HHS Public Access

Author manuscript

Headache. Author manuscript; available in PMC 2024 March 01.

Published in final edited form as:

Headache. 2023 March ; 63(3): 309–321. doi:10.1111/head.14487.

Functional Magnetic Resonance Imaging of Headache: Issues, Best-Practices, and New Directions, A Narrative Review

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Abstract

Objective: To ensure readers are informed consumers of functional magnetic resonance imaging research in headache, to outline ongoing challenges in this area of research, and to describe potential considerations when asked to collaborate on functional magnetic resonance imaging research in headache as well as to suggest future directions for improvement in the field.

Background: Functional magnetic resonance imaging has played a key role in understanding headache pathophysiology, and mapping networks involved with headache-related brain activity has the potential to identify intervention targets. Some investigators have also begun to explore its use for diagnosis.

Methods/Results: The manuscript is a narrative review of the current best practices in functional magnetic resonance imaging in headache research, including guidelines on transparency and reproducibility. It also contains an outline of the fundamentals of magnetic resonance imaging theory, task-related study design, resting-state functional connectivity, relevant statistics and power analysis, image pre-processing, and other considerations essential to the field.

Conclusion: Best practices to increase reproducibility include methods transparency, eliminating error, using a priori hypotheses and power calculations, using standardized instruments and diagnostic criteria, and developing large-scale, publicly available datasets.

Keywords

fMRI; functional magnetic resonance imaging; migraine; cluster headache

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Conflict of Interest Statement:

Jennifer A. Hranilovich declares no conflicts of interest. **Kristina T. Legget** declares no conflicts of interest. **Keith C. Dodd** declares no conflicts of interest. **Korey P. Wylie** declares no conflicts of interest. **Jason R. Tregellas** declares no conflicts of interest.

Introduction

Our understanding of headache is constantly evolving and is subject to ongoing investigation and debate. In the 2022 Nature Reviews Disease Primer on Migraine, authors noted that "[a]lthough understanding migraine attack pathophysiology has substantially improved, the pathogenesis of the disease migraine…remains poorly understood"[1]. The authors go on to outline what is known, including the phenomenon of spreading depolarization[1]. For several of the agreed-upon aspects of headache pathophysiology, functional magnetic resonance imaging (fMRI) has played a key role[2].

Neuroimaging has made these contributions in part because it is a non-invasive method for bridging our understanding between basic science findings in animal models and the human brain. An example is the role of fMRI (which in essence tracks changes in oxygenated blood flow) in our understanding of spreading cortical depolarization[3]. As with many other avenues for scientific discovery, the most compelling use for fMRI is in parallel with other techniques. In the example of spreading cortical depolarization, this phenomenon was initially observed in animal models using electrophysiologic methods. These observations have since been confirmed and ultimately strengthened with imaging methods, including magnetoencephalography in animal models, positron emission tomography (PET) in humans, and of course, fMRI in humans.[2, 4] The use of fMRI has expanded in part because of its ease of use, as it does not require a contrast agent, is non-radioactive, can be performed on most existing MRI (magnetic resonance imaging) scanners, and has finer spatial resolution than PET or electroencephalography (EEG).

The primary use of fMRI in headache remains in the investigation of pathophysiology and not, at this time, individual diagnosis. This is an area under active investigation, and several attempts have been made to use fMRI to develop a biomarker or classification algorithm for migraine, either using fMRI data alone[5–7] or in combination with structural MRI data[8]. Only one of these, by Tu et al., validated study findings against independent datasets. Tu et al. achieved an accuracy comparing migraine without aura to healthy controls of 84.2% and comparing to a combination of healthy controls and those with chronic lower back pain or fibromyalgia of 73.1%[5]. With a sensitivity of 77.8% and a specificity of 75.9% in this later analysis, for a combined value greater than 1.5, this test could be considered useful.[9] However, non-methodological barriers also slow implementation of fMRI biomarker as a diagnostic tool, including concerns about the ethics and liability issues surrounding the use of artificial intelligence in radiology on the part of radiologists and pain clinicians and researchers[10, 11], and regulatory issues[12].

While understanding migraine pathophysiology is an important goal, one might question fMRI's importance if it's not being used for diagnosis. A key contribution is fMRI's ability to map networks involved with headache-related brain activity, which could potentially identify intervention targets, including targets for neuromodulation using techniques such as transcranial magnetic stimulation[13]. Given the growth of fMRI for headache research, the objectives of this review are to ensure this journal's readers are informed consumers of this area of scientific inquiry, to outline fMRI's ongoing challenges for headache specialists, and to describe potential considerations when asked to collaborate on fMRI research, as well as

suggesting future directions for improvement in the field. This review is intended to cover essentials and due to length constraints is not meant to be comprehensive.

Methods

This manuscript is a narrative review of current best practices in using fMRI for headache research, including the application of international guidelines on Best Practices in Data Analysis and Sharing in Neuroimaging using MRI[14]. Literature reviewed included published primary research and review articles indexed in PubMed, and textbooks and reports, written in English, inclusive of years 1936–2022. Relevant online publications were included when known to the authors and when representing primary source material.

