# Functional brain imaging in stroke patients

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## Introduction

Neuroimaging already plays a crucial role in the initial assessment of stroke patients. Different types of scans can reveal different properties of the brain that can be combined synergistically to guide initial treatment. In addition, these different modalities hold the potential to impact the long term prognosis. While traditional structural scans locate the extent of the injury, other modalities such as functional imaging can help assess the extent of disruption which may be far more extensive than the core lesion visible on anatomical scans. For example, a brain region that appears structurally intact following injury may be functionally compromised because it has insufficient blood flow to function correctly (misery perfusion), has been disconnected from other regions, or relies on information from a distant region that has been injured. In all these cases, functional imaging can provide information not available from structural scans.

Indeed, functional imaging acquired in healthy individuals has already transformed our understanding of the human brain. Therefore, one might expect that functional imaging will have the same transformative impact in our understanding of stroke. Here, we temper this enthusiasm, noting some of the challenges and limitations associated with functional imaging of stroke. The aim is to provide a balanced and informed foundation for understanding the potential for this method. While we clearly recognize the potential for this modality in stroke patients, the method must be used carefully and the findings must be interpreted in context of the inherent limitations of this technique.

Brain imaging has had a profound impact on acute stroke management. Both computerized tomography (CT) and Magnetic Resonance Imaging (MRI) can help differentiate ischemic and hemorrhagic injury, and both from other neurological events that may have similar behavioral presentation to a stroke. Further, acute stroke management already leverages the fact that different imaging modalities provide complementary information regarding injury. A concrete example of this synergy comes from the combination of diffusion and perfusion measures in hyper-acute stroke management. Diffusion images acquired at the patient's admission can

reveal lost tissue. On the other hand, perfusion images that measure blood flow can identify tissue at risk. Taken together, these two modalities can suggest tissue that might be salvaged by intervention as well as helping to more accurately predict eventual lesion size. For this reason, the diffusion-perfusion mismatch has helped refine standard of care, leading to, e.g., the rationale for thrombectomy for large vessel occlusion.

Beyond these well established benefits for acute care, brain imaging in stroke holds great promise for both theoretical and clinical questions. Analyzing the territory of brain lesions can provide intrinsic knowledge into the function of the human brain. Since Broca's time many of our insights regarding cognitive function have come from observing the consequences of brain injury. Textbook descriptions of human language, memory, emotion, motor control and perception have all had their foundation in neuropsychology. A skeptic could argue that brain injury had an impact in Broca's era simply because it was the only method available. Today, we have numerous methods that allow us to observe the healthy human brain directly and non-invasively. Thus, it seems that modern neuroscientists should focus on healthy humans to make inferences about brain function. However, even in modern times analyses of brain lesions still provides an indispensable method (Rorden and Karnath, 2004). Brain injury can provide a perspective of normal brain function that is not possible merely from observing healthy brain function. First, brain injury helps identify regions that are required rather than merely associated with cognitive functions (Rorden and Karnath, 2004). In contrast, brain activation techniques – such as functional MRI – in healthy humans are unable to distinguish between regions that are correlated versus those that are necessary for a given function. As an analogy, observing the brain is like listening to a highly trained orchestra, where it can be hard to distinguish the contribution of a single musician because the whole network of the symphony is working together in concert. In contrast, if one section (say, violins) stops playing we can become aware of their contribution to the network. Observing how a network is disrupted can prove a powerful tool for understanding the interactions of the network. While there are some non-invasive methods that can transiently disrupt normal brain function (e.g., Transcranial Magnetic Stimulation

[TMS] and Transcranial Direct Current Stimulation [tDCS]), these methods can only target certain regions and have a relatively subtle and brief effect (for review, e.g., Shin et al., 2012; Horvath et al., 2015; Woods et al., 2016; Lage et al., 2016; Beaulieu et al., 2017). While we do not claim that stroke provides a superior method to understand the healthy brain, we do think it provides a complementary tool. Contemporary cognitive neuroscience relies on numerous tools, each with a unique set of strengths and limitations. In this context, understanding the consequences of brain injury can fill an important niche.

Beyond these clear theoretical insights, neuroimaging can impact clinical care. First of all, one of the most salient questions of patients, their family and their doctors is the amount of recovery expected. Brain imaging can improve the quality of prognosis, identifying which individuals are likely to get better spontaneously, those that will benefit from a specific treatment and those where compensation may prove more beneficial than attempts to recover lost skills (e.g., Naeser et al., 1998; Karnath et al., 2011; Basilakos et al., 2014; Lunven et al., 2015; Hope et al., 2017). In the same way that contemporary genetics can help optimize cancer treatment, we envision that brain imaging will help select optimal therapy. Better prognosis can also improve clinical trials, more accurately matching patients who are receiving different treatments based on their expected level of recovery (and thereby increasing statistical power). Finally, brain imaging can help guide brain stimulation, ensuring that stimulation is applied to intact portions of eloquent cortex. Since the location of injury varies across patients, one may want to maximize the likelihood that the stimulation is being applied to a spared region that is involved with the task, rather than destroyed tissue. Alternatively, one might want to target regions that are distant from the injury but where changes in response are predictive of good outcome. While functional imaging can aid all of these questions, this final implication uniquely relies on measuring the residual brain's function because this information cannot be inferred directly from other modalities.

