Functional Magnetic Resonance Imaging - An Insight into the Imaging Trends

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Abstract: ***The application of Functional Magnetic Resonance Imaging (fMRI) has passed through various stages of evolution ranging from research-based imaging to clinical imaging techniques. As a primary indication, it is commonly used for pre-surgical mapping of functional areas around the tumour and areas of resection. In the early 1990s, Ogawa et.al initially described the Blood Oxygen Level Dependent (BOLD) imaging technique with animal studies. In recent years, the methodologies of visualizing the human brain have passed through an enormous evolution. Whenever there is a stimulation of neural activity, there is an increase in blood flow by capillaries adjacent to the region which reflects the increased synaptic activity in that specific area. During this process, the cerebral blood flow (CBF) and cerebral blood volume (CBV) are increased with a collateral increase in oxygen than necessary. The difference in susceptibilities between oxy and de-oxy hemoglobins can be identified by using MRI with their different susceptibility effects. This imaging approach by MRI is called BOLD imaging. The functional MRI can be performed in either of two ways, task dependent fMRI and resting state fMRI. The tasks vary depending on motor or language with dedicated adult and pediatric protocols. The acquired images were subjected to various post-processing steps like image realignment and motion correction, Coregistration, spatial normalization and smoothening, statistical analysis, and activation map generation. The usual clinical applications of fMRI include language lateralization, brain plasticity evaluation, and motor region evaluation. Though there are some technical challenges, fMRI is considered a powerful imaging tool to visualize the neural correlation of subject behaviour in higher resolution non-invasively.***

Keywords: Functional MRI, Post Processing in fMRI, fMRI

1. Introduction

The functional magnetic resonance imaging (fMRI) technique uses a powerful magnetic field and magnetic gradients to create, detect, and record the MRI signal with an adequate image processing method. This is used to effectively map the functional imaging of the brain with related neuronal activity. (1) The clinical application of fMRI is improvised with the help of combining it with routine MRI imaging which gives structural information and the acquired fMRI gives functional information from the sensory, motor, and cognitive processes as shown in figure 1. The combination of these two results in increased spatial and temporal resolution. The main reason a patient can be referred for an fMRI is to localize the areas in the brain responsible for motor, language, and memory functions before performing the surgery. (2) The fMRI imaging technique is based on the cerebral blood flow changes related to the energy demand and neuronal activity. (3)The traditional methods of acquisition are in two dimensional (2D) slices and manipulating it into surface models etc. (1)

2. History

The application of BOLD fMRI has passed through various stages of evolution ranging from research-based imaging to clinical imaging technique. As a primary indication, it is commonly used for pre-surgical mapping of functional areas around the tumor and areas of resection. In the early 1990s, Ogawa et.al initially described the BOLD imaging technique with animal studies. (4) Ogawa observed the effects induced by BOLD in T2* weighted imaging in rodent examination. This initial imaging is based on the concepts of Linus Pauling in 1935 and Keith Thulborn in 1982 who described about the magnetic property changes in hemoglobin by oxygenation. (5) In 1991 it came to human imaging when Belliveau et al, imaged the human visual cortex with the help of fMRI. These days, fMRI has emerged as one of the most beneficial research techniques in the modern age of cognitive neurosciences with its clinical application in neurology, psychology, and linguistics. (4) Belliveau demonstrated fMRI imaging with the use of contrast media in the annual meeting of the Society of Magnetic Resonance in Medicine in San Francisco. After that, contrast-free fMRI was described by R. Turner. Then several researchers contributed to the evolution of fMRI thus resulting in a 2% BOLD effect in the visual cortex achieved with the usage of Echo Planar Imaging (EPI) pulse sequence by flashlight simulation. This research was published by Kwong and Turner. During this time, the clinical usage of fMRI was quickly recognized as PET-based functional imaging and was researched by most researchers. The disadvantage in that is due to the usage of ionizing radiation which prevents longitudinal observations. In 1992 Frahmet.etal, first described the onset, rise, and duration of the BOLD signal using visual stimulation by using fast low-angle shot (FLASH) imaging. But compared to FLASH, EPI enabled higher slice acquisition with considerable prone to artifacts and distortions. In addition to this many researchers from Germany have joined the research team of Harvard-based research group and developed the spin echo imaging optimized for fMRI acquisition. This technique shows increased magnitude of BOLD signal, reduced image noise, and optimized spatial and temporal resolution. (5) In the past years, tumor imaging in MRI has various enhancement methods namely fluid attenuation inversion recovery (FLAIR) sequence, post-contrast imaging, etc**.** (6)The greatest advantage to neurosurgery in recent days is to preoperatively image the functional part of the brain that is responsible for a cognitive process using BOLD imaging and fMRI has benefitted neurosurgeons in determining the surgical intervention of brain cortex.(7) The imaging can be done for functional mapping in both healthy as well as preoperative assessment patients.(8) The major disadvantage of fMRI was the time taken to post-process the data to get adequate clinical outcomes.(2)

Today's fMRI

In recent years, the methodologies of visualizing the human brain have passed through an enormous evolution.(1)The versatility of MRI is its ability to map the cerebral cortex as well as the functioning of the brain with the help of cognitive and sensory stimulation.(9) The adequate field of view, and high resolution facilitated by fMRI have made it an excellent tool for mapping the brain non – invasively.(3) In clinical practice, fMRI is done by performing some tasks inside the magnetic bore of an MRI scanner to generate an fMRI signal. To attain this, proper patient cooperation and effective task performance are mandatory to avoid spurious results. Also, the concern that needs to be considered is compliance with the tasks and adapting to the tasks, particularly in pediatric and pathological patients. (8)

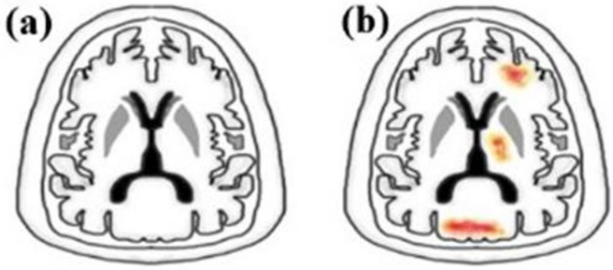

Figure 1: Schematic representation of routine MRI image with fMRI image. The diagram (a) – represents axial brain MRI image. (b) – represents fMRI brain axial image with functional activations (coloured regions).

Choice of mapping technique

As each patient represents unique pathological, structural, and functional properties of the brain, it is not optimal to generalize a specific brain mapping technique for all. Hence it must be made according to the pathological condition and expected clinical outcome. The mapping techniques can be widely categorized into two types namely, invasive and non-invasive techniques.(10) In the olden days, pre-surgical brain mapping was done by Intraoperative Electro cortical Stimulation (ECS) and Intra Carotid Amobarbital Test (WADA). However, due to their invasiveness, their clinical implementation was lesser compared to the advancements in non-invasive brain mapping techniques.(8) The American Society of Functional Neuro Radiology (ASFNR) was established in 2004 to report the concerns in the clinical usage of non-invasive techniques like BOLD fMRI, Diffusion Tensor Imaging (DTI), MRI perfusion imaging, and Spectroscopy.(4)

<u>Invasive Technique</u> –It includes ECS and WADA. These techniques were considered the golden standard for mapping brain functions and determination of dominant hemispheres invasively. However, the expected clinical information is minimal compared to the imaging techniques.(10)

Electro cortical Stimulation Technique(ESM)– This method is used to localize the language functions done by the direct application of electric current to the cerebral cortex of an awake patient. This produces a response or disrupts the function. This technique is considered a golden standard imaging for language localization in patients with temporal lobectomy. This method can be done extra operatively in implanted electrode patients and intraoperatively with cortex-exposed patients. Though this method is considered a golden standard, it has potential disadvantages that include invasiveness, tendency to cause epilepsy, prolonged operation time, etc.(11)

WADA(11–14)–The sodium amobarbital was injected intra arterially (Internal Carotid Artery) by Wada and Rasmussen to anesthetize the cerebral hemispheres for the lateralization of language dominance. When one side hemisphere is anesthetized, the contralateral hemisphere can be evaluated. Initially before anaesthesia injection, the baseline acquisition is acquired. After anesthetized, the patient performs expressive and receptive language tasks like number counting, naming objects, etc. along with responding to verbal commands activates the frontal and posterior brain regions. In case the dominant hemisphere of speech is anesthetized, the patient cannot deliver speech temporarily, and vice versa in case of non-dominant hemisphere anaesthetization. This procedure is also used in the assessment of hemispheric memory competence. In most epileptic surgical cases, WADA is done prior to the surgical resection of the epileptic foci. This test is done by anesthetizing each hemisphere and observing the disruption degree of language production and comprehension.

This information is crucial for planning the surgery and also to rule out whether additional mapping technique like cortical stimulation is needed or not. But the procedure has disadvantages like,

1) Carotid catheterization has a morbidity risk of 5%
2) Higher cost
3) It provides lateralization of language but does not localize the speech or other functions.
4) Discomfort during the procedure
5) It does not provide more specific information about the language localization in the hemispheres. (lesser spatial resolution)
6) It highly depends on patient cooperation verbally which is challenging in cases of mentally challenged patients and young children.

All the disadvantages led to the advent of fMRI which is a non-invasive imaging technique with more specific information on brain activations.