Methods of fMRI Research

The BOLD contrast

Magnetic resonance imaging takes advantage of the differing tissue responses to electromagnetic fields in order to image the body noninvasively and without radiation. It can be used to examine numerous tissue properties, such as structure[15], diffusion patterns[16, 17], and blood flow[18, 19] by varying patterns of electromagnetic field pulses within what is typically a 1.5–7 Tesla magnetic field. In the case of fMRI, pulse sequences allow researchers to indirectly measure activation of small scale neuronal ensembles, such as cortical columns[20, 21] or layers[22–24] through changes in regional blood flow[25].

The main signal of interest in fMRI is referred to as the blood-oxygen-level-dependent (BOLD) contrast, or signal[25]. This signal depends on the ratio of oxygenated to deoxygenated hemoglobin within the blood[26]. Oxygenated hemoglobin is lightly repelled by magnetic forces (weakly diamagnetic) while deoxygenated hemoglobin is attracted to magnetic forces (strongly paramagnetic)[27, 28]. These differences lead to detectable magnetic susceptibility differences when the ratio of oxygenated to deoxygenated hemoglobin changes, leading to the BOLD signal[3].

When neurons become active, there are two main phenomena that are relevant to BOLD imaging. First, active neurons uptake oxygen to meet their metabolic needs, which converts nearby oxyhemoglobin to deoxyhemoglobin[25]. Second, blood flow to the area of neuronal activation is increased in excess of metabolic needs (hemodynamic uncoupling)[25, 29]. Because the increase in oxygenated blood is greater than metabolic oxygen consumption, the ratio of oxygenated to deoxygenated hemoglobin increases within the area of increased neuronal activity[30]. These changes in the BOLD response are characterized by the hemodynamic response function[31, 32] as seen in Figure 1. The hemodynamic response function often begins with an initial dip in signal, possibly due to initial oxygen uptake to meet metabolic demands[33]. After 3–6 seconds post-stimulus[32], a large peak follows due to regional blood flow increase. Finally, a small potential post-stimulus undershoot may be observed as the ratio of oxygenated to deoxygenated hemoglobin returns to baseline[34]. Altogether, this type of fMRI utilizes changes in the hemodynamic response function to track regional blood flow differences related to neuronal activity changes. This signal is sensitive to both increases and decreases in neuronal activity[35]. It is important to recognize

that the BOLD signal indirectly measures brain activity through blood flow changes related to metabolism[3]. Activity is measured at the level of the "voxel," or three-dimensional version of a pixel, as determined by slice thickness.

Arterial spin labeling

A different type of contrast from BOLD is perfusion-based contrast, focused on intravascular tissue perfusion by tagged blood flow. This is accomplished by using electromagnetic field pulses to label protons in upstream blood and then detecting where it travels in the brain or labeling based on blood flow velocity[36]. The mechanics of this technique have been summarized for clinicians by Grade et al[37] and includes mention of the use of phased array coils. Surface, volume, and phased array coils are additional hardware placed around the head to improve the signaling and detection[3] with this method and others. The benefits of this method include changes in blood flow that are quantifiable, e.g. with units such as mL blood/100 g tissue/min[38]. It was recently used to investigate interictal blood flow differences in a small group of patients with migraine with or without aura in the area of the extrastriate visual cortex and found hyperperfusion in area V5.[39]

Task-based fMRI

Theory—One of the two primary uses of fMRI is to assess brain activity during the performance of a "task." Tasks can include sensory, motor, or cognitive activities[40, 41]. Task-based fMRI studies most commonly compare BOLD signals between two or more different conditions or states[42], in which one or more conditions are designated as the control condition. An example is comparing brain responses during painful stimuli to those during nonpainful stimuli. In this case, the task is sensory, and the experimental condition might be painful heat versus a nonpainful warmth control condition[43, 44]. Differences between these two conditions are thought to represent brain activity specific to the pain stimulus administered.

Task selection—Selecting specific and narrowly defined stimuli is necessary to precisely target brain functions of interest (e.g., mapping thalamic neurons involved in allodynic response to heat or light touch during migraine[45]). Historically, many if not most task-based fMRI studies in headache have involved sensory stimulus exposure[46]. Overall, task/stimulus selection should involve careful consideration of factors such as feasibility, external validity, generalizability, and reproducibility. In pain investigations, fMRI studies comparing brain function during painful heat compared to rest allow a fairly well-controlled investigation of the pain response; however, observed effects may not be truly generalizable to the headache-specific pain response. If instead, brain response during periods with headache is compared to periods without, the results may better reflect mechanisms underlying headache pain, but study design becomes much more difficult. Appropriately timing periods of headache during fMRI is substantially more challenging than administering a controlled painful stimulus task.