Modern MRI can acquire a broad range of modalities that are able to reveal different properties of the human brain. Diffusion measures can not only detect acute injuries, but it can also be used to assess the integrity of the white matter connections.

Structural measures like fluid-attenuated inversion recovery (FLAIR) can reveal the structural extent of a brain injury. Furthermore, FLAIR imaging can help identify if the individual has signs of other pathology such as, e.g., white matter hyperintensities that can be predictive of poor stroke recovery (e.g., Bahrainwala et al., 2014). These general measures of brain health may provide biomarkers for cognitive reserve, which can aid prognosis and treatment.

The focus of this chapter will be on neuroimaging measures of brain function, which can identify brain regions where activity levels are modulated by specific tasks demands. For example, if we ask an individual to read a text while we acquire functional scans we can identify brain areas that respond to visual stimuli and language comprehension.

## 1. Measuring Blood Flow in the Human Brain

There are several neuroimaging modalities that can measure blood flow in the human brain. In the acute stroke, contrast enhanced perfusion methods are popular for both magnetic resonance imaging (MRI) and computerized axial tomography (CT, CAT). In these methods a contrast agent (e.g., gadolinium) is injected into the bloodstream. One can then trace the speed and concentration of this bolus as it enters the brain. This can show regional cerebral blood flow as well as the amount to time it takes the bolus to transit through the tissue. In addition, one can measure the latency for the bolus to reach different parts of the brain (time to peak). These methods often reveal acute pathological perfusion that can be distant from the site of the injury (diaschisis). Since these measures are directly related to the functional disruption, they supplement the information deriving from structural imaging in their attempt to identify the neural basis of behavioral disorders after stroke. Thus, contrast perfusion not only can provide reasonably accurate measures of eventual lesion extent; it also may play a pivotal role in understanding acute brain injuries.

The bolus used for these measures generates a strong signal, so one can rapidly acquire an image of perfusion, which – in a clinical context – is ideal for eligibility for thrombolysis/thrombectomy in which the interventions are time

limited. Despite these benefits, these contrast based methods essentially only provide a snapshot of blood flow and thus do not allow us to measure subtle changes that occur as the brain switches from doing one behavioral task to rest or to a different behavioral task. For this latter purpose, which typically arises from research questions in cognitive neuroscience aiming at understanding normal brain function, we need to rely on methods that allow us to continuously acquire a relatively stable measure of blood flow. Specifically, arterial spin labelling (ASL) and blood oxygenation level dependent (BOLD) MRI provide us this possibility, i.e. to infer task related brain activity.

For arterial spin labeling (ASL), a radiofrequency pulse is used to tag blood in the extracranial carotid artery. We can compare this brain scan to an identical brain scan where the tag is not applied, and continuously acquire these pairs of labeled and unlabeled scans. The only difference between these paired scans is whether a label was applied in the carotid artery, so differences between the images reflect blood that has moved from the neck to the brain. Note that this is conceptually similar to the gadolinium bolus: we now leverage the fact that our label will influence the image signal when it arrives in the brain. One advantage of ASL, however, is that this labeling method uses the blood itself as a tracer and does not require a contrast agent, allowing continuous acquisition of these images. ASL has started to prove its value in acute stroke care, but it can also be used to observe changes in blood flow that occur following brain activity.

Another blood flow measure allowing us to infer task related brain activity is blood oxygenation level dependent (BOLD) MRI, which is often referred to as functional MRI (fMRI). Like gadolinium, deoxyhemoglobin has a local influence on the MRI signal. Changes in metabolic demand modify the relative concentration of deoxyhemoglobin. Therefore, one can infer that changes in MRI signal reflect changes in brain activity. Unlike ASL, we do not need to create a label: we leverage the fact that the concentration spontaneously varies with metabolism. Instead of a label, we acquire a scan where the image intensity is influenced by oxygen levels (often referred to as a T2* contrast). We thus can detect increases and decreases in oxygenation levels.

If measuring task related changes in brain activity, ASL and fMRI measure largely the same signal. The main challenge with ASL in that context is that one must carefully pace the labeled and unlabeled images: one must provide a sufficient delay for the tagged blood to get to the brain, yet not wait so long that it has already left. The ideal post-label delay varies with many factors including age. In contrast, fMRI scans can be acquired much more rapidly, which is useful for modeling dynamic changes in the brain and is the reason why BOLD fMRI remains more popular than ASL in that context. While there are different strengths and weaknesses for fMRI and ASL, we wish to emphasize that each of these techniques is measuring the same core changes for task-related activity. For brevity, and due to its current relative popularity, we tend to use 'fMRI' to describe functional brain imaging in general (and 'BOLD fMRI' when we are attempting to distinguish T2* fMRI from ASL).

Contrast enhanced MRI tells us how much blood is getting to parts of the brain, and how long the blood requires to get there. In contrast, fMRI is interested in how blood flow changes in response to brain activity. Note that these two can dissociate. While enhanced MRI identifies regions with abnormal blood flow, fMRI identifies areas where the region modulates flow based on task demands. A region might have normal perfusion, but may not show a fMRI signal for several reasons. For example, the region may never have had any role with that task, such as primary somatosensory areas that are involved with touch are not involved with low level visual perception. Therefore, one would not expect to see activation changes in somatosensory regions in an fMRI task we compare activity during rest to a visual task. In this case, the somatosensory cortex may be intact, but its activation is not modulated by the task. Furthermore, a region may not generate fMRI signal because it is destroyed. On the other hand, a region that is intact may not generate a fMRI signal if it is disconnected from its network or if it relies on information from a distant node that has been injured. Therefore, when we do observe significant regional activation changes we can infer that these regions are not only receiving sufficient blood flow to function, but they are connected to a task-relevant network. A crucial aspect to fMRI is that we will see different patterns of activity based on the task used. So memory, language, finger tapping, perceptual, etc. tasks will each make unique

demands on the brain and elicit different networks to respond. Therefore, any inference drawn from fMRI requires examining the task(s) used to elicit responses.