<u>Non-Invasive Technique</u>**:** It includes fMRI, Positron Emission Tomography (PET), Magnetoencephalography (MEG), Electroencephalography (EEG), and Transcranial magnetic Stimulation (TMS). The MEG and EEG have good temporal resolution but it has very poor spatial resolution.(15)

DTI: It is the quantification of diffusion properties of the water molecules which is based on the principle of unrestricted diffusion of water molecules along all the directions in cerebrospinal fluid (CSF). But in white matter (WM) the diffusion increases parallel to the fiber bundles (anisotropic diffusion) and is restricted axially. Thus DTI is done by diffusion-weighted imaging acquired in various gradient directions in many encoding steps and directions. Fractional anisotropy (FA) is an indirect measure of the structural integrity of fiber tracts. If the FA is higher it reflects greater integrity of WM whereas lesser FA shows demyelination, reduced axons and their diameter, etc. DTI provides direct information regarding the axonal tracts thereby ruling out the functional connectivity between brain areas.(15–17)

Other imaging evaluation techniques include imaging of the hippocampus by visual estimation or by volumetric assessment. The morphometry is based on the analysis of local deformity and voxel size attenuation values by statistical parametric mapping (SPM) that is used for the evaluation of grey matter (GM).(17)

Functional Magnetic Resonance Imaging (3,7,9,11,15,16,18–21, 22–24)

Physiological Process and its Biophysics

The brain is the controller of all the functions in the body. The brain is divided structurally as well as functionally into different regions. This functional area is responsible for the complex functions that reflect human behaviour. Neurons are the structural and functional units of the brain. These neurons require a continuous supply of glucose or oxygen from the blood as there is no reservoir for energy. The total volume of the brain is approximately 2% of the body weight but it needs 20% of glucose as energy for functioning. Whenever there is a stimulation of neural activity, there is an increase in blood flow by capillaries adjacent to the region which results in increased delivery of oxygen and glucose by vasodilation of the vessels. This process is mathematically called as "hemodynamic response function" (HRF) which is due to neurovascular coupling. The increase in blood flow reflects the increased synaptic activity in that specific area due to a specific stimulation. The signal generated by this activity is not only from the localized neuron firing rate but also subthreshold activations like simultaneous excitation of axillary regions. The HRF starts at 200 – 400ms after neuronal activity and it takes approximately 2-5 seconds delay for the blood to travel from the artery, capillary, and to the vein. During this process, the cerebral blood flow (CBF) and cerebral blood volume (CBV) are increased with a collateral increase in oxygen than necessary. This occurs secondary to the neuronal stimulation and results in changes in oxyhemoglobin (hemoglobin with oxygen) and deoxyhemoglobin (deoxygenated hemoglobin) levels, where oxyhemoglobin is diamagnetic or isomagnetic and deoxyhemoglobin is paramagnetic relative to brain tissue. The oxygenated blood causes very little or no distortion in the field whereas the veins and capillaries distort the magnetic field. This change reflects the increased oxyhemoglobin arrival in the area under stimulation beyond the necessary demand which paves the way for increased oxyhemoglobin in post capillary bed (vein capillaries). The difference in susceptibilities can be identified by using MRI with their different susceptibility effects (increased oxyhemoglobin). Thus the degree of blood oxygenation (i.e.) deoxyhemoglobin is used as an endogenous MRI contrast for measuring the neuronal activity related to CBV changes indirectly and creates functional mapping of the brain. This imaging approach by MRI is called Blood Oxygen Level Dependent (BOLD) imaging. The fMRI contrast mechanism is based on the T2* relaxation time. The resultant signal is an overall averaged signal from the changes between intravascular and extravascular water molecules. BOLD fMRI is the imaging approach that is used to detect the functional analysis of the brain in healthy as well as diseased individuals by detecting smaller changes in the relative ratios of oxy and deoxyhemoglobin. In short, the reduction in inhomogeneity with the extra oxy-hemoglobin signal in the postcapillary bed gives the signal as shown in figure 2. This type of imaging is preferable in higher magnetic field strength equipment like a 3T scanner rather than 1.5T as the BOLD signal is 4 – 5% higher with lesser scan time in 3T. The functional MRI can be performed in either of two ways task dependent fMRI and resting state fMRI.

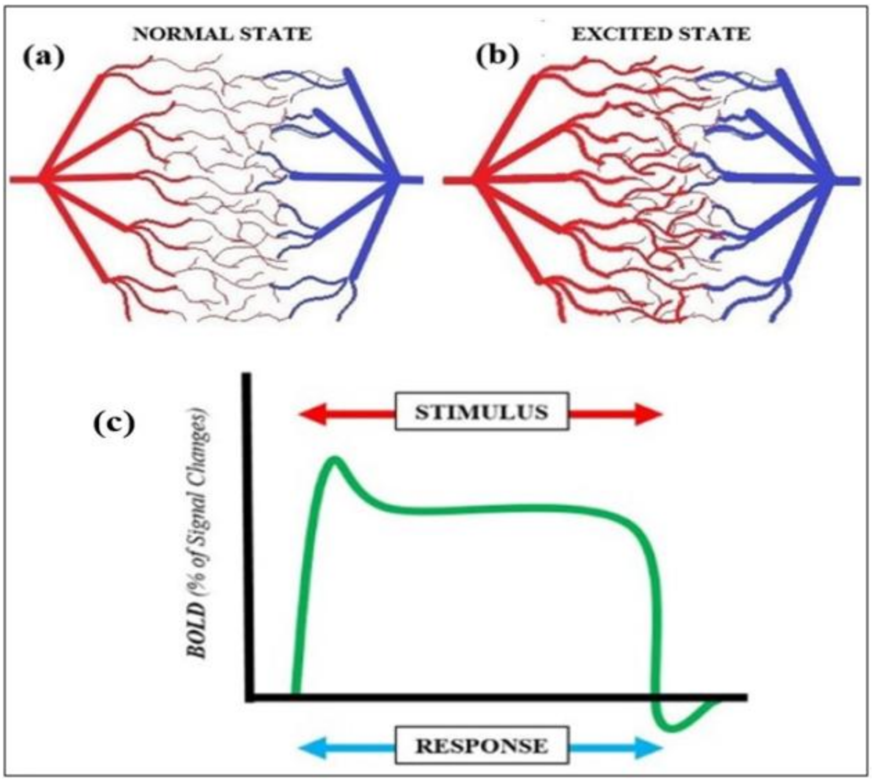

Figure 2: Schematic illustration of BOLD activity changes in the capillary bed and the mechanism behind it. The diagram (a) shows normal blood flowing through artery (thick red line), capillary (thin line), and draining to vein (thick blue line). The diagram (b) shows higher level of oxy hemoglobin in the artery and capillaries due to a specific activity in the respective area. The diagram (c) shows the graph of BOLD response with the presented stimuli / task. There is an initial overshoot in the curve followed by a respond plateau. Finally there is an undershoot dip in the signal below baseline after task completion. The diagram clearly shows that the duration of the stimuli is directly proportional to the duration of the BOLD response.

Task Based fMRI(6,8,10,11,17,19,25)

The task-based fMRI imaging uses changes in blood flow to map the areas of cortical activation by performing certain tasks like visual, motor, and language. This is based on the mechanism of changes in the BOLD signal during a resting state (baseline) versus a particular task (activity). This reflects the changes in the CBF which is an indirect measurement of synaptic activity in a specific area. Whenever a task is performed, the neuronal activation is higher in a specific area related to the task stimulation leading to the increase in regional CBV and CBF. The signal intensity is directly proportional to the perfusion rate and hemoglobin ratio in the area under evaluation by a specific task. This mechanism has wider acceptance in clinical applications. The tasks are performed with the help of the fMRI paradigm that is displayed inside the scanner during acquisition. The tasks are performed several times repeatedly during the task acquisition period to eradicate noise in the signals. The human language system mainly consists of expressive Broca's area in the postero-inferior frontal lobe and receptive Wernicke's area in the postero-superior temporal lobe. In some language tasks, Broca's area is more highly activated than Wernicke's area.

Resting State fMRI (10,16,17,20,26–28)

The resting state fMRI serves as the tool of choice for mapping of brain's neural architecture and its development. The resting state fMRI is the imaging of spontaneous BOLD fluctuations and alterations with the patient in a resting state (i.e.) no task is given or done during the acquisition that is useful for mapping functional brain network architectures. The main consideration in this type of approach is the abundance of data (activations) which needs sophisticated analysis methods. The resting state fMRI is helpful for the evaluation of connectivity of the brain in a resting state and can be imaged even in uncooperative patients like young children, altered mental status patients, or patients under anesthesia. In addition to this advantage, resting state fMRI is useful to map various neural networks at the same time compared to task-specific activations by routine fMRI. The term connectivity is coined by the "temporal correlation of neurophysiological index measured in different parts of the brain". This neurophysiological index is mapped by the low-frequency fluctuations ranging from 0.01 – 0.1 Hz which can be differentiated from respiratory and cardiovascular signal frequency that ranges between 0.1 – 1.2 Hz. These fluctuations were crucial for the evaluation of neural networks such as visual, auditory, sensory-motor, and cognitive performance. This fluctuation's functional importance was presented by Biswal et al, in the year 1995. This method of mapping has the advantage of simultaneous mapping of multiple regions with the minimal protocol that needs to be followed compared to task-based fMRI for signal generation. To improve the Signal – to – Noise Ratio (SNR), spatial smoothening and low pass filtering (0.1Hz) are used to null the non-neuronal peripheral signals.