Study design—Stimulus presentation timing and arrangement are also important study design considerations. The majority of task-based fMRI studies use either "block" or "event-related" designs. In *block* designs, stimuli belonging to one condition (e.g., visual

stimulation) are presented in a group in an alternating fashion with another condition (e.g., rest from visual stimulation), resulting in an additive effect on the BOLD response across stimuli in each block[42, 47, 48].

Event-related designs consider individualized trial responses and typically involve more rapid change between conditions compared to block designs[42, 47–49]. This design was used in a painful trigeminal nerve stimulation study, in which puffs of room air, gaseous ammonia, or rose odor, were presented in random order[50]. While block designs can provide increased statistical power, higher reliability, and greater observed BOLD signal change, event-related designs can allow analyses to take individual responses to each trial into account (e.g., response speed or accuracy) and can facilitate the use of adaptive paradigms that adjust on a trial-by-trial basis based on individualized performance[42, 47, 48, 51–53]. It is important to recognize that block designs may increase the likelihood that participants will be able to anticipate, or predict the timing or pattern of stimulus onset, which can in turn influence their pattern of brain activity[54, 55]. Varying the block sizes, the order of stimuli, and the amount of time between stimuli can help minimize this effect. Event-related designs can further do so, as they allow for more random stimulus presentation, reducing the potential for prediction or habituation[52, 56]. Event-related designs may also support better estimation of the hemodynamic response compared to block designs[56, 57]. Mixed block and event-related designs are used to simultaneously assess both event-related brain activity and sustained activity across blocks of stimuli, but this approach presents unique challenges in analysis, such as the potential to misattribute brain activity across conditions[51, 52].

Resting-state fMRI

Theory—Resting-state fMRI (rs-fMRI) scans are most often acquired when the brain is in a relaxed and unfocused state, as opposed to performing a focused cognitive task or reacting to external stimuli[58, 59]. In practice, subjects are instructed to simply let their mind wander freely within the scanner for several minutes[59]. In this relaxed state, the brain is primarily driven by internal processing demands. Study of the brain at rest allows examination of intrinsic circuitry and connectivity without the confounding effects of a task or external stimuli[59].

Resting-state fMRI has greatly advanced the study of the brain's connectivity and networks. It relies in part on the theory that brain areas that are active at the same time are in some way functionally connected to each other[60]. The analysis of these dynamics describes what is termed "resting state functional connectivity" (rsFC). This differs from assessing the structural connectivity of white matter tracts, termed tractography. Broadly speaking, tractography investigates the brain's structural wiring while functional connectivity focuses on neural activity being transmitted along this wiring[61]. Tractography is performed using a different type of MRI called diffusion tensor imaging (DTI), which examines the direction of water flow in a voxel, and is particularly well-suited to mapping the integrity and direction of myelin sheaths[3]. In practice, fMRI and DTI are complementary methods that differ in their acquisition of data and can be integrated for analysis and interpretation. For a comparison of roles of various neuroimaging modalities (including fMRI, structural MRI,

tractography, magneto-, and electrophysiology) in brain connectivity characterization, see the review by Bassett and Bullmore[62].

Hypothesis-driven analysis

Seed-based: In the most straightforward method, a single location or brain region (the "seed region") is hypothesized to be involved in the clinical symptom of interest, such as processing noxious sensation. The fluctuating BOLD signal in this region is correlated with activity in every voxel within the brain[59]. The resulting statistics for all voxels are then displayed as a whole-brain spatial map, showing functional connectivity between the seed region and other cortical and subcortical regions. Anatomical boundaries of the seed region or region of interest (ROI) could be delineated using anatomical atlases[63–65] such as a Brodmann's Area, or defined using functional activation coordinates from independent task-based datasets[59]. This design can examine clinical population connectivity differences, such as patients with migraine with and without aura, or headache treatment intervention effects on connectivity. A limitation of seed-based functional connectivity is the choice of seed region, which must be selected carefully based on study hypotheses. Different seed regions will yield different results for the same study.[66] Importantly, using datasets that include the same subjects for both seed region selection and the subsequent connectivity analysis should be avoided, as outcomes and conclusions are limited by the resulting dependencies in the analysis, termed statistical "double-dipping"[67].

Data-driven analysis

Independent component analysis

The most widely used data-driven method is independent component analysis (ICA)[68–70]. This method estimates a set of statistically independent signals, each with an associated spatial map and timing of their spontaneous fluctuations. Each ICA component summarizes a connectivity pattern, often termed an intrinsic connectivity network (ICN). Spatial maps of resting state ICNs can be compared to detect connectivity pattern differences between groups or treatment conditions, or correlated to investigate differences in large-scale coordinated activity patterns throughout the entire brain.

As a part of this analysis, it is necessary to classify the resulting components into different ICNs and noise components[71]. Identifying individual networks by comparing them to atlases of known networks is a common practice[72]. Additionally, ICA requires specifying the number of networks and noise components in advance. This number strongly influences which networks are estimated and their spatial extent, with higher numbers potentially resulting in subnetworks of a larger ICN[73–75]. Even with the specified network number held constant between studies, different noise levels can result in different network sets, potentially limiting replicability.