Indeed, an optimal fMRI experiment is not designed to generate the maximum sustained brain signal. Rather, the goal is to generate the most predictable change in brain activity. Again, we are not attempting to determine whether a region is getting blood flow (recall that the BOLD fMRI is an inherently poor measure of overall signal). Rather, we want to measure the dynamic changes that occur in response to task demands. A nice analogy is to think of a key – one could easily pick a lock for a key that is completely flat with no grooves. However, it is unlikely that a random key will fit a lock with a complicated shape of grooves. Likewise, an efficient fMRI study is designed to generate large, predictable changes in brain activity – brain areas that show precisely this complicated pattern of fluctuations are likely to be involved with this task. So, the next obvious question is how does blood flow change with the response to a task? From first principles, one might intuitively think that brain activity cause metabolism, and metabolic demands would cause a local decrease in blood oxygenation (e.g., neural firing depletes the oxygen in the blood). Surprisingly, this effect is not what we look for in fMRI studies. Rather, blood supply to a particular brain area increases in response to demand, so about five seconds after brain activity we find that previously-activated regions become oxygen rich. While paradoxical, empirically this effect is remarkably reliable and forms the basis for our statistical predictions. Specifically, to 'cut our key' we simply look at the different times when an individual was performing a task and assume that brain regions related to this task will show increased oxygen about five seconds later (the 'hemodynamic response'). This relationship is often referred to as the neurovascular coupling. Our model is driven by two empirical observations: the hemodynamic response is sluggish (it peaks several seconds after brain activity) and that it is additive (more brain activity generates a bigger response). Therefore, an optimal fMRI task has a person do a specific cognitively-intensive task for a period of a few seconds, and contrasts this with periods where the person rests or executes a different but similarly demanding task. We can then look throughout the entire brain to identify regions that fit our predicted pattern of sluggish and additive response.

## 1.1. The Dilemma with Measuring Hemodynamic response in individuals with a stroke lesion

The discussion of the hemodynamic response outlined above has crucial implications for translating fMRI paradigms to stroke patients. First of all, the fMRI signal is inherently a very indirect measure of brain activity. Techniques like single cell recording, electroencephalogram, and magnetoencephalogram can rapidly and directly detect the firing of neurons. In sharp contrast, with fMRI we are measuring a physiological response that occurs seconds after the firing. An important question is whether the alterations in blood flow following stroke can disrupt the neurovascular coupling. Whereas we typically see more signal in healthy humans in seconds after brain activity, stroke injury often leads to chronic changes in blood flow. The interpretative dilemma that arises from this fact is that we cannot decide whether the failure to detect the hemodynamic response in these individuals is clearly attributable to the inability to perform the task. Given the indirect nature of the hemodynamic response, one needs to ask whether absence of evidence is evidence of absence. Considering our key analogy, it is possible that stroke disrupts the amplitude of the hemodynamic response, analogous to the grooves not being as distinct or deep. With more subtle effects, it will be harder to detect regions that are truly responding. Likewise, one could expect that the hemodynamic response may exhibit abnormal latency, analogous to the grooves being cut in the wrong place so our prediction (key) does not match our observed data (the lock). One could also conceive of interactions between these effects. For example, if a cognitive task requires a few seconds to complete, the initial firing would be normal, but if the magnitude of the hemodynamic response is insufficient to meet the sustained metabolic demands over a longer period (due to misery perfusion), then function will start to degrade (with subsequent changes in demands). Therefore, while fMRI has proved remarkably reliable in healthy adults, from first principles it may prove problematic in stroke patients. In the next section we examine evidence that directly investigates this concern.

Consider commute times as an analogy for the neurovascular coupling. In most normal cities, commute times are modulated by demand, with it taking longer to get

to work during periods of peak demand. However, consider a situation where several lanes of a freeway are closed, restricting traffic flow. In this case, the restriction may be the rate-limiting factor for the commute time, rather than the demand. Conversely, consider a city where most citizens are evacuated due to a storm threat. In this case, the freeways have an excess capacity, and the remaining individuals may have similar commute times regardless of time of day. These same principles may influence neurovascular coupling. In situations of misery perfusion, the chronically restricted blood flow may not be able to increase to meet demands. In the case of increased perfusion, the death of neighboring regions may mean that there is an overabundance of blood flow in the remaining intact areas, regardless of behavioral task performed. These possibilities suggest the need for empirical evidence regarding whether brain injury can attenuate the traditional fMRI signal.