Thus the fMRI mapping done by either task-based fMRI or resting state fMRI is acquired as a series of images with the time frame in seconds for task-based acquisition to generate an image and in resting state acquisition the entire scan time may range from 5 – 15 minutes. The regions with significant activation are calculated by the statistical analysis by a linear model with multiple linear regression algorithms.

fMRI Procedure

The routine anatomical MRI images in three dimension were acquired initially as reference images. Then the fMRI study is done initially with a gradient field mapping sequence to ensure field homogeneity as fMRI is sensitive to inhomogeneities. Then it is followed by the EPI-based fMRI BOLD sequence planned the same as the reference images. The acquisition is done by various methods as mentioned below. During the BOLD acquisition, the patient performs tasks based on the paradigm shown which is chosen with the clinical indication as mentioned in table 1. The additional sequences like (DTI or Perfusion) are performed based on necessity.(22) In an fMRI study, the whole brain BOLD functional images were acquired once in 2 – 3 seconds resulting in several time series images (i.e. datasets) which have around 1,00,000 voxels.(18,19) The functional mapping of the brain's activated areas will be co-registered with the anatomical MRI for effective interpretation of cortical maps.(21) Recently many advancements like dedicated pulse sequences, peripheral devices for presenting stimuli, and devices for recording the response, statistical analyses, etc. make the fMRI procedure even easier with highly efficient results.(15)

Indication (4,7,10,14,23,27,29–32)

The clinical fMRI are usually done for patients with,

1) Preoperative language lateralization and assessment for patients with epilepsy, vascular malformation, and tumors. The lateralization is useful for assessment for cognitive functional analysis.
2) Especially for preoperative epilepsy patients, the localization of hemispheric language dominance is crucial in cases of focal and drug-resistant epilepsy. Compared to the mapping of language area, ruling out the hemispheric dominance is important.
3) Preoperative imaging of lesions in the brain that are closer to the eloquent cortex for functional risk assessment. The region of assessment includes areas of *motor, sensory, language, memory, and visual*. Precise imaging is mandatory for ruling out functional anatomy and language dominance around the pathological area to give effective neurosurgical outcomes.
4) Post-therapeutic follow-up examination for the evaluation of preserved eloquent cortex.

As most of the treatments/indications are based on the surgery, the preoperative assessment of surgical feasibility, approach, and risks are crucial considerations. Thus the assessment of areas by fMRI is useful for guiding the neurosurgeon in surgical planning as well as resection of pathological areas without damage to the surrounding healthy tissues. The surgical planning is decided mainly by the mapping of the anatomical location of the tumor and the surrounding functional areas. It also provides additional information to proceed with the surgery in high-risk areas like *Broca's and Wernicke's areas*. However, in cases of drug-resistant focal epilepsy, surgical intervention leading to the resection of the area is the only treatment to attain long-term control of seizures.

The fMRI has also extended its application in hippocampal imaging along with the other regions involved in schizophrenia and Alzheimer's disease. The role of fMRI analysis in stroke imaging is still in research and evolution for clinical practice. Also, the resting state fMRI is in its evolution and early stage of implementation in preoperative functional assessment and language dominance. It may have a role in the future for diagnostic and prognostic value in neurology and psychology.

Patient Preparation (7,10,22)

The additional consideration compared to routine MRI examination patient preparation was the task familiarity with the patient prior to the examination with detailed communication. The examination accuracy is improved by adequate task familiarity. Familiarity refers to the practice of the paradigm that is to be displayed during the acquisition. The paradigm must be in the patient's own mother language as it gives higher robust activation and also use of alternate language leads to eloquent region's signal overlapping leading to slight differences in signal intensity. Also, the patient's handedness must be confirmed prior to the examination. Apart from normal patients, care must be given to patients with weakness, cognitive disorders, deafness, and neurological deficits whose performance will be compromised compared to normal patients resulting in optimal performance and signal generation during the procedure. In such cases, the paradigm and task must be tailored according to the patient's limitations. For patients with motor deficits, sensory paradigms will yield the localization of the motor cortex. Likewise, the routine tasks can be replaced with an alternate task that yields similar clinical information. For patients who are unable to perform any task, is examined with the help of resting state fMRI.

Tasks and Paradigms

During the initial clinical application for fMRI BOLD imaging, the U.S. Food and Drug Administration (FDA) strictly restricted the examination to research only as the stimuli (task) and its related paradigm for a specific regional mapping were at the beginning stage of research and very lesser literature were done on that which paved the way to conduct even more researches on that in many institutions. After many analyses, the recent FDA-approved commercial MRI scanners are packed with the latest paradigm designs that ease the task delivery during acquisition and relevant updated post-processing packages.(4) As already stated, BOLD signal generation is a slow response due to the inter stimuli intervals. Hence the paradigm designing must be taken care of to avoid overlapping of signals from rest and tasks.(9) The resting state baseline tasks must be designed or selected with utmost care as the signal differentiation between rest and tasks is important for fMRI. There is no standardized paradigm for rest states.(7) In many parts of the world, fMRI is clinically done for the pre-surgical mapping of language functional assessment in cases of brain tumor, and epilepsy surgery, and still counting on its additional implementation in various other studies.(29) The motor tasks are done to evaluate the motor cortical homunculus which is done by active motion performed either unilaterally or bilaterally or a combination of both. (22) The tasks and their relevant paradigm were listed below as per the ASFNR in table 1.

Table 1: List of Tasks and its relevant paradigm used in fMRI as per the ASFNR recommendations. (29)

Sl. No	Task	Stimuli Generation	Clinical Usage	Paradigm
LANGUAGE				
(1)	**Sentence Completion (SC)**	• An incomplete sentence is shown in the display. • The patient has to sub vocally think about the word that completes the sentence. • If the task is done quickly during the acquisition phase, then the patient can continue to think for any alternate words.	Language Localization and Primary Language Cortex identification *Activates – Wernicke's area and less robust Broca's area.*	The sentence is presented in *Times New Roman* font with 42 in size.
(2)	**Silent Word Generation**(SWG)	• A single word is shown in the display. • The patient has to sub vocally think about the words starting with the displayed letter as much as possible during the acquisition phase.	Pre surgical mapping of Language Lateralization and Localization. *Activates – frontal lobe cognitive and language areas.*	The Letter is presented in *Times New Roman* font with 117 in size.
(3)	**Rhyming**	• The display is presented with two words one below another. • A response pad (shown in figure 6c) is given to the patient and advised to press the button in case the two words rhyme each other.	It is done along with the SWG for effective Language Lateralization. *Activates – Broca's area and less robust Wernicke's area.*	The word is presented in *Times New Roman* font with 200 in size.
(4)	**Object Naming** (ON)	• The display is shown with an object image in white outline and black background. • The patient has to name the object silently.	It is done along with SC and SWG for activation of critical language areas. *Activates–frontal gyrus, ventral occipitotemporal cortex.*	Optimal sized images will be displayed as not text is present.
(5)	**Antonym Generation** (AG)	• A single word is presented on the display. • The patient is advised to think the opposite term of the given word.	To assess Speech and Language systems. *Activates – Speech areas and Broca's area (compared to baseline).*	The word is presented in *Times New Roman* font with 44 in size.
(6)	**Passive Story Listening** (PSL)	• The patient is given the MRI compatible headphones and background story is played during acquisition. • This makes easier task performance even for pediatric patients whose ability to perform other language based tasks harder.	To assess syntactic processing and words recognition. *Activates – superior gyrus*	A 20 seconds story will be played that will have selected passages from a tale.
(7)	**Breath Hold Task**	• The patient will inspire air in a slow controlled manner for a time frame of approximately 4 seconds. • Then a breath hold of 16 seconds is done. • Finally 40 seconds of normal breathing is carried out.	To assess the functional analysis changes between normocapnia and hypercapnia conditions.	The task can be intimated in a *Times New Roman* font with font size of 44.
MOTOR (22)				
(8)	**Hand Clenching**	• The patient will initially clench the hand. • Then the hand will be opened.	Assessment of upper Extremity function	
(9)	**Finger Tapping** *(Simple and Complex)*	• *Simple* – A single finger is tapped with the thumb either single time or continuously. • *Complex* – Multiple fingers is tapes as same as simple.		
(10)	**Foot tapping & Ankle –** *Flexion and Extension*	• The patient flexes and extends the foot. • This is done either single time or repeatedly.	Assessment of lower Extremity function	
(11)	**Lip punckering**	The patient puncker and unpuncker the lips repeatedly.	Activates bilateral inferior motor cortex.	
(12)	**Tongue thrusting**	The patient moves the tongue side to side and thrust it against the teeth repeatedly.		

The tasks and their combinations were discrete for adult and pediatric patients. The combinations are mentioned below.

Adult Protocol - The combination of SC, SWG, and rhyming tasks is clinically implemented as an adult language assessment protocol. For higher language area activations SC task is preferred compared to SWG. In cases where the patient is unable to perform SC and SWG, the preferable tasks were ON or PSL. The main drawback of PSL is that there is no route to identify whether the patient is listening or not. In some cases, the patient is advised to recall the story heard which could act as an indirect task performance.(29)

Pediatric Protocol–The pediatric patients in the age group between 5 to 11 years of age were designed with this protocol. This protocol uses a combination of SC, rhyming, and AG. For patients unable to perform rhyming, PSL is recommended. The choice of the task depends on the individual assessment of the patient with relevant task-performing capability prior to the study. In pediatrics, the lateralization areas are highly visible when performing SWG and rhyming tasks compared to SC. Though SC gives robust overall activations. In addition to this BH tasks can also be used which helps in detecting false negative language activations that lead to accurate diagnosis. However, due to the atypical post-processing technique for the BH task, it is not preferred. (29)

As per the ASFNR criteria, the pre-surgical mapping of language areas must be done considering the mentioned tasks and relevant paradigms. Also, ASFNR recommends that at least three combined tasks must be done for various language areas assessment.(22) The algorithm combination and individual tasks were framed depending on the areas that needed to be activated. The paradigms are made according to the sensitivity and specificity of the signal enhancement as well as its role in the localization and lateralization of areas. The areas of activation and relevant tasks were given below,

- Frontal / Expressive regional activation – SWG, ON, and AG.
- Temporal / Receptive regional activation – SC and PSL.