Local vs. global connectivity

Local connectivity is frequently investigated using a metric termed regional homogeneity (ReHo)[76], which indicates whether a voxel is coherent and synchronized with its neighbors

and they with it. Limitations of ReHo include its exclusive focus on hyper-local connectivity, connections no more than the width of an fMRI voxel.

Global connectivity, along with local connectivity, is often investigated using measurements provided by the mathematical study of networks, termed graph theory[77]. To apply graph theory, the brain is first parcellated into individual regions using an ROI atlas, termed nodes. Fluctuating activity at each node is then estimated by averaging encompassed voxels, and nodes' activities are correlated to estimate the whole-brain network. In contrast to previously described methods, the resulting network encompasses many more connections, potentially only limited by the ROI atlas' resolution. This whole-brain network is then analyzed by calculating summary metrics, and quantitatively measuring abstract network features. The most widely applied graph theory measures include the following.

1. Clustering: the tendency of a node's connected neighbors to themselves share a connection.
2. Characteristic path length: the number of intermediary connections between nodes.
3. Degree: the number of a node's connections.
4. Modularity: the extent to which the entire network can be partitioned into relatively unconnected parts.

Networks in graph theory are commonly displayed using circles representing nodes, connected by lines. Node arrangement can include ordered circular displays, spring-based layouts grouping similar nodes, or more abstract visualizations. For a sound introduction to graph theory, including an overview of its main concepts and measures, see Sporns' review on this topic[78]. For a more detailed but still accessible understanding of the neurobiology and physics of complex networks, see the review by Bullmore and Sporns[79].

Measuring low-frequency fluctuations and local activity

Resting-state fMRI does not allow an inherent measure of the "activity" occurring at any voxel within the brain. As a surrogate measure of activity during the resting state, amplitude of low-frequency fluctuations[80] (ALFF) or fractional ALFF[81] (fALFF) are commonly used. These measures calculate the degree of fluctuations at the individual voxel level with results displayed as spatial maps. This approach was recently used to predict response to transcutaneous auricular vagus nerve stimulation in patients with migraine without aura[82]. The study's primary outcome measure was improvement in the visual analog scale score in the fourth week of treatment, and while their patients did achieve this, it was not significantly associated with changes in fALFF, though a secondary outcome of duration of migraine attack was, with change occurring in the bilateral precuneus. For further helpful introductory information on ALFF and ReHO as well as a good primer on neuroimaging, see Lv et al.[83]

Interpretation of rs-fMRI results—For methods resulting in whole-brain spatial maps, such as seed-based FC, ICA, ReHo, and fALFF, results are interpreted according to relevant neuroanatomical regions highlighted by the analysis. For instance, using a seed in the

nociceptive periaqueductal gray (PAG) increased connectivity with somatosensory cortical voxels in subjects with migraine has been interpreted as impairment of the descending pain modularity system centered on the PAG[84]. Correlations between network time series from ICA, i.e. wide-spread patterns of coordinated neural activity, are interpreted in terms of whole-network effects. An example of analyzing connections between pre-specified ROI is the improvement seen in connectivity between a subset of regions within Executive Control and Salience Networks in patients with chronic migraine after sphenopalatine ganglion block, suggesting the procedure modifies specific circuitry underlying the cognitive and salient aspects of pain[85]. Graph theory measures are interpreted as rearrangement in the overall network topology, such as widespread disconnections throughout the entire brain network.

Image preprocessing: Two main issues surrounding initial fMRI data collection must be addressed with preprocessing before group analyses can be performed: (1) the shape and position of scanned brain regions are not aligned across scans and patients, and (2) the data comprise both the signal of interest (BOLD) and noise and artifacts. Preprocessing addresses both issues, significantly increasing the signal-to-noise ratio and allowing comparison across patients and scans. Many studies preprocess data with “out-of-the-box” established major software packages, such as the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library (FSL), Analysis of Functional NeuroImages (AFNI), Statistical Parametric Mapping (SPM), or fMRIPrep. This may be completed via a hosted pipeline (e.g., University of Southern California Laboratory of Neuro Imaging – LONI[86]) or in-house. Numerous studies combine subsets of major packages or develop custom tools to address various preprocessing aspects, which may impact reproducibility.

While there are many methods, theories, and software that can be utilized for preprocessing, there are numerous commonly applied themes, listed below.

Distortion correction—Common fMRI sequences assume homogeneity of the MRI machine’s magnetic field within the scanned region; however, placing a patient’s body within the scanner creates field inhomogeneities, leading to geometric distortions[87]. Distortion correction addresses this. Commonly, magnetic field inhomogeneities are mapped out with initial scans[88]. From there, distortion correction methods can utilize this field map to “unwarp” these distortions.

Slice time correction—Magnetic resonance imaging data are typically collected in slices, often one slice at a time, with a delay between each slice. Slice time correction involves using the calculated time offset of each slice to realign the slices in time to each other[89].