## 2. fMRI in Stroke Patients

In recent years, several studies have investigated acute/subacute stroke patients with different fMRI paradigms (e.g., Corbetta et al., 2005; Saur et al., 2006; He et al., 2007; Fridriksson et al., 2009, 2012; Cater et al., 2010; Baldassarre et al., 2014; for reviews see Crosson et al., 2007; Thompson & den Ouden, 2008; Hamilton et al., 2011; Karnath et al., 2018). Some of these studies were designed to compare BOLD activity in different regions of interest between the patients' lesioned and the non-lesioned hemisphere. A common observation in such studies has been an imbalance of BOLD signals in the structurally intact tissue of the damaged relative to the non-damaged hemisphere. For example, Corbetta et al. (2005) examined 11 stroke patients with profound spatial neglect in the acute period of a right hemisphere stroke and observed reduced BOLD signal in intact attention specific regions of the damaged hemisphere relative to homologous regions of the non-damaged hemisphere. Likewise, Saur et al. (2006) examined 14 patients who recovered from acute aphasia after a left hemisphere stroke. Acute and subacute fMRI indicated an initially decreased signal in language specific areas of the damaged hemisphere, followed by increased BOLD signal in both the damaged and the intact hemisphere. fMRI of these

patients in the chronic period showed a reduction of this abnormal BOLD response pattern that was accompanied by language improvement. On the basis of their respective findings, both studies thus concordantly concluded that the patients' disrupted behavior (i.e. the impairments in attentional orienting [Corbetta et al., 2005] or in language processing [Saur et al., 2006]) depended on more than the neuronal loss at the site of injury; they assumed that, in addition, the patients' disrupted behavior was causally linked to this abnormal BOLD signal in distally located, structurally intact tissue (via connections to the infarcted tissue). As noted above, however, a general concern for fMRI-based studies in stroke patients is that the local hemodynamics (i.e., the neurovascular coupling) might be abnormal in a damaged brain, i.e. that abnormal BOLD responses might not only reflect functional disruption. fMRI relies on a BOLD measure. The increased metabolic demands triggers a net increase in local oxygen. As we have described, this relationship between brain activity and subsequent oxygen influx may be disrupted following brain injury. First of all, consider the case of misery perfusion, where the injury leaves a very constrained blood supply. In this case, the blood flow may not be able to increase following metabolic demands. On the other hand, consider increased perfusion, where the destruction of neighboring regions may result in a blood supply that far exceeds the needs of the remaining tissue. In this case, neural activity might not require a change in blood flow, as the basal state is in excess of the demands of the tissue. In both these cases, neurovascular coupling may not function as it does in a healthy brain. As the BOLD response fundamentally relies on an increase in regional blood flow after a transient increase in neuronal activity (Ogawa et al., 1990,1992), BOLD responses have unsurprisingly been shown to be abnormal in stroke patients with impaired cerebrovascular reactivity (e.g., Carusone et al., 2002; Röther et al., 2002; Krainik et al., 2005; Murata et al., 2006; Amemiya et al., 2012).

A recent study analyzed the anatomical localization of these effects in relation to lesion location. de Haan et al. (2013) explored the BOLD signal in acute stroke patients while they performed a simple visual orientation judgment task. Each patient's normalized lesion shape was dilated into 12 adjacent 3mm perilesional regions expanding 39mm beyond the structural brain lesion's rim (Fig. 1). Analysis

highlighting voxels that observed significant task related changes thus resulted in 12 (perilesional) regions reflecting task responsive voxels for both the intact left and the damaged right hemisphere. For each patient, this percentage signal change was compared to the percentage signal change in the same voxels in the control subjects. The authors observed an abnormal interhemispheric balance consisting of reduced signal change in perilesional areas of the damaged hemisphere relative to homologous areas in neurologically healthy controls, unrelated to the patients' behavior. This suggests that the physiological changes and corresponding interhemispheric imbalance detected by fMRI BOLD in acute stroke observed close to the lesion border may not necessarily reflect changes in the neural function, nor necessarily influence the individuals' behavior. In other words, abnormal BOLD responses in stroke patients could not only reflect functional disruption but also a decoupling of the neurovascular response (without changes in neuronal functioning and/or in the individuals' behavior), or a combination of these two effects. Compounding these effects, studies in chronic patients using ASL demonstrate reduced perfusion not only in perilesional regions but also extending further into the ipsilesional hemisphere (Richardson et al., 2011). This reduced perfusion may be associated with misery perfusion, but also suggests there may be a weaker baseline signal for fMRI to detect. While we have focused on our own findings, it should be noted that other teams have also reported attenuated BOLD response in individuals with stroke (for review see Lake et al., 2016).