In the above-mentioned tasks, SC and SWG must be performed first as they are highly robust. For a language-based task, the combination of tasks is preferred for a patient and not an individual due to the reason that a specific task does not activate all the regions involved. The language consists of orthographic, phonologic, pragmatic, semantic, and discourse dimensions areas for complete processing. Hence multiple tasks are done during the acquisition to enhance more areas of the entire process which would result in an effective mapping of the region.(29) In the case of younger or developmental delay patients, the tasks must be tailored with lesser and shorter span tasks with relevant paradigms.(22) The ASFNR conducted a poll regarding the choice of a task in the language task battery. The results show that SWG and SC are the most commonly used due to their reliability and usefulness. (29) A study by Pillai et al., has shown that SWG and rhyming are more efficient in language lateralization.(10) In the preoperative mapping of the human brain, language, and motor tasks are the commonly performed fMRI examinations.(7) For the mapping of motor areas near interhemispheric fissure, tasks and paradigms related to leg or foot is used as their motor areas are in that region.(7) The fMRI of motor cortex evaluation in sedated patient with passive motion of limbs have shown successful results in some reports.(8)

Various studies and meta-analyses showed that for Language Lateralization, there is a strong correlation between fMRI and WADA test in all patients. In addition to this Janecek et al, ruled out that fMRI is superior to WADA in the postoperative evaluation of language function tests. A guideline from the American Academy of Neurology suggested that fMRI can be replaced with WADA in epilepsy patients.(10) The correlation between EEG and fMRI has shown good agreement in functional localizations.(15) But in the case of ECS, fMRI is not a potential replacement due to the discordance in the results between ECS and fMRI.(10)

A study of 10 patients with TLE by Lehericy et. al, was done with language paradigms of SWG, PSL, and sentence repetition. From this study, the SWG is effective compared to other tasks and more correlative to WADA. A study by Rutten et.al was done with 7 unilateral TLE and 6 bilateral TLE patients for fMRI language lateralization. The results have shown that a combination of verb generation, verb fluency, picture naming, and sentence comprehension showed better language lateralization. The largest study for correlation between fMRI and WADA was done by Woermann et al with 100 patients with epilepsy and 64 of them have TLE. The only task and paradigm used was SWG which yielded a 91% concordance rate.(11)In a study, the fMRI for a musician was conducted with a separate algorithm other than the recommended tasks as routine protocol does not activate areas of musical processing which is crucial for the patient rather activates speech and memory lateralization. The tasks framed for this type of patient is called as ***Musical Paradigm***.(33) They are,

- ***Listening*** – This task involves hearing of music during the acquisition which rules out auditory and extra auditory substrates of areas of musical processing. This task activates different degrees of auditory lateralization in primary auditory cortex.
- ***Humming***–This task involves the humming of a specific music by the patient without involvement of external stimuli for assessment of motor areas of musical involvement and memory. The activated areas were auditory cortex in left side *(the patient is left handed)* eloquent to tumour region.
- ***Thinking –*** This task involves thinking of a specific musical piece during the acquisition which stimulates mental imaginary in music.
- ***Musical Tones***(22) – This task involves listening to musical tones of varying frequencies during the acquisition.

Apart from the routine motor, language, and musical paradigm and tasks, ***Visual Paradigms*** were also used in fMRI which involves,(22)

- ***Expanding Checkerboards*** – This task involves watching the expanding checkerboards from centre to peripheral with high contrast.
- ***Rotating Checkerboards*** - This task involves watching the ring rotating through multiple angles.

Task cooperation is one of the most important parameters during fMRI acquisition. The occurrence of False Positive results is incident in cases of superlative activities like bilateral limbic motions (motor coactivation) or mouthing words (tongue activation). The False Negative results are incident in case the patient fails to perform the task or falls asleep during the scan.(22)

Recently, respiratory challenge fMRI has been implemented clinically which is done by a short-term breath hold that increases the CBF leading to vasodilation. In cases of tumor evaluation, the BOLD response can be mapped which will be reduced or completely eradicated. Also, fMRI may miss or underestimate the BOLD response of eloquent regions which can be overcome by using intraoperative cortical stimulation.(22)

fMRI Paradigm Designing Tools(7,19,22,34)

As discussed above, a paradigm is used to provoke the hemodynamic response for fMRI imaging that is used to examine various functions like sensory, motor, visual, language, and auditory functions. This can be presented either visually, auditory, or both. Hence for acquiring effective examination results, the paradigm has to be designed accordingly. The paradigm must be designed and tailored according to the patient's maturity and cooperation in performing the task as well as specific mapping of the eloquent cortex to the pathology in anatomical MRI images irrespective of peripheral motor activations. Currently, there are many paradigm designing packages available which have their respective advantages and disadvantages. The available software packages are A Simple Framework (ASF), BOLDsync, Cogent, E-Prime, FLX lab, Inquisit, Nordic Aktiva, Paradigm, Presentation, Psychophysics Toolbox, and Superlab, etc. Among these, any software program can be used for a variety of stimuli presentations and synchronization with the scanner during acquisition.

Pulse Sequence and Acquisition Design (6, 7, 9,10,13,15,19,22,23,34)

The pulse sequence is based on the Gradient Echo acquisition with parameters sensitive to the changes in hemoglobin levels. The Echo Planar Imaging (EPI) technique is used which has a shorter scan time (i.e.) faster acquisition which collects the entire brain dataset in fewer seconds. The Spiral k-space filling is preferred instead of routine Cartesian filling to overcome motion artifacts and faster image acquisition. However, the compromise in this imaging technique is the degradation of temporal and spatial resolution as well as the prone to susceptibility artifacts. This is improved by the introduction of higher field strength magnets. The mapping of the brain's neural activity over a period of time across the whole brain needs higher spatial and temporal resolution. In fMRI, the temporal resolution is based on the time it takes to acquire one entire brain volume. The lesser temporal resolution is due to the time taken for the hemodynamic response event to occur after a stimulus is presented. The spatial resolution is lesser compared to the routine anatomical MRI. In addition to this, the temporal and spatial resolution are opposite poles (i.e.) increasing the spatial resolution (increased scan time) will collaterally decrease the temporal resolution. This increase of any one parameter will also contribute to a decrease in SNR. Hence to overcome these obstacles, multiband and multi-echo sequences were designed which also increases the Contrast-to-noise ratio (CNR).

The susceptibility artifact is highly noted in the frontal and temporal regions of the brain where air/sinus/tissue interfaces are present. These artifacts are seen higher due to the pulse sequence being prone to the susceptibilities which will increase with higher field strength magnets. The speed of the image acquisition in EPI is in such a way that it acquires the entire brain coverage both with the patient performing the task and in a resting state. A typical fMRI investigation has thousands of images generated and acquired at various time points and multiple brain levels. Each image has a matrix ranging from 64 – 256. Then the voxel level correlation between the time course of the task as well as resting state signals were correlated and analysed. Before starting the fMRI scan, the MRI scanner stabilizes the gradients for some time before performing the procedure to excite the tissues being imaged to reach the necessary excitation. On considering this, it is optimal to leave the initial volumes of 10 seconds. The acquisition design is mainly classified into two main categories namely, *Block design* and *Event related design*.

Block Design: The fMRI signal is very small that it needs repeated stimuli triggers for signal generation and the design has interleaved equitemporal blocks in which one single block has a signal with tasks performed and the other block with baseline or resting state or event which is opposite and not relevant to the task paradigm. Each block ranges from 15 – 30 seconds of activity or rest. The duration between the rest and the task may be equally divided duration or unequal duration (non-periodic task delivery) as shown in figure 3. The Unequal duration has proven to be effective in reducing noise from the scanner. These signal differences from the task to the baseline are compared and analysed for effective signal visualization. Due to the higher duration of the task, increased BOLD response is seen with higher statistical power. During a task block, several images that trigger the hemodynamic response are recommended to avoid habituation to a single repeatedly displayed image to avoid boredom leading to an adaptation effect. The factor that needs to be considered is the time duration of each block. The time must be long enough to facilitate the hemodynamic response to be triggered high and the signal intensity is at its peak whereas it must also be short enough to perform a post-processing filter ***(High Pass Filter)*** that is applied simultaneously after the acquisition to remove low-frequency noises that degrade the image quality. The first few acquisitions were not analysed to aid magnetization equilibrium and to make the patient adapt to the environment.

Event Design: In this design, the stimuli are presented randomly in a shorter period of time which facilitates a single event to be presented repeatedly leading to a shorter signal period generation.The time duration between the rest and task are same. These considerations make this design harder as the smaller signal must be collected in a correct time to maximize the statistical power. As the time duration of stimuli and the peak signal intensity is lesser compared to the block design, an adequate number of repeated triggers is preferred for optimal signal generation for imaging. This individual episodes were evaluated which the BOLD signal has generated transiently during the task that is not possible in block design as shown in figure 4. The advantages includes sensitive localization of language areas in brain tumour patients, lesser motion artifact incidence, estimation of time to peak, full width half maximum, etc. This is the commonly used paradigm design in fMRI clinical setting. The hybrid paradigms like combination of both event and block designs has an advantage of mapping both transient and sustained functional activations. Ping both transient and sustained functional activations.