Motion correction—Patient head motion is the largest contributor to artifacts and noise in MRI. Motion correction involves aligning each collected slice to a reference slice (e.g. the first collected slice)[90]. This alignment process is iterative and utilizes a cost function to determine which steps in the transformation process should be applied to reach optimal alignment[90]. Movement confounds are more problematic in rs-fMRI as compared to task-fMRI, requiring more stringent protocols to avoid false positive results.

Normalization to a template—Comparing patient scans to each other is challenging due to differing scanner head positioning, as well as anatomic brain structure variations. Spatial normalization overcomes these issues by aligning scans across patients to the same established template brain. This allows for similar relative regions or stereotaxic coordinates to be compared both within and across studies (e.g., using neurosynth.org). Two of the most commonly utilized coordinate systems include Montreal Neurologic Institute (e.g., MNI152 2009[91, 92]), and Talairach[93]. Whereas motion correction typically only uses a linear transformation[90], normalization often includes a non-linear transformation[94, 95].

Coregistration with structural scans—With a typical resolution of 2–4 mm^3, functional imaging often lacks the level of detail necessary to identify fine neuroanatomic landmarks. In order to improve visualization of results, higher-resolution structural scans are often obtained along with the functional scans.[3] Many image preprocessing protocols also use coregistration, or alignment with a subject's structural scans in addition to normalization to a template for greater accuracy.[3]

Spatial smoothing—Spatial smoothing involves averaging signals across small sets of neighboring voxels across the whole image. As the true BOLD signal of closely neighboring brain voxels is inherently similar due to shared blood supply and function, smoothing significantly increases the signal-to-noise ratio[96]. It is typically performed by choosing a Gaussian kernel with a full width at half maximum (FWHM) that is approximately the same diameter as the expected activated clusters of voxels, perhaps 6–8mm, for group studies. While improving the signal-to-noise ratio and enhancing the validity of common statistical assumptions underlying subsequent analyses, it is useful to note that the size of the smoothing kernel can also bias analysis results – larger kernels will tend to increase sensitivity to detect larger areas of activation, while smaller kernels will have the opposite effect.[3]

Denoising—Even after the above preprocessing steps, scans will still include non-neuronal noise. The most prominent sources include motion-related and physiologically-induced signal changes such as those from cardiac or respiratory pulsations[97]. Commonly employed techniques to maximize signal-to-noise ratio include nuisance regression of motion parameters and global signal, filtering out signal not related to the timescale of task-based stimuli in task-based fMRI (temporal filtering), nuisance regression of principal components of regions outside grey matter-related BOLD signal (e.g., cerebral spinal fluid), or use of independent component analysis to identify various noise types[97]. Physiological artifacts may be corrected by recording cardiac and respiratory fluctuations during scanning then using retrospective denoising algorithms (e.g. RETROICOR[98]) to remove noise correlated with these. Noise artifacts may be especially challenging to remove when their frequency correlates with the fMRI task[99], and the optimal denoising strategy may depend on study design[100]. For instance, in studies of pain, heart rate may increase with painful stimuli[101]. Denoising is particularly important when analyzing small ROI, for example, when performing brainstem functional imaging of hypothalamic activation in cluster headache[102].

Statistics

General linear model—Functional MRI studies often model BOLD response in two-level designs incorporating subject and group-level information. At the individual subject level, task-related timing is used to predict BOLD activity at each voxel in a multiple regression model. This results in a test statistic, such as the beta value in multiple regression, for each subject and each voxel. Group results are then obtained by combining subject-level results for each voxel in a general linear model (GLM). Associated p-values are calculated for each voxel, and displayed as a whole-brain spatial map, or statistical parametric map[103, 104]. Importantly, due to the large number of statistical tests involved, false positive results can be found in isolated voxels due to random noise. As such, it is critically important to control for this by correcting for multiple comparisons, as described below.

Multiple comparisons—For typical fMRI analyses, comparisons can be made at each of the ~100,000 voxels in the brain, in each subject, leading to a risk of false positive results. Therefore, failure to adequately correct for multiple comparisons will very likely result in false, or as in the case of a famous fMRI experiment on a dead salmon, deliberately absurd conclusions[105]. Absence of multiple comparisons correction in an fMRI study is a red flag and resulting conclusions should be treated with appropriate skepticism.

Multiple comparisons correction can be applied either to individual voxels or to clusters of adjacent voxels. Methods include Bonferroni correction to control familywise error rate (FWE), the probability of any false positive result, by dividing the uncorrected p-value significance level by the number of tests in the analysis[3]. Another commonly-used method is the false discovery rate (FDR), which controls the proportion of false positives by ranking all p-values and applying a sequential cutoff[106]. In contrast to the stricter Bonferroni correction, FDR has increased sensitivity to true positive results, with the potential tradeoff of including more false positives. Either method is acceptable for fMRI studies and both are preferable to even strict uncorrected p-values.

