subjects demonstrate different HRF between subjects, between regions, and over time. Patients' HRF in particular can be expected to evolve over time.

Perhaps the most illustrative case is a patient who had recurrent transient ischemic attacks (TIAs) and no activation in ipsilateral motor cortex although he appeared behaviorally normal. It is possible that lack of activation in some patients during normal behavioral performance is related to impaired vasomotor reactivity, rather than carotid stenosis per se. This could create a lack of sensitivity to the BOLD response that is not related to hypoperfusion in the absolute sense, but rather to the fact that increased blood during the resting state changes the relative amplitude. Migraine studies have also shown a reduced BOLD response during flashing checkerboard paradigms.To assess the impact of hypoperfusion it may be better to evaluate the activity in the opposite, non-hypoperfused hemisphere, shown to be increased in the setting of chronic carotid disease (Marshall et al JCBFM 2006)[2] and to return to normal in the setting of revascularization (Chmayssani et al Neurol2007(abs))[4].

It is also possible that accuracy in localization may be "off" in terms of so-called perilesional activation, e.g., whether the region selected by BOLD response is related to maximum neural activation or whether it may be cms away from the actual centroid of neural activation. It may possibly be related to the degree of latency in TTP. Perilesional activation could also just reflect restored blood flow in the region, rather than a marker of neural activation. It's an empirical question, but the study hasn't been done.

Other methods have been used to predict recovery including examining secondary degeneration using diffusion tensor MRI ([DTI]; e.g., Liang et al., 2007)[7]. Twelve patients w/ subcortical infarct involving the posterior limb of the internal capsule underwent DTI at 1, 4, and 12 weeks post-stroke. Correlations between per cent changes in DTI measures (mean diffusivity and fractional anisotropy [FA]) and clinical scores (NIH Stroke Scale, Fugl-Meyer scale and Barthel index) were assessed. Low FA values were correlated with relatively impaired recovery, suggesting that FA may be useful as a physiological marker for recovery. Questions remain, however, regarding individual vs. group level predictability and the timecourse of degeneration, and the degree to which edema may change the amount of free water effecting FA. These questions hamper the current clinical usefulness of DTI.

Magnetoencephalography (MEG) is another non-hemodynamic dependent measure that has demonstrated the ability to predict longterm effective recovery (Tecchio et al., 2007)[8]. Thirty-two patients were studied at two timepoints: acutely and once stabilized. Clinical outcomes were grouped at three levels: worsening, partial recovery, and complete recovery. Two variables were observed to predict recovery or worsening of symptoms. In addition to lesion volume, an increase in delta activity in the contralateral hemisphere was predictive of worsening of symptoms. It was hypothesized that the contralateral correlation may be due to neuromodulation, i.e., the perilesional region's release of inhibitory effects on the unaffected homologous region. EEG could also be used to obtain similar delta range data. Although this is speculative, there are plans to improve on this study by refining the electrophysiological data and providing more integration with the anatomical data.

Recommendations:
These studies demonstrate potential clinical applications for predicting early poststroke patterns of activation (or physiological markers) that map onto later functional recovery or impairment. Currently, a lot of studies exclude patients with hypoperfusion due to imaging issues but these patients make up approximately 15% of stroke patients. Perhaps this exclusionary criteria needs to be reconsidered. At the same time, the BOLD response and its relationship to hypoperfusion is not well understood. Therefore, converging evidence from different imaging methods may help to assess the validity of the coupling between neural activity, blood flow, and the BOLD response. One could compare FDG PET to BOLD activation, or alternatively, one could use a modality that doesn't depend on blood flow, e.g., DTI, MEG or EEG and then compare and contrast between modalities. Multiple collection of data over the timecourse of evolution of the stroke is recommended. Examination of individual patients' HRF is also recommended, rather than relying on the canonical HRF during statistical analysis.

Alternative methods in fMRI analysis
There may be a performance confound when comparing patients to controls. When looking at the relationship between recovery and activation, the performance-related activation can't be disambiguated from reorganization per se. Often statistical localization of activations is abused by subjecting it to reverse inference, i.e., "brain area, *x*, was activated, therefore cognitive process, *y*, was engaged". Brain imaging can be useful even without statistical localization: 1) to test for existence of brain-behavior correlations; 2) to test for cognitive or other physiologic dissociations (via qualitative differences); or 3) to predict behavior, clinical diagnoses, or outcomes.

Multivariate testing in neuroimaging is not valid when the number of voxels is greater than the number of subjects. Using the multivariate linear model (MLM; Worsely et al., 1997)[9], sensitivity for detection of diffuse effects is much greater than for localization. The typical trade-off between specificity and sensitivity is reflected in the power of localization vs. detection. The MLM uses a global test, i.e., testing all voxels simultaneously. It is much more sensitive to spatially diffused effects.

Analyses often rely on qualitative differences, e.g., we decide whether two conditions (or groups) engage the same or different brain circuits by examining whether the same (suprathreshold) blobs appear. But there is no formal statistical test for inferencing "blob pattern similarity". What if the same circuit is engaged but simply to a greater degree in one condition than another? Typically, we would miss this pattern, because we would be missing all the information below the (arbitrary) spm threshold.

The MLM can test whether activation patterns in stroke patients are qualitatively different from controls, e.g., by testing for qualitative difference between left and right motor/sensory activation, or a squeeze test at different force rates. For example, one experiment asked if there were correlations between motor recovery at 3 months and brain activation in the first 48 hours poststroke (Krakauer, Zarahn, Lazar, Marshall 2007, unpublished data). To avoid the performance confound, motor recovery was represented as a change in score, i.e., the difference between the 3 month motor score minus the acute 48 hr motor score (Fugl-Meyer scale). Analysis included a 48 hr motor score covariate. The MLM tested whether the best combination of voxels to predict change does better than what is expected under the null hypothesis. Results detected a correlation between the delta score and activation. The pattern of recovery was qualitatively different from the motor execution pattern. The authors suggest that neural plasticity underlying recovery doesn't simply involve an amplification of the motor execution network. In a traditional analysis, very few voxels would have survived the spm threshold for correction of multiple comparisons, in spite of a very strong global effect. Instead the MLM analysis yielded a prediction of the recovery pattern, accounting for 87% of the variance. Patients were included who were unable to move their affected hand at all. They weren't outliers, but rather fit well into

the curve. The unaffected hand also showed correlation with recovery. It is possible that derangements in CBF could have contributed to the conclusions of the study, but simply an overall lowered CBF would not have. Patients with large vessel disease were excluded. There are no claims being made about the mechanism underlying the recovery pattern, simply that the pattern is qualitatively different from that of normal subjects.

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