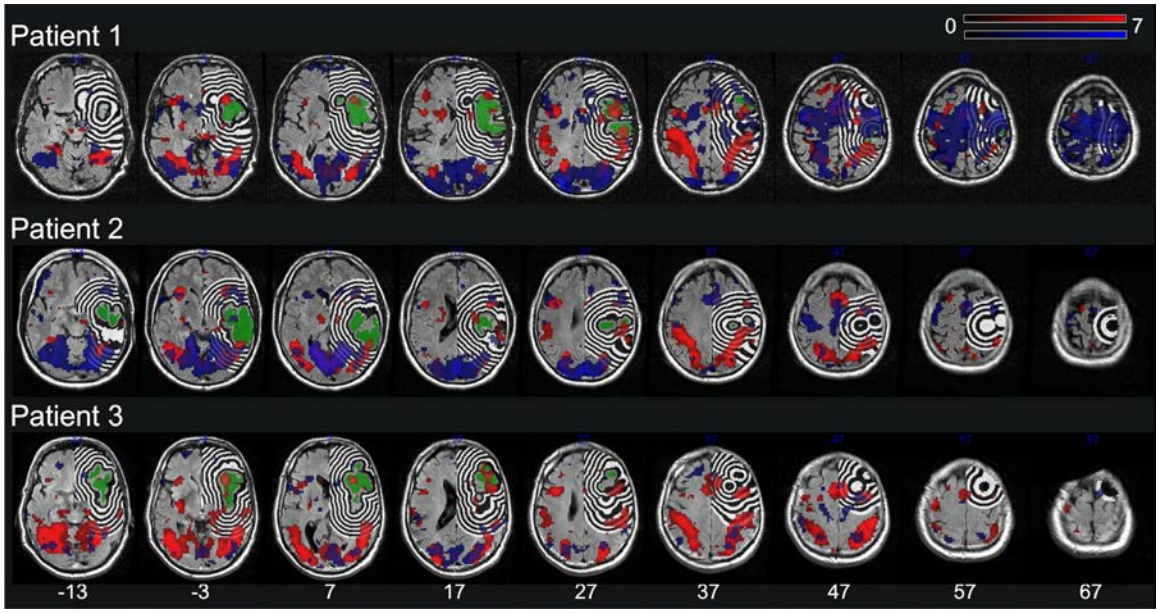


**Figure 1.**
Illustration of the fact that abnormal BOLD responses in stroke patients do not only reflect functional disruption but also a decoupling of the neurovascular response (without changes in neuronal functioning and/or in the individuals' behavior). BOLD signal in acute stroke patients while they performed a simple visual orientation judgment task. Each patient's structural brain lesion (green) was dilated into 12 adjacent 3mm perilesional regions expanding beyond the structural brain lesion's rim. Additionally shown are the results of the statistical analysis highlighting the voxels showing significant task related changes in the individual patient (blue) as well as the group of control subjects assigned to the respective stroke patients (red). Results revealed an abnormal interhemispheric balance consisting of reduced signal change in perilesional areas of the damaged hemisphere relative to homologous areas in neurologically healthy controls, unrelated to the patients' behavior. (From de Haan B, Rorden C, Karnath H-O. [2013]. Abnormal perilesional BOLD signal is not correlated with stroke patients' behavior. Frontiers in Human Neuroscience, 7, 669.)

## 3. Consequences of Disrupted Hemodynamic Response by Stroke

The finding of attenuated BOLD response in individuals with stroke unrelated to the patients' behavior, has clear consequences that should be carefully considered. First, neuroscientists need to exercise caution when interpreting BOLD data acquired in stroke patients; fMRI protocol cannot be executed as if the data were acquired from

healthy subjects. Second, absence of BOLD activity is only meaningful in areas of the brain that are clearly distant of the structural brain lesion.

This latter concern has profound implications. Effectively, the work outlined above (Richardson et al., 2011; de Haan et al., 2013) suggests that we will often have poor perilesional sensitivity in stroke patients if using fMRI. Yet, the perilesional regions are typically the most critical to recovery. For example, an individual who enrolls in aphasia treatment has damage to portions of their language system. One could expect that recovery is often mediated by the surviving parts of the damaged module. For this reason, for transcranial brain stimulation studies, we often want to target the perilesional eloquent cortex. Unfortunately, we may have very poor ability to detect fMRI activation in precisely these regions, due to the attenuated BOLD response in these regions (see above).

A challenge in describing these shortcomings is that they must be weighed against the clear potential offered by functional brain imaging to impact stroke. Indeed, despite our concerns that fMRI has low sensitivity in perilesional regions, we have used fMRI to, e.g., guide transcranial brain stimulation (Fridriksson et al., 2018). However, it may be that relying on fMRI biased us to select more distant targets for the application of tDCS in that study. Perhaps this is not an important concern in the specific context of guiding brain stimulation in a large network, namely the language network: stimulation applied to any portion of the distributed network may propagate to other regions. In this latter case, identifying the any  nodes may be sufficient. According to this model, different brain regions work in concert, and it might not matter which node we stimulate, rather what is important is we stimulate some functional node somewhere even if this node is located more distant to the structural brain lesions. From this perspective, the role of neuroimaging is to ensure we do not target a destroyed area, rather than select between the residual nodes. In this case and in the specific context of guiding the locus of application in transcranial brain stimulation, the poor perilesional sensitivity of fMRI (Richardson et al., 2011; de Haan et al., 2013) is not so much of a concern, as long as we are working with a large, distributed network (as with the language system) that has other distant nodes we can identify.

So far, the issues with the influence of lesion on fMRI signal have been described in the context of studies that focus on individuals, such as choosing a personally tailored stimulation site for applying transcranial brain stimulation. However, challenges also arise when using fMRI in group studies that attempt to find general patterns of stroke patients with a deficit. Before describing some of the dangers for applying these approaches to stroke populations, we will describe the traditional approach of group fMRI analyses performed with healthy individuals. This approach would be as follows: First, we conduct statistics within each individual, identifying how likely each location is involved in a given task. Second, we warp every participant's brain to have the same size and shape as a common template brain. Third, after all individuals' images have been warped to the same space, we can compare whether the activity in each area of the brain is consistently involved with the task across our group. The first step involves the same concerns regarding the neurovascular coupling that we have already addressed. The second and third steps face their own concerns with regards to stroke populations. We will discuss these steps in turn.