Cluster-level methods to control for multiple comparisons group neighboring voxels into clusters and then test the overall cluster size. Individual voxel-level p-values are first grouped into clusters by applying a cluster defining threshold (CDT) using a p-value cutoff. As a result, clusters of adjacent voxels with similarly low p-values are grouped together. For fMRI analyses, the recommended CDT is at least $p<0.01$, and is likely more conservative in many cases[107]. For example, if the voxel-level test statistic is from the gaussian distribution of z-statistics, or z-scores, applying a CDT of $p<0.01$ corresponds to thresholding all the significance level of voxels at $z>2.05$ or $z<-2.05$. Subsequently, the size of each cluster is tested for significance and reported as a cluster-level p-value that is then corrected for multiple comparisons. When displaying results in figures or tables, clusters or voxels that are non-significant after correcting for multiple comparisons should either not be displayed or clearly marked as non-significant.

Power

While more challenging for fMRI studies, a priori power analyses can help to estimate the necessary sample size for a study[108–111], with available analytical toolboxes designed

for fMRI power calculations (e.g., Neuropower[112]). Increasing sample size can improve reliability and reproducibility, but there are often constraints on study sample size due to cost, time, or limitations relating to the participant population. Multi-site studies and data sharing are potential approaches to this in addition to facilitating meta-analyses[109, 113]. The development of large-scale, publicly available neuroimaging datasets reflects recent efforts across the field to provide larger samples than most investigators could feasibly collect independently (e.g., Human Connectome Project[114]). Although large samples are advantageous for improving power, smaller-scale datasets still play a key role in neuroimaging research, particularly in studies involving repeated-measures intervention studies, or in assessing novel MRI paradigms[115, 116]. Repeated measures at the individual level can be powerful in driving data-driven discovery and can address unique research questions[117–121]. For example, a study by Schulte and May included daily scans of a single patient with migraine for one month to capture fMRI changes preceding migraine attacks[122]. Increasing the amount of data collected per person (e.g., by increasing the number of trials in task-based fMRI[111] or increasing resting-state scan length[123–125]) can also improve power and reliability[126].

Reproducibility concerns

Concerns regarding the reproducibility of neuroimaging research results (i.e., the "reproducibility crisis" or "replication crisis") have garnered much attention in recent years[109, 110, 116, 127–130]. Many of these can be addressed through careful attention to approaches outlined elsewhere in this article, such as best-practice pre-processing, appropriately addressing multiple comparisons, and statistical power; however, questionable research practices (e.g., testing different analytical approaches until a significant p-value is found, or "p-hacking"[131, 132]), suboptimal study design, publication bias favoring positive results, variability in analytical methods, and lack of transparency in methods may also play a role[113, 128, 130, 133]. Although sometimes termed a "crisis," the dialogue in this area has actually served to bolster ongoing efforts across the neuroimaging research community to promote the adoption of research practices supporting improved rigor and reproducibility, including a strong momentum towards increasing transparency and open science practices in MRI research[109, 134–137]. One such practice is pre-registering studies, in which planned hypotheses and analyses are described prior to conducting the study, which can increase confidence in study results by reducing positive publication bias, p-hacking, and instances of hypothesizing after results are known ("HARKing" [138])[109, 128, 132, 136]. Published manuscripts should also outline which analyses were planned ahead of time and which were exploratory and/or data-driven[109, 128].

Putting it all Together: Quality fMRI Headache Research

Given what is described above, one may already discern some of the issues to consider when reviewing this literature. Although there is no reporting guideline checklist, such as STROBE (Strengthening the Reporting of Observational Studies in Epidemiology)[139], listed by the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) Network[140] for fMRI studies, there are other resources. The Organization for Human Brain Mapping's Committee on Best Practices in Data Analysis and Sharing (COBIDAS) issued a report in 2016 on MRI research called the Best Practices in Data Analysis and Sharing in

Neuroimaging using MRI[14]. Its Appendix D, consisting of "Itemized lists of best practices and reporting items," can serve this purpose.

In addition to subject selection, participant consent, and other reporting criteria common to all clinical research, the following checklist features elements particular to fMRI of a well-performed and well-reported study.

For an excellent review of overall study design considerations in functional connectivity studies specific to migraine, see Maleki and Golub's review, which discusses questions of task vs resting-state and related concepts for this population[13]. For a review of pain neuroimaging in general with good attention to task design related to nociceptive stimulation, see Moayedi et al.[141] The following sections will focus on some additional items.

Ictal vs interictal—In the Schulte and May study of a single individual's daily evolution of fMRI over a one-month period and three migraine attacks, the authors found differences between ictal and interictal patterns of activity, with changes even during the prodrome period.[122] Other studies have shown differences between ictal and interictal fMRI as well[142]. As a result, most studies attempt to image patients that are either ictal or inter-ictal, without combining the two as noted in Maleki and Golub's review[13]. To accomplish this, studies may require that patients have been migraine-free for [72] hours prior to scanning if they are trying to capture the interictal period.[5]

Age—Functional MRI study design considerations could include how age is handled in pediatric studies. Of note, there has been a call to use age-specific atlases or templates in neuroimaging, particularly in younger children, given the evolving myelination patterns into adulthood[143, 144]; however, many important pediatric studies use software packages for analysis such as FreeSurfer that in turn use atlases based on older adults. This includes the enormous and still-ongoing longitudinal Adolescent Brain Cognitive Development Study (ABCD) and its Brain Imaging Data Structure (BIDS) standards-compliant Community Collection[145].