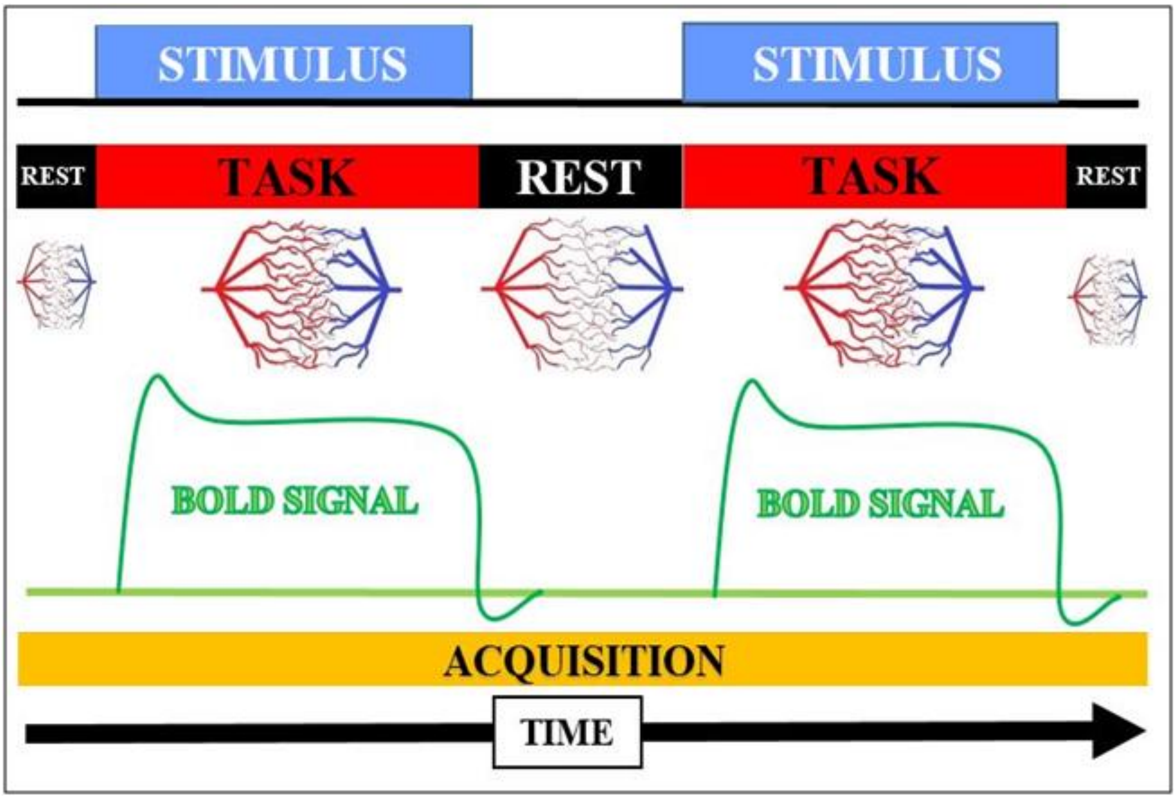

Figure 3: Working mechanism of Block design acquisition technique. In this technique, the tasks are performed for an approximate of 30seconds continuously with an interleaved rest period.

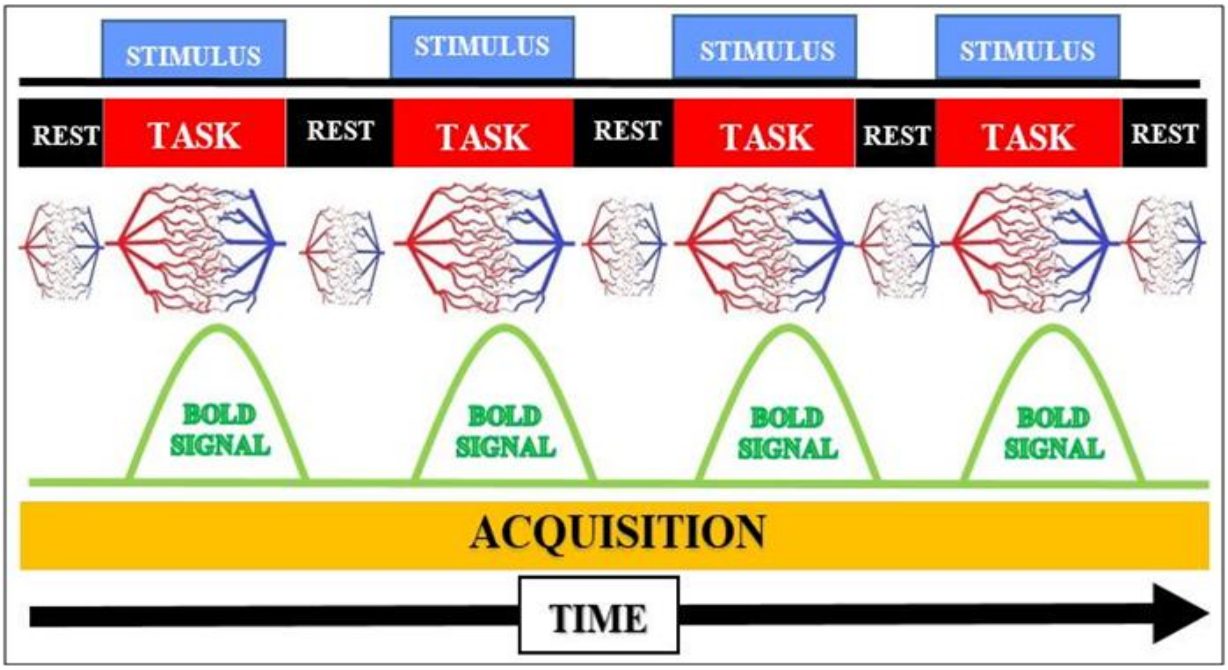

Figure 4: Working mechanism of Event design acquisition technique. In this technique, the tasks are performed for a shorter period of time frequently in the given time with an interleaved rest period.

fMRI Equipment Setup (22, 29, 35)
The fMRI setup initially starts with an adequate field strength MRI scanner as shown in figure 5 with fMRI acquisition peripherals like as shown in figure 6,

- ***Image or Video Projector (Behind the Scanner)***
- ***Central computer for paradigm designing and display.***
- ***Reflecting mirror attached to Head coil***
- ***Synchronization Device*** - During fMRI acquisition, the paradigm presentation and the MRI scanner must be synced for effective signal classification which can be achieved by synchronization device.(19)
- ***Response Pad****(special cases)*
- ***Binocular lens***
- ***Post Processing Software Packages.***

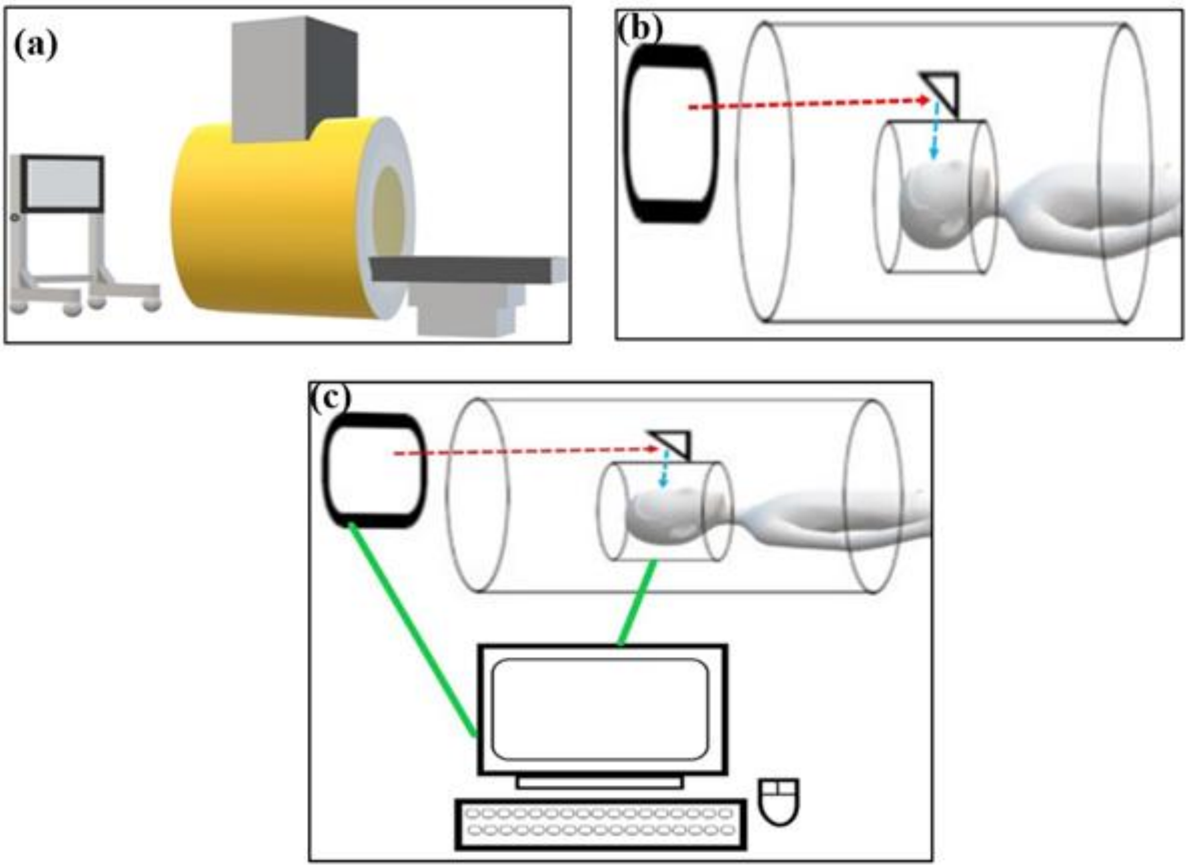

Figure 5: Schematic representation of fMRI equipment setup. Figure (a) shows an fMRI gantry setup with a MRI scanner and stimuli presenter (monitor) behind it. Figure (b) shows the reflecting mirror (triangle shape) attached to the MRI head coil that reflects the stimuli presented in the screen to the patient in the bore. Figure (c) shows the fMRI central computer that controls the stimuli presented and the relevant BOLD activity from the patient in scanner.

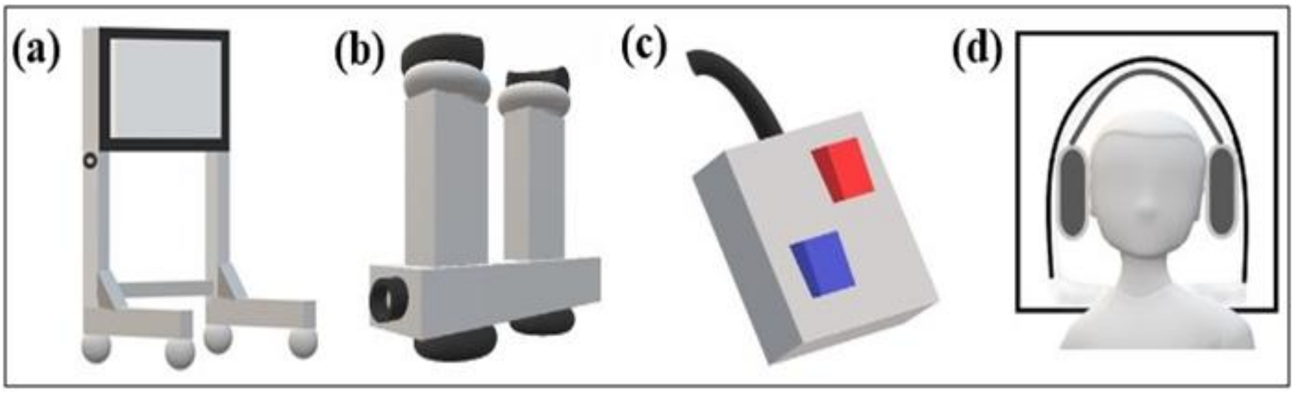

Figure 6: Schematic illustrations of fMRI peripheral devices. The figure (a) shows stimuli presenter monitor which is MR compatible and linked with the central computer and synchronization box. Figure (b) shows binocular lens used for myopic patients to enhance visualization of stimuli presented. Figure (c) shows response pad which has variety of button configurations. The buttons are pressed by the patient according to the stimuli presented. Figure (d) shows MR compatible headphones that delivers audio tasks like musical paradigm which can also be used for ambience.