As noted, group analyses require us to 'normalize' the size and shape of each person's brain so that they are all in alignment. Here, all of the images are co-registered into a common space, allowing us to compare the same anatomical location across our participants. This step can be disrupted by the lesion characteristics of the brain injury itself. Automated methods of normalization attempt to make a brain look like a 'normal' brain. This normalization procedure is straightforward in a healthy human brain but can be disrupted by a structural defect. For example, an automated method might shrink a lesion and expand the surrounding intact perilesional regions into the lesion territory. Mathematically, this approach does indeed make the brain appear more 'normal', but it artificially distorts the size and location of the brain injury. There are several modifications to the normalization step that can address this problem. First, if working in the acute setting one can leverage the fact that brain injuries do not appear on all modalities of the admission scan. Specifically, T2-weighted B0 scans are often included as part of an admission diffusion sequence, yet recent injuries will not appear on these scans (only injuries that are at least one or

two days old will be observed on T2 scans). If one has these acute scans, one can simply compute how to warp the healthy appearing scan to match the normal template image. Once these transforms are computed, they can be applied to the other modalities (Mah et al., 2014). This approach of using the healthy appearing T2 scan for warping, however, is not possible for studies of patients with a post-stroke interval of more than 2 days (the infracted brain does no longer appear 'healthy' on T2 scans). There are two other approaches that can be considered. Since brain injury is typically unilateral we can estimate what the person's healthy brain looked like if it is assumed that the brain is roughly symmetrical in humans (which it is actually not exactly). Under this assumption, healthy homologous tissue is inserted in the damaged territory of the opposite hemisphere (Nachev et al., 2008) and we can use this 'simulated healthy' brain for the normalization process. Again, once computed, these transforms can be applied to the real scans that show the injury. A final approach for normalization of individuals with stroke is to mask the lesion so it does not contribute to the warping estimates, ideally using a template from an aged-match population that has similar anatomical features (Rorden et al., 2012). To summarize, while the normalization step can be disrupted by lesions, there are several available methods that can allow robust normalization.

The final step of group analyses is to identify brain locations that reliably respond to the behavioral performance in a given task across a population of individuals. While there are variations of this approach (e.g., Saxe et al., 2006), this base strategy remains the bread and butter of most fMRI group analyses. There are a couple of potential problems faced when adapting this approach to stroke participants. First, since each individual has a different pattern of brain injury, at some locations we will be examining locations that are intact in some of our participants and damaged in others. We need to expect that destroyed tissue will not show a fMRI BOLD response to our task. The challenge is that we are confounding the location of the structural brain injury and its functional consequences. This makes inference difficult: Does reduced fMRI activation in a group at a given location reflect changes in structure or function? While methods have been described that attempt to identify individual variations in module location for group studies (Poldrack, 2007;

Saxe et al., 2006), these methods are rarely applied in practice. At the very least, one expects low statistical power in group fMRI studies of stroke participants, so that we will need to conduct large studies and may often miss real effects. We do suggest two approaches to tackle this issue. First, one could conduct an analysis in which each voxel is restricted to include data only from those with intact tissue at that location. The structural scans can help map the lesion and look for lesion-related effects, while the masked fMRI data could provide information about brain function. To date, we know of no fMRI studies in stroke patients that have applied this approach. Another solution is specific to longitudinal studies that map changes in brain function. Here, one can compute a regression analysis to detect voxels that change their activation in response to training. For example, finding voxels where increased activity from baseline to follow-up indicates behavioral improvements. In this case, one expects that an individual with injury at a specific location will show little task-based activity at either time point, and therefore statistically significant effects are driven by those who have intact cortex at the given location. This approach is described by Fridriksson (2010), but has not been widely adopted yet.

One more popular response to this conundrum is to restrict group-based fMRI analyses to the intact hemisphere. This has two potential advantages. First, one expects the hemodynamic response to be less altered, at least in the subacute/chronic phases of stroke. Once acute diaschisis has resolved, the hemodynamic response in the contralesional hemisphere should be relatively normal (though see Lake et al., 2016). Second, individual variability in lesion location does not necessarily mean that a particular area is unable to show a hemodynamic response simply due to the fact that the neural tissue at this location got infarcted. If fMRI analyses are restricted to the intact hemisphere, none of the voxels examined are destroyed. Likewise, while the location of injury will certainly influence the pattern of recovery and brain response, none of the voxels entered into the statistical test are at locations with structural injury. This is certainly a principled approach to the issue, and can yield insights regarding the extent of plasticity. However, it does necessarily mean abandoning attempts to make inference about functional changes in the injured hemisphere. However, even then, findings from such studies can prove tricky because

changes in fMRI signals in the undamaged hemisphere might reflect either inhibitory or excitatory mechanisms. For example, is the increased activation observed in the right hemisphere of aphasia patients maladaptive or beneficial for recovery? This is a general problem in fMRI: brain activation reveals areas involved or related to, but not necessarily required for a given task. However, it becomes amplified in situations where we are seeing stroke related changes of fMRI activity in regions not classically associated with successful behavioral performance of the task under study. As we emphasize in the next section, the proper response is that fMRI should be used in conjunction with other neuroscientific methods (in general, but in particular) if investigating patients with stroke lesions.