Sex and gender—Sex and gender are emerging areas of importance in headache medicine and research[146]. The NIH has begun to focus on reporting these variables with new requirements for considering sex as a biologic variable[147], though there is still progress to be made on reporting gender[148]. There are known sex differences in post-pubertal brain structure, including the amygdala[149], a region shown to have altered connectivity in migraine[150]. Considering sex and gender separately is also important, as transgender individuals who have undergone gender-affirming hormone therapy have reported alterations in headache with this therapy[151]. Given findings of sex differences in migraine patients with task-based fMRI[152] and resting state fMRI[153], it is reasonable to address this difference in study design or analysis in fMRI research, e.g. matching controls and cases for sex, or covarying for sex in statistical analysis.

Handedness—Resting-state functional connectivity patterns have been shown to differ by handedness[154]. As such, it is reasonable to gather information on handedness and use this as a covariate in fMRI studies.

Scanner

Multisite acquisition: In the case of relatively rare diseases, such as cluster headache, it may be desirable to recruit and scan individuals at multiple sites across a geographic region to gain statistical power for analysis; however, intra-individual differences in rsFC pattern when acquired in different scanners and different sites has been demonstrated.[155] The Functional Biomedical Informatics Research Network has published recommendations on how to reduce inter-site variability that should be considered when designing a multi-site study.[156]

Magnet strength: Although higher magnetic field strength, e.g. 3 Tesla (T) or 7T MRI as compared to 1.5T improves sensitivity to detect smaller areas of functional activation, higher field strengths cause additional artifacts that must be considered. This tradeoff is likely worthwhile, however, for smaller regions of interest such brainstem nuclei, e.g the trigeminal nucleus.[157]

Safety: Certain subjects may need to be excluded from any MRI study due to risk from exposure to the magnetic field, such as those with certain implanted or onplanted devices, e.g. cardiac pacemakers. For a more complete reference on this topic, see the American College of Radiology's current ACR Manual on MR Safety.[158]

Significance: In assessing the significance of findings beyond p-values, some have begun to explore the use of sensitivity and specificity[5] and others have suggested that activation clusters of voxels should be reported using a confidence interval[159–161] or confidence set[162]. It can also be helpful to consider whether data from other experimental methods support an fMRI study's conclusions. For example, altered connectivity in the PAG as a feature of migraine pathophysiology seems reasonable given its demonstrated role in reducing pain when stimulated in vivo[163]. As with many other avenues for scientific discovery, the strongest use for fMRI is in parallel with other techniques.

Considerations if asked to collaborate on fMRI research: If asked to collaborate on an fMRI study, consider whether the above have been addressed in the study design as well as the degree to which potential imaging collaborators understand headache or how early in the study design headache expertise was included. It is also important to consider the goals of potential collaborators in using fMRI to study headache, namely, the development of new experimental methods vs disease characterization. An example of new methods development is a recent paper demonstrating a novel method of identifying functional networks, dubbed functional areas of unitary pooled activity by the authors, in the visual cortex in migraine patients vs healthy controls[164]. It is often desirable to demonstrate a new imaging method on a well-characterized disease state such as migraine. In contrast, an example of disease characterization is a recent task-based fMRI study of trigeminonociceptive stimulation with

ammonia in cluster headache patients, using established methods for brainstem imaging and an established ammonia stimulation protocol[102].

Separate considerations may pertain to studies in special populations, such as children. For instance, braces are commonly found in pediatric subjects. Although braces are MR-safe, they have long been known to cause imaging artifacts, primarily signal loss or frontal lobe distortion in certain imaging sequences[165, 166]. Most imaging artifacts, including hardware-related, are more noticeable at higher field strengths[167], an important consideration for fMRI at 7 Tesla. Expense is also a limiting factor in data collection; at most institutions, hourly scanning costs for investigator-initiated NIH-funded studies are typically $600-$700.

Conclusions

The methods outlined above describe several attributes of research that can be applied to fMRI headache research to increase reproducibility, including methods transparency, eliminating error, using a priori hypotheses, and using power calculations. We could add to this use of standardized instruments such as the National Institute of Neurologic Disorders and Stroke Common Data elements[168] for covariates, use of the current International Classification of Headache Disorders[169] criteria for diagnosis, and development of large datasets, such as the American Registry for Migraine Research[170]. The promise of the field for understanding headache and potentially identifying biomarkers for treatment response or diagnosis is high.

Acknowledgements:

JAH would like to thank Angeliki Vgontzas, who suggested I write on this topic after Todd Schwedt's presentation at the Graham Headache Center as Visitans Scolaris Capitis Dolor.