As per the ASFNR guidelines 3T MRI scanner is preferred for fMRI acquisition. This is due to the appreciable SNR with adequate spatial and temporal resolution. The 1.5T scanner has lesser sensitivity to vascular bed changes and it can be used in the case of implanted patients due to magnetic field-related safety considerations. The higher magnetic field strengths may cause magnetic field susceptibility-related inhomogeneities which can be overcome by usage of parallel imaging techniques and shimming optimization. The pulse sequence used for acquisition must be acquired in 4mm slice thickness. The parameters include an approximate time of repetition of RF Pulse (TR) of 2000 ms, time of Echo collection (TE) of 30 ms, etc.

Post Processing (2, 7, 31, 34, 9, 10, 15, 16, 18, 19, 21, 23)
The BOLD signal is extremely low which needs strategies to maximize its useful signal from noise i.e. Motion artifact, collateral non-task related neuronal activations, etc. The necessity of a dedicated team of Neuro-Imaging Physicists, neuroscientists, radiographers, and radiologists is due to the limitation of fMRI (The presence of activation is not always

true as it may be a collateral artefactual one. The absence of activation is not always true as a pathology may compress the area's blood vessel leading to no activation).

The basic concept of this post-processing technique is to rule out the voxels that show BOLD signal changes due to the neurovascular coupling over time that changes with the brain's varying state of interest (Task and Resting states). To attain successful fMRI end results, factors like experimental design and good data analysis methods are required. The need for a data analysis package is due to the lesser strength signal from the study i.e. 0.5% - 5% (False Negative Results) and collateral imaging of larger volumes at a stretch (False Positive Results). Hence a Statistical Analysis is needed to correlate signal changes with the BOLD response. The respective MRI manufacturers have their own inbuilt fMRI processing software (Real-time fMRI Processing) which is restricted to limited-level post-processing for simple fMRI studies in busy centres for ruling out eloquent cortex and language lateralization. In addition to that, there are several third-party fMRI post-processing packages developed by many vendors that run in a common platform based on the SPM (Offline Processing). The SPM-based processing platforms are commonly used in research setups. Whereas for clinical use, manufacturer-based processing software was used as the above-mentioned one demands labour dependent analysis method. For effective post-processing of fMRI data, dedicated computers and relevant programs with mathematical and statistical analysis software packages are necessary. The fMRI data is processed and analyzed according to the following steps,

(i) ***Image realignment and patient head motion correction***: The fMRI is extremely sensitive to motion either by the entire head motion or involuntary movements like pulsations, respiratory, and cardiac cycles which reduces the sensitivity of end results. Hence to avoid these effects on the end results, fMRI data is initially subjected to realignment (realigns the movement image to normal) and automated motion correction to reduce these effects on the subsequent images. The realignment is done based on the six parameters (3 translations – X, Y, Z and 3 rotations – Pitch, Roll, and Yaw). The voxel misregistration of less than 2- 3 mm is considered acceptable to lead for further analysis.

(ii) ***Coregistration***: It is the merging of a structural image (3D anatomical MRI like T1-3D etc.) with a functional image which gives an anatomical image with functional mapping that improves the spatial resolution and localization of mapping areas easily. The functional images have lesser spatial resolution compared to anatomical images. This process enhances the image's spatial information prior to data normalization.

(iii) ***Spatial normalization and Smoothening***: The normalization of data refers to organizing the acquired to the standard functional mapping templates like the Talairach template, Montreal Neurological Institute (MNI) Template, and International Consortium for Brain Mapping (ICBM) Template. Additional techniques like the removal of non-brain areas in the FOV with skull masking and striping can improve the normalization process. Following these, the visual quality control must be performed by comparing fMRI data with reference EPI template. The smoothening refers to the image quality enhancement leading to increased SNR (increased detection of activations) and reduced noise. However, it collaterally reduces Spatial Resolution and increased spatial smoothening can lead to activated region suppression in the images. The optimal smoothening is based on the size of the stimulus, the size of the tasks, etc. The smoothening is done by using a Gaussian Kernel of 8 mm^3. The HRF tends to have the shape of a Gaussian filter, hence it is optimal to use the kernel double the size of voxel for effective smoothening.

These three factors were considered as the pre-processing steps that control the effect of artifacts in the image that may deteriorate the end results. Thus these steps lead to precise statistical analysis with homogenous data as an input. The fMRI data robustness also depends on the pre-processing steps. The slice timing correction is also done which adjusts the time lag between slices and automated skull striping contours the skull bone region for effective data normalization and analysis. (9, 34)

(iv) ***Statistical Analysis***: The primary goal of statistical analysis is to identify voxels that show response to stimulus (i.e. correlating signal changes between control and stimulated states over time). The statistical analysis performs simple averaging with magnitude subtraction by correlating time or frequency domain analysis to modeling changes in signal intensity changes and SPM. This is achieved easily by generating a map by correlating the signal time course between the individual voxels and the relevant time course of the tasks. The SPM allows interventions at each step of the data processing to enhance the end statistical analysis results which is crucial for ruling out false positive and negative activations. The recent advances in computer programs aids way for correcting artifacts like patient motion and susceptibility artifact. The result of this analysis will be a functional mapping of the brain which shows statistically significant changes associated with brain function.

(v) ***Activation Maps***: The generation of activation maps that are calculated with SPM with two contrast types namely activations vs. baseline. To visualize the statistically significant areas of activations, a significant Threshold based on the spatial extent and probability of clusters based on the radiologist is mandatory. For example, A threshold of $p<0.05$ and clusters of <10 is used for any motor cortex activation visualization. These images were fused with the patient's anatomical images for better visualization of the lesion margin and location of activations. The oxygen uptake's various levels were illustrated in a pseudo-colour scale map with the acquired images. The commonly used pseudo scale has colours like Red (Highest Oxygen uptake), Yellow (Intermediate uptake), Green (Normal uptake), and Blue (Lesser uptake than normal). Using this scale, the interpreter can easily rule out the volumes of differential colored regions (i.e. various levels of oxygenations) in a localized region or the whole brain. (25)

The fMRI data analysis is the most challenging step in image processing. (9) The ultimate goal of the fMRI dataset must be to identify all the eloquent regions by the task of collaterally eliminating the noise in the datasets. If the data set is robust, the higher statistical threshold of p-value < 0.00001 can be used to rule out all eloquent cortices. (22) As the FOV used in fMRI is large, a statistical threshold p-value must be 0.001 to avoid false positive signals. The data analysis software packages include SPM, Analysis of Functional Neuroimages (AFNI), and FMRIB software analysis which has been used by many users since the 1990's. Other packages include Analyze 4D, Analyze fMRI, BioImage Suite, Brain Connectivity Toolbox, Brain VISA, Brain Voyager, Cambridge brain Analysis (CamBA), Functional Connectivity Toolbox, Freesurfer, Marsbar, Neurolens, Nitime, The Decoding Toolbox, and Nipy.(34)

Statistical Parametric Mapping (19)

The SPM was initially developed for the statistical analysis of PET functional imaging by Karl Friston and John Ashburner in the functional imaging laboratory in London. The software was clinically implemented in 1991. The first version of the software package was SPM'94 which was technically updated in the forthcoming years- as follows SPM'96, SPM'99, SPM2, SPM5, and SPM8. The SPM is a voxel-based technique that works on mapping the regional changes in the brain in response to the tasks by generating a parametric map on a voxel basis across the brain. Each voxel gives a statistical value (t-value) by means of a statistical approach called the ***General Linear Model (GLM)***. The end result analysis in SPM is done by Cognitive Subtraction Logic where the brain's activated areas are given a positive value (+1) and baseline activations are given a negative value (-1). Both values were subtracted to give a statistical parametric map. In the pre-processing step, the data is subjected to random Gaussian field corrections by Gaussian Smoothening Filters. Due to the univariate approach of SPM, the voxels must be analyzed individually which may show minimal false activations in group data. To overcome that, multiple comparison corrections were necessary. However, applying multiple comparisons like GLM and statistical tests can lead to false positive results. To correct this, steps like Threshold of p<0.001, ROI analysis, etc. were used. The correction method used was, Bonferroni Correction *(Traditional Method)*which is independent of statistical analysis and reduces false positives but yields false negatives as the time course at each voxel was not considered as an independent sample (*Neighbouring voxels may also have the same time course as it relies in the same region*). The SPM statistical inference is made by considering the 3D anatomical MRI image, BOLD time series, experimental conditions, hemodynamic response, and data corrections.