## 4. Future Directions

Task-based functional imaging relies on neurovascular coupling: we assume that brain activation evokes a delayed but large change in local blood oxygenation. There is clear evidence that this fundamental relationship can be disrupted in stroke, in particular for perilesional brain tissue. Unfortunately, this fact reduces the impact for using fMRI to understand stroke. It is difficult or can even be impossible to infer if observations of reduced signal reflect reduced activity related to the task of interest or simply is due to reduced neurovascular coupling. Despite these concerns, we feel that fMRI can still aid our understanding of stroke. For example, BOLD activity can be meaningfully interpreted if (i) only voxels are included in the analysis which correspond to structurally intact brain tissue and if (ii) inferences regarding reduced signal are only meaningful for voxels are clearly distant of the brain lesion (e.g., are from the undamaged hemisphere). Moreover, task-based fMRI can be a reasonable approach for identifying brain stimulation targets in stroke patients. Regions where the image brightness is significantly correlated with the task of interest remain good candidates for successful treatment. In this case, we have clear fMRI evidence that these regions are involved in the task of our interest, although we cannot be certain that this region is also necessary for this task. However, as we have noted, nodes that

are merely involved with a task may provide good conduits for modulating task critical nodes.

A crucial realization is that each method used in neuroscience has its own set of strengths and limitations. We espouse honestly identifying the weakness of each modality and leveraging different modalities with complementary strengths and weaknesses. For example, we can conduct multivariate analyses by using machine learning algorithms that look for unique patterns within each modality. These methods can use multiple sources of information to generate accurate prognoses. For example, information such as age at time of injury, time since injury, genes and different imaging modalities can be combined. The classifier can implicitly learn the independent information described by each of these biomarkers. This view suggests that despite limitations, fMRI in stroke patient may provide an independent predictor for outcome. By leveraging the unique information from each biomarker, we can generate better predictive models than using each in isolation. The primary concern with this approach is that such studies are necessarily expensive and time consuming. The breadth of predictors can require a large sample size to ensure a sufficient training set for the machine learning algorithms.

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## References

Amemiya S, Kunimatsu A, Saito N, Ohtomo K. (2012). Impaired hemodynamic response in the ischemic brain assessed with BOLD fMRI. Neuroimage, 61, 579-590.

Baldassarre A, Ramsey L, Hacker CL, Callejas A, Astafiev SV, Metcalf NV, Zinn K, Rengachary J, Snyder AZ, Carter AR, Shulman GL, Corbetta M. (2014). Large-scale changes in network interactions as a physiological signature of spatial neglect. Brain, 137, 3267–3283.

Bahrainwala ZS, Hillis AE, Dearborn J, Gottesman RF. (2014). Neglect performance in acute stroke is related to severity of white matter hyperintensities. Cerebrovasc Dis., 37, 223-230.

Basilakos A, Fillmore PT, Rorden C, Guo D, Bonilha L, Fridriksson J. (2014). Regional white matter damage predicts speech fluency in chronic post-stroke aphasia. Frontiers in Human Neuroscience, 8, 845.

Beaulieu LD, Flamand VH, Massé-Alarie H, Schneider C. (2017). Reliability and minimal detectable change of transcranial magnetic stimulation outcomes in healthy adults: A systematic review. Brain Stimul., 10, 196-213.

Carter AR, Astafiev SV, Lang CE, Connor LT, Rengachary J, Strube MJ, Pope DL, Shulman GL, Corbetta M. (2010). Resting interhemispheric functional magnetic resonance imaging connectivity predicts performance after stroke. Ann. Neurol., 67, 365–375.

Carusone LM, Srinivasan J, Gitelman DR, Mesulam MM, Parrish TB. (2002). Hemodynamic response changes in cerebrovascular disease: Implications for functional MR imaging. Am. J. Neuroradiol., 23, 1222-1228.

Corbetta M, Kincade MJ, Lewis C, Snyder AZ, Sapir A. (2005). Neural basis and recovery of spatial attention deficits in spatial neglect. Nat. Neurosci., 8, 1603–1610.

Crosson B, McGregor K, Gopinath KS, Conway TW, Benjamin M, Chang YL, Moore AB, Raymer AM, Briggs RW, Sherod MG, Wierenga CE, White KD. (2007). Functional

MRI of language in aphasia: a review of the literature and the methodological challenges. Neuropsychology Review, 17, 157–177.

de Haan B, Rorden C, Karnath H-O. (2013). Abnormal perilesional BOLD signal is not correlated with stroke patients' behavior. Frontiers in Human Neuroscience, 7, 669.

Fridriksson J, Baker JM, Moser D. (2009). Cortical mapping of naming errors in aphasia. Human Brain Mapping, 30, 2487–2498.

Fridriksson J. (2010). Preservation and modulation of specific left hemisphere regions is vital for treated recovery from anomia in stroke. J. Neurosci., 30, 11558-11564.

Fridriksson J, Richardson JD, Fillmore P, Cai B. (2012). Left hemisphere plasticity and aphasia recovery. Neuroimage, 60, 854–863.

Fridriksson J, Rorden C, Elm J, Sen S, George MS, Bonilha L. (2018). Transcranial direct current stimulation vs sham stimulation to treat aphasia after stroke: a randomized clinical trial. JAMA Neurol., 75, 1470-1476.

Hamilton RH, Chrysikou EG, Coslett B. (2011). Mechanisms of aphasia recovery after stroke and the role of noninvasive brain stimulation. Brain and Language, 118, 40–50.