Financial Support:

NIH/NINDS Child Neurologist Career Development Program (CNCDP) grant K12NS098482 subaward to Jennifer Hranilovich

Abbreviations

fMRI	functional magnetic resonance imaging
MRI	magnetic resonance imaging
PET	positron emission tomography
EEG	lectroencephalography
BOLD	blood-oxygen-level-dependent
rsFC	resting-state functional connectivity
DTI	diffusion tensor imaging
ROI	region of interest
ICA	independent components analysis

ICN	intrinsic connectivity network
ALFF	amplitude of low frequency fluctuations
fALFF	fractional amplitude of low frequency fluctuations
ReHO	regional homogeneity
FSL	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library
AFNI	Analysis of Functional NeuroImages
SPM	Statistical Parametric Mapping
LONI	Laboratory of Neuro Imaging
MNI	Montreal Neurologic Institute
ANOVA	Analysis of Variance
FWHM	full width at half maximum
GLM	general linear model
FWE	familywise error
FDR	false discovery rate
CDT	cluster defining threshold
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
EQUATOR	Enhancing the QUAlity and Transparency Of health Research
COBIDAS	Committee on Best Practices in Data Analysis and Sharing
ABCD	Adolescent Brain Cognitive Development
BIDS	Brain Imaging Data Structure. 2

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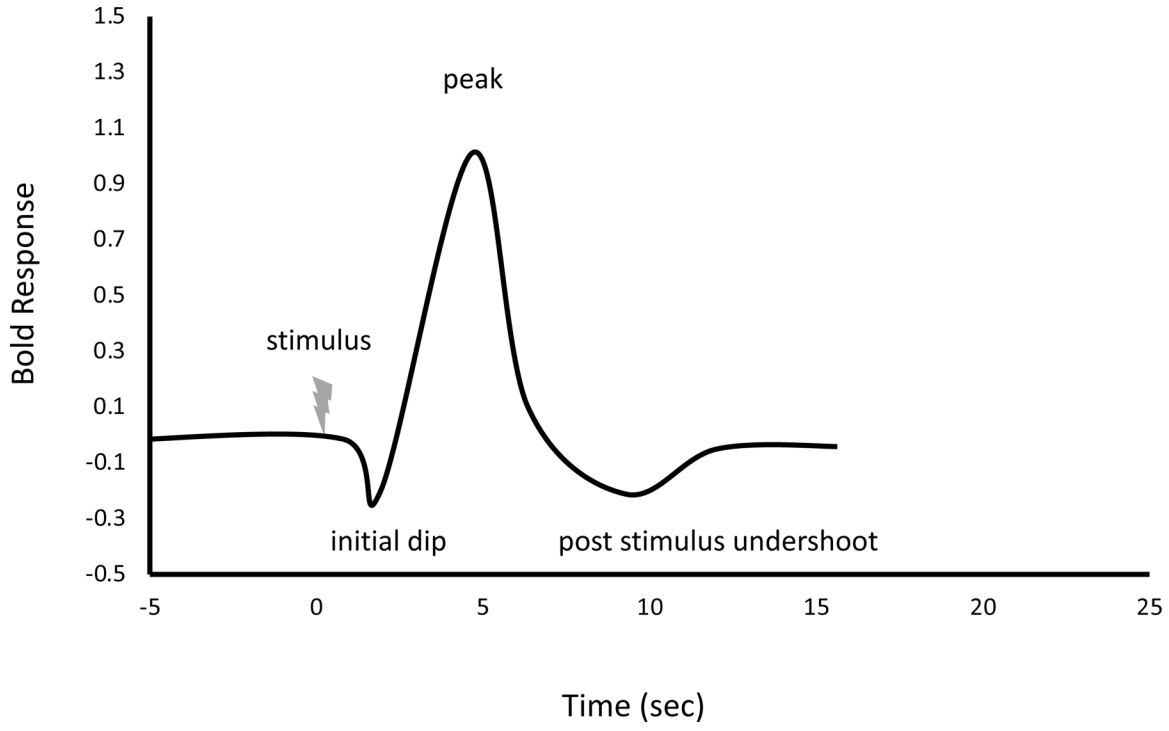

Figure 1:

Hemodynamic Response Function

Representative of averaged response after ~1 second stimulus at 7 Tesla strength. Curve normalized by the height of the hemodynamic response function as a function of the time after stimulus.[171], [172]

Table 1.

Checklist of fMRI study essentials

	Are the Figures thresholded or unthresholded?
	Have the authors addressed multiple comparisons in some way?
	Have the authors reported areas of peak activation coordinates for task-based fMRI?
	Did the authors have pre-specified hypotheses and/or primary outcome(s)?
	If multiple scanners and/or image acquisition sites, has this been addressed, particularly if the project is longitudinal?
	Have scanner noise and related field inhomogeneities been addressed?
	Was handedness reported and addressed?
	Has physiologic noise been addressed?
	Has subject motion been corrected?