The General Linear Model (9)

There are two different levels of inferences that characterize the statistical analysis of fMRI datasets. At first, the data is analyzed on task-related effects in an individual. Secondly, the datasets were compared across subjects to examine experimental effects across groups. These are made to rule out specific and random effects etc. The beta values associated with each voxel were represented as beta images. These beta images were subjected to statistical analysis initially. These beta values differ significantly between tasks and baseline conditions. The usual fMRI software uses group-level analyses as default but it depends upon the vendors in estimating subject variance. After group analysis, hypothesis testing is conducted (t-test, etc.). Then the final image is visualized with areas of activation maps in different colour codes related to the statistical significance of the BOLD response. The coloured areas reflect the results of hypothesis testing. The GLM is a hypothesis-driven statistical testing for mapping the task activations. However, the acquired fMRI data cannot be correlated with the assumptions of GLM which may lead to end-result bias or false positive results. Alternatively, GLM is also programmed in an alternative way in non-parametric approaches and Bayesian inference for better statistical inference outcomes. While others were based on analysis like t-tests, etc.

Role of Thresholding in SPM (7, 10, 11, 15, 18, 22)

From the data analysis perspective, the final step is Thresholding the SPM data. The threshold makes the data homogeneous without any false positive results. As SPM is based on spatial autocorrelations, the number of regions with activations above the threshold values is always lesser than the number of voxels with activations above the threshold. Also there is no specific threshold that is mandatory to interpret BOLD fMRI signals as the laterality and eloquent cortices extent varies with different statistical thresholds and also varies from patient to patient. The lower the threshold, the higher the activations in a wide range of areas – These signals are contributed by weaker signals with background noise and may lead to false positive results. The higher the threshold, the lesser the activations in lesser areas – These signals are contributed by higher intensity signals The thresholding is also useful in managing the patient's with inability to perform the task well, as their BOLD response will be very low or absent. Thus this approach balances the sensitivity and specificity of the procedure irrespective of the patient's condition in performing the tasks. An adequately performed task will reflect robust BOLD activation which produces large areas of activations in higher threshold.

This clinical application of thresholding in clinical fMRI has complicated the statistical inference. To overcome this, threshold-independent approaches were developed. However, the role of thresholding in statistical analysis is crucial as it is used to rule out the extent of language activations. The choice of threshold level depends on the subject as well as the data obtained from previous studies.

SPM based Statistical Analysis Methods

***ROI Analysis*:** This method is used where the neural activation is strongly expected. As the region is smaller, the incidence of false positives is also low. To avoid errors in area selection, the following steps were used obtaining areas of optimal activations based on previous studies, selecting regions relevant to the task-specific anatomy, etc. While performing this analysis, the average activity within the prescribed voxel is obtained and tested against the baseline.

***Cluster-based Thresholding*:** This method works on a cluster of voxels (i.e.) user-dependent selection of voxels that form a cluster. The extent of clusters was determined by Monte Carlo simulations or random methods. This reduces the number of test steps and false positives. The disadvantages are, the clusters are sensitive to data smoothness which may sometimes wrongly imply false positive results.

***Non-Parametric Tests*:** The non-parametric tests were available in most of the fMRI analysis which allows access to most researchers. For a group inference, the Permutation Test is done to accept or reject the null hypothesis.

***Multivariate Approaches*:** The fMRI datasets have numerous voxels which makes the question arise in the mind whether the individual voxels are analysed. The multivariate approach is used to reveal the correlations that are not detectable with the classic univariate approach. In the conventional approach, each voxel was analyzed as individual time courses whereas, in the multivariate approach, each voxel was analyzed as each dimensional space in one data point that moves as per experimental conditions. The multivariate approaches were, Principal Component Analysis (PCA) – This technique has the advantage of eliminating the axillary activity signals in the datasets thereby improving the SNR, Independent Component Analysis (ICA), and Multivariate Pattern Analysis (MVPA) – Machine learning algorithm. The ICA and PCA are combined occasionally to give hypothesis-driven analysis.

The ICA is done by separating the BOLD datasets into spatially independent components (i.e. Functional Connectivity) without prior knowledge of time.The degree of thresholding has a direct effect on the visualization of activation maps which can be variable to maintain sensitivity and specificity and may also be fixed for a given patient.(22) Compared to SBA, ICA has the advantage of manual selection of components which aid in distinguishing stimulation signals from background noise. Some studies have progressed to automate the manual process of identifying noise signals from BOLD. Apart from these there are other analyses like Connectivity analyses which focus on the functional connection between brain regions of behavioural responses, sensory stimuli, cognitive processes, etc., and reflect the functional as well as structural networks of the regions. The DTI is one of the examples for the analysis. Also, there are commercial packages like FSL, AFNI, Brain Voyager, etc.(11)

***Brain Voyager*:** The Brain Voyager QX is a software package that is programmed in C++ for analysing the fMRI datasets and displaying them in an easier user interface. Basically, this platform is a multimodal package that allows post-processing of DTI, EEG, MEG, fMRI, etc. The software supports automatic brain segmentation, surface reconstruction, cortex flattening, inflation, etc. This software supports advanced data analysis methods like ICA, Multi Voxel Pattern Analysis (MVPA), and Probability maps. The latest update on this platform is the Turbo Brain Voyager where real-time image processing analysis is done and the fMRI data is set for visualization.(19)

Resting state fMRI analysis methods

The clinical application of ICA will be very useful for the resting state fMRI as no data of time course is necessary. It will improve the sensitivity and specificity of task-based fMRI.(10) Like ICA, Seed Based Analysis (SBA) is also used in some studies.The resting state fMRI uses a Cluster Algorithm for data analysis which works on grouping the datasets based upon the similarities in signal characteristics (BOLD activations vs. Baseline activations) within the region of interest with respect to their time courses by a distance metric like Pearson correlation, etc. The Multivariate Pattern Analysis is another method for data analysis of resting state fMRI. (27)

The resting state fMRI data is analysed mainly by two steps, (20)

(i) ***Functional Segregation*:** Used for mapping of localized brain function in specific region(functional activity). The data analysis methods used were *Amplitude of low Frequency Fluctuations (ALFF) and Regional Homogeneity (ReHO)*.

ALFF Analysis

This method measures the BOLD signal with power between 0.01 – 0.1 Hz. The fractional ALFF is a variant of this method which rules out the low frequency oscillations in the datasets by dividing the low frequency BOLD signal with total power in the detected frequencies.

ReHO Analysis

As ALFF, this method also measures low frequency BOLD signal levels. But it is subdivided into different level frequency bands that is more sensitive to cortical activity. The ReHO is a voxel based analysis method that measures the correlation between time series of a BOLD signal with its neighbouring regions by Kendall Coefficient of Concordance.

(ii) ***Functional Integration*:** Used for assessing brain's integral neural network (functional connectivity and integration) by measuring the BOLD time series of each voxel in different brain regions. The functional integration is the foundation of brain's physiology in transferring information to different regions. It has various methods as follows.

Functional Connectivity Density Analysis (FCDA)

This method is the basic analysis method in ruling out the highly connected brain's functional hubs with a given voxel. This method works on the principle of correlating the BOLD time series between voxels in the region. The short and long-range FCD maps were generated in which short-range regional functional connectivity of a voxel and 75mm around it and long-range reflects long range connectivity.

Seed based Functional Connectivity Analysis

It is also called ROI based functional connectivity analysis based on the "Seeds" that were derived from the ALFF, Reho, or with prior determination by hypothesis or previous results. The analysis is done by correlating the seed region with the rest of the brain regions by BOLD time series. This analysis is susceptible to data variables due to task performance or physiological motions. The major advantage is that the computation of data is simple.

Independent Component Analysis (ICA)
The functional connectivity of a voxel with a region is converted to separate spatial maps with Z-score derived from the variation in time series of BOLD signals. The Z-score indicates the magnitude of brain's functional connectivity network.

Graph Analysis
This analysis is used in mapping of complex neural networks. The analysis is done by dedicated software with no prior assumption data with lesser bias. But the results were difficult for interpretation. For patients with Alzheimer's disease, the fMRI with graph analysis between Alzheimer and control patient group showed significantly lower cluster coefficient in Alzheimer group with sensitivity of 72% and specificity of 78% in hippocampus.(1)

Thus, in resting state fMRI the data can be analysed by different methods (more than one) for effective data processing, analysis, and visualization.

As fMRI is one of the growing scientific queries, many public/community-driven guidelines were framed to route the researchers for best practice outcomes by the software packages like *OpenNeuro and NeuroVault*.(9)

fMRI Reporting Considerations (22)
The reporting must include points as follows,

- Mentioning the paradigm used for acquisition
- Patient task performance ability and relevant image quality.
- Before transferring the acquisition images to the fMRI data analysis package and PACS server, the radiologist must evaluate the quality of the images and areas of BOLD activations. In case of any head motion is seen, the direction of angular and linear displacement with the maximum degree of motion should be reported for individual tasks.
- It is advised to avoid mentioning the size or distance between the activations and eloquent cortex. Rather the activation area's centroid must be mentioned as overlapping. (with the same gyrus (or) with a different gyrus (or) distal to the surgical pathology)

Clinical Application of fMRI (7, 15, 18, 19, 22, 27, 36)
The clinical use of fMRI is mainly focussed on pre surgical mapping of brain's cortex and post-surgical follow-up of treatment response.Pre surgical mapping is analysing the language localization, brain's neuro plasticity, localization of sensory and motor functions, etc.

- ***Language Lateralization*** – Language lateralization is to evaluate whether the language dominant hemisphere is targeted for surgery as surgical intervention in that hemisphere may cause a higher risk associated with language impairment. For patients with temporal lobe epilepsy, surgical intervention offers the most reliable control over the epilepsy. But mapping of the eloquent neural cortices surrounding the pathology is important before and after surgery especially due to the presence of language area in that region. The mapping techniques were initially done by WADA which was replaced by fMRI due to its non-invasiveness and higher specific information about the anatomy.
- ***Brain Plasticity*** – Brain plasticity is also called cortical plasticity or cortical reorganization. For patients with any instance of brain injury and diseases, the brain has the tendency to alter the regional functional organization of the brain which is called "Adaptive Plasticity" which is due to the assignment of intact brain regions for the compensation of neural regional functional deficit by the injury or disease which is a general response done by the brain. Usually, this occurs in the opposite hemisphere. This reorganization occurs when the pathologically affected area does not have the tendency to complete the neuronal function. Thus fMRI plays a major role in the mapping of cortices especially in the localization of neural networks in these patients where the neuronal activations have been displaced to another region. This makes fMRI of language area identification challenging as the region can be variable in location due to plasticity.
- ***Memory Testing*** – patients with clinical conditions like Alzheimer's Dementia or Schizophrenia and Seizure disorders may experience loss of memory functions. Compared to language functional mapping, memory processing functional analysis is quite complex due to its differential components' involvement in neuronal activation and relevant paradigm designing.