He BJ, Snyder AZ, Vincent JL, Epstein A, Shulman GL, Corbetta M. (2007). Breakdown of functional connectivity in frontoparietal networks underlies behavioral deficits in spatial neglect. Neuron, 53, 905–918.

Horvath JC, Forte JD, Carter O. (2015). Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. Neuropsychologia, 66, 213-236.

Hope TM, Leff AP, Prejawa S, Bruce R, Haigh Z, Lim L, Ramsden S, Oberhuber M, Ludersdorfer P, Crinion J. (2017). Right hemisphere structural adaptation and changing language skills years after left hemisphere stroke. Brain, 140, 1718-1728.

Karnath H-O, Rennig J, Johannsen L, Rorden C. (2011). The anatomy underlying acute versus chronic spatial neglect: a longitudinal study. Brain, 134, 903-912.

Karnath H-O, Sperber C, Rorden C. (2018). Mapping human brain lesions and their functional consequences. Neuroimage, 165, 180–189.

Krainik A, Hund-Georgiadis M, Zysset S, von Cramon, DY. (2005). Regional impairment of cerebrovascular reactivity and BOLD signal in adults after stroke. Stroke, 36, 1146-1152.

Lage C, Wiles K, Shergill SS, Tracy DK. (2016). A systematic review of the effects of low-frequency repetitive transcranial magnetic stimulation on cognition. J. Neural Transm., 123, 1479-1490.

Lake EM, Bazzigaluppi P, Stefanovic B. (2016). Functional magnetic resonance imaging in chronic ischaemic stroke. Philos Trans R Soc Lond B Biol Sci., 371, 1705.

Lunven M, Thiebaut De Schotten M, Bourlon C, Duret C, Migliaccio R, Rode G, Bartolomeo P. (2015). White matter lesional predictors of chronic visual neglect: a longitudinal study. Brain, 138, 746-760.

Mah YH, Jager R, Kennard C, Husain M, Nachev P. (2014) A new method for automated high-dimensional lesion segmentation evaluated in vascular injury and applied to the human occipital lobe. Cortex, 56, 51-63.

Murata Y, Sakatani K, Hoshino T, Fujiwara N, Kano T, Nakamura S, Katayama Y. (2006). Effects of cerebral ischemia on evoked cerebral blood oxygenation responses and BOLD contrast functional MRI in stroke patients. Stroke, 37, 2514-2520.

Nachev P, Coulthard E, Jäger HR, Kennard C, Husain M. (2008). Enantiomorphic normalization of focally lesioned brains. Neuroimage, 39, 1215–1226.

Naeser MA, Palumbo CL, Prete MN, Fitzpatrick PM, Mimura M, Samaraweera R, Albert ML. (1998). Visible changes in lesion borders on CT scan after five years poststroke, and long-term recovery in aphasia. Brain and Language, 62, 1-28.

Ogawa S, Lee TM, Kay AR, Tank DW. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc. Nat. Acad. Sci. USA, 87, 9868-9872.

Ogawa S, Tank DW, Menon R, Ellermann JM, Kim SG, Merkle H, Ugurbil K. (1992). Intrinsic signal changes accompanying sensory stimulation: Functional brain

mapping with magnetic resonance imaging. Proc. Nat. Acad. Sci. USA, 89, 5951-5955.

Poldrack RA. (2007). Region of interest analysis for fMRI. Social Cognitive and Affective Neuroscience, 2, 67–70.

Richardson JD, Baker JM, Morgan PS, Rorden C, Bonilha L, Fridriksson J. (2011). Cerebral perfusion in chronic stroke: implications for lesion-symptom mapping and functional MRI. Behav. Neurol., 24, 117-122.

Röther J, Knab R, Hamzei F, Fiehler J, Reichenbach JR, Büchel C, Weiller C. (2002). Negative dip in BOLD fMRI is caused by blood flow - oxygen consumption uncoupling in humans. Neuroimage, 15, 98-102.

Rorden C, Karnath H-O. (2004). Using human brain lesions to infer function: a relic from a past era in the fMRI age? Nature Reviews Neuroscience, 5, 813-819.

Rorden C, Bonilha L, Fridriksson J, Bender B, Karnath H-O. (2012) Age-specific CT and MRI templates for spatial normalization. Neuroimage, 61, 957-965.

Saur D, Lange R, Baumgaertner A, Schraknepper V, Willmes K, Rijntjes M, Weiller C. (2006). Dynamics of language reorganization after stroke. Brain, 129, 1371–1384.

Saxe R, Brett M, Kanwisher N. (2006) Divide and conquer: a defense of functional localizers. Neuroimage, 30, 1088-1096.

Shin J, Yang E, Cho K, Barcenas CL, Kim WJ, Min Y, Paik NJ. (2012). Clinical application of repetitive transcranial magnetic stimulation in stroke rehabilitation. Neural Regen. Res., 7, 627-634.

Thompson CK, den Ouden D-B. (2008). Neuroimaging and recovery of language in aphasia. Current Neurology and Neuroscience Reports, 8, 475–483.

Woods AJ, Antal A, Bikson M, Boggio PS, Brunoni AR, Celnik P, Cohen LG, Fregni F, Herrmann CS, Kappenman ES, Knotkova H, Liebetanz D, Miniussi C, Miranda PC, Paulus W, Priori A, Reato D, Stagg C, Wenderoth N, Nitsche MA. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. Clin. Neurophysiol., 127, 1031-1048.