3. Clinical Conditions

Hyperthyroidism (30): The thyroid hormone supports the brain development. Hyperthyroidism is a condition in which the hormone circulates excessively in association with emotional and cognitive impairments. The fMRI with reduced functional connectivity between the hippocampus, bilateral anterior and posterior cingulate cortex, and right medial orbitofrontal cortex suggests emotional and memory circuit dysfunction in hyperthyroid patients.

Epilepsy (11, 18, 27, 36): About 50% of the patient population with refractory epilepsy gets benefitted only by surgical resection of the epileptogenic zone. To perform this resection, pre-surgical mapping of the zone with the help of MRI (Anatomical information) and fMRI (Functional analysis) is crucial for preserving a healthy eloquent cortex surrounding the zone. For patients with Temporal Lobe Epilepsy (TLE), the pathological areas show lesser activation compared to the normal lobe. The distance between the eloquent language cortices to the resection boundary is the most important criterion that influences postoperative language deficits. In resting state fMRI, the spatial resolution is higher compared to EEG for epileptic network mapping. The patients with successful localization of language lateralization with fMRI do not require WADA testing as they both are clinically concordant, especially in the case of left dominant language areas.

Treatment Monitoring (12): Some studies reported that patients undergoing rehabilitation and medication showed potential changes in areas of activation, especially in language-related disorders.

Stroke (23): The stroke fMRI is targeted at ruling out healthy brain tissue by cerebral blood flow instead of damaged brain tissue as it cannot measure the damaged cells. This is ideal for a healthy brain but challenging in the case of an ischemic brain. Hence there is having lesser possibility of performing stroke fMRI in hyper acute phase (less than 6 hours) due to the scan time and paradigm choice. The results of stroke fMRI also have variabilities in the recovery patterns of the brain.

Psychology (35): The important region to be mapped for the psychological application was the *Amygdala* which is challenging. It also has a part in the limbic system as it is connected to the hippocampus. The Amygdala is responsible for controlling emotion, especially fear. The fMRI paradigm chosen for the psychological evaluation of the amygdala was fearful faces and negatively affected images. In healthy subjects, the amygdala showed remarkable activation whereas for patients with anxiety, it showed higher activation which is used to represent the disease pattern. For patients with depression, paradigms showing sad faces were used to differentiate normal (nominal activation) and depressive patients (hyper activation).

Fetal fMRI (28): The fetal brain mapping has opened doors for evaluating the architecture of the brain while it is in its growth state. The resting state fMRI has paved the way for the mapping of the fetal brain in utero. Due to technical challenges like inadequate image analysis tools dedicated to fetal fMRI, the further progression to its clinical application is made slow. Also, the research on the post-processing techniques was very few and mostly focused on structural imaging. Recently the Convolution Neural Network (CNN), a deep learning algorithm has been used for post-processing of fetal fMRI.

4. Technical Challenges

The technical challenges range from various factors like task selection and its design, data acquisition, and analysis which has the tendency to introduce false positive and false negative results.(10) Though these can be resolved by various methods, other crucial challenges are as follows.

(i) *Temporal Resolution*(9,20)
The fMRI has many potential advantages in neuroimaging but it has challenges that need to be faced technically. The first challenge is that the fMRI signal has a poor spatial resolution as the BOLD event lasts only in the millisecond range. Also, the BOLD signal is dependent on the vascular response to the task which takes some seconds to trigger. This is due to the connectivity between the neural activity and the hemodynamic response. Thus it reduces the temporal accuracy of the BOLD imaging. Also, the signal is altered in the areas of altered blood circulation (pathological condition). A start-up method to overcome this is to combine fMRI with other methods like EEG.

(ii) Artifacts *(7, 10, 18, 22, 35)*
The artifact in BOLD imaging can be from any kind of source. For instance, the patient's head movement can move or mix the high-intensity voxels with the low-intensity voxels. This paves the way for false positive or false negative results. The excessive head motions higher than 2mm in any direction invalids the entire scan date as 2mm is the limit of acceptable neurosurgical error. However, due to the advancements in the software like inter-sequence realignment, this was eliminated to a minimal extent. The physiological artifacts from breathing and cardiac motion may cause considerable signal fluctuations.

Another type of artifact is *susceptibility artifact* which can arise from both exogenous and endogenous sources that contribute to fMRI signal. This creates false negative results leading to inaccuracy in imaging analysis. This artifact leads to decreased SNR by localized signal loss. Also, this artifact originated from post-operative patients with titanium implants, staples, and skull drills. In these types of imaging situations, it is optimal to conclude that there will be a lack of useful signal from the site which is uninterpretable. In normal patients also these artifacts were visible in the tissue-air interface, especially in hippocampal fMRI (mesial temporal structures). This artifact has the potential to mask possible areas of activation leading to misinterpretation. The occurrence of this artifact in clinical fMRI may be due to the use of echo planar imaging technique where different materials' spin frequencies (air and bone) within a voxel cause dephasing. This can be solved by using the Parallel Imaging Techniques. A study by Devin et al shows that discrepancies were noted in language localization in fMRI and PET due to signal loss by susceptibility artifact in the temporal region which can be compensated by using the smaller voxel size despite increased noise. Recent advancements show that the use of the spin echo technique potentially reduces susceptibility artifacts and improves image quality even at higher-strength magnetic fields (7T). For smaller regions like *the amygdala*, small voxel sixes of 1.5mm x 1.5mm x 3mm were used along with high-resolution protocol and higher field strength magnets to increase SNR.

(iii) Patient's Physiological Condition *(10)*
The patients with ischemic cerebrovascular diseases like stenotic blood vessels, the vascular bed reflects lesser blood flow leading to hypo vascularization which results in lesser BOLD signal intensity. Also for patients with arterio-venous malformation, the vascular bed shows hyper vascularization which needs to be concentrated during examination and image evaluation. In addition, the age-related changes in the neuronal level cause loss of vasodilatory response with neural activation. Hence it requires multiple trials to compensate for the reduced Signal–to–noise ratio (SNR).

(iv) Patients with Pain *(1)*
As pain is unique for every clinical condition and alters from one patient to another, this could lead to non-cooperation during the scan acquisition. To overcome this, faster imaging techniques like Simultaneous Multislice acquisition were developed to complete the scan as early as possible.

(v) *Reverse Inference* (9)
As the fMRI is based on the reverse inference paradox, (i.e.) data interpretation in a backward direction from the brain activity to related cognitive process. It is not accurate that the specific area activated in fMRI is responsible for that specific task trigger only. So, interpretation of data with expertise is important in fMRI.

5. Current Perspectives

- ***Multimodality Approach***(9) – The multimodal approach of fMRI is that combining it with different other imaging techniques like DTI, EEG, etc. can increase the specificity of end results without loss of information due to the respective modality's limitations. The lesser temporal resolution of fMRI can be compensated with the addition of fMRI with EEG and the neuronal tract connectivity patterns can be visualized with the combination of fMRI with DTI.
- ***Lie Detection***(37) – In a study, the clinical application of fMRI in lie detection was done. The study has a liar who responds with a lie and simultaneously withholds a factual event (truth). The factual event information forms the baseline of the scan and the additional information (lie) demands an extra cognitive function of prefrontal systems. The statistical analysis of the data reveals, higher signal activations in one anterior cingulate and two prefrontal regions on Lie compared to the truth. The study has limitations like subject willingness to enter the scanner, cooperation during the scan, responding to the paradigms, etc. as simple motion artifacts can highly spoil the useful end data.
- ***Advent of Peripheral Devices***(9) – Many scanners and third-party vendors have designed various peripheral devices like respiratory sensors, cardiac triggers, pupil responses, etc. for synchronization with MRI scanners during fMRI to provide additional diagnostic information in response to BOLD data.
- ***Human Connectome Project***(21,27) – The project is the collection of large volumes of functional imaging data like white matter tracts and correlating it with behavioural and genetic information. In psychiatry, it is also useful in the follow-up of drug treatment response with neuronal rehabilitation.
- ***fMRI of Memory Function***(7) – It is currently limited in clinical application due to the susceptibility artifact from the air-bone interface.

6. Future Directions

Fetal fMRI (28) – Fetal fMRI is challenging, especially in cases of data processing and analysis. For effective processing and data analysis, deep learning methods may be used in brain segmentation which is challenging. There may be many upcoming improvements in three-dimensional convolutions with model's probability masks in the future making the easier application of fMRI in fetal imaging.

Resting-state fMRI (27) – Though the results from various resting-state fMRI studies were promising, adequate focus is mandatory for the comparison of various data analysis methods and their respective diagnostic accuracy in pathology detection both in clusters of patients and individuals.

Biomarkers (5) – In the cases of follow-up recovery studies, the fMRI application is lesser which needs to be considered for future research. Effective treatment delivery can be achieved by neuroimaging biomarkers. These biomarkers can be concentrated for a specific disorder and easier mapping of treatment delivery.

7. Conclusion

To date, fMRI is considered as a powerful imaging tool to visualize the neural correlation of subject behaviour in higher resolution. The fMRI has evolved as the pre-operative method of choice for neurosurgical interventions due to its effectiveness in mapping the brain's activity in the eloquent regions. The future directives of fMRI clinical application need maximum benefit with minimal limitations irrespective of the subject's physiological and cognitive states that derive accurate end results. This can be made possible by the usage of optimal imaging parameters with automated evaluation strategies, etc.
Thus, fMRI is the field that deserves more upcoming innovations to step in.

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