Abstract

In this article, we first review some aspects of functional magnetic resonance imaging (fMRI) paradigm designing for major cognitive functions by using stimulus delivery systems like Cogent, E-Prime, Presentation, etc., along with their technical aspects. We also review the stimulus presentation possibilities (block, event-related) for visual or auditory paradigms and their advantage in both clinical and research setting. The second part mainly focus on various fMRI data post-processing tools such as Statistical Parametric Mapping (SPM) and Brain Voyager, and discuss the particulars of various preprocessing steps involved (realignment, co-registration, normalization, smoothing) in these software and also the statistical analysis principles of General Linear Modeling for final interpretation of a functional activation result.

Key words: Functional magnetic resonance imaging; general linear modeling; statistical parametric mapping

Introduction

Functional magnetic resonance imaging (fMRI) is an effective tool for analyzing brain functions. It is a breakthrough imaging modality which is non-invasive and is quite precise in tracking functional responses of brain through Blood Oxygen Level Dependent (BOLD) contrast.[1] The BOLD effect was first explained by Seiji Ogawa.[2] This technique relies on difference in the magnetic properties of oxyhemoglobin and deoxyhemoglobin (dHb).[3] When we perform a task, the neuronal activity in the brain area specific to that particular task increases. Enhanced neuronal activity leads to a local increase in energy and oxygen consumption in functional brain areas, which is followed by an increase of regional cerebral blood volume (rCBV) and regional cerebral blood flow (rCBF). Since increased neuronal activity results in an increase in blood flow beyond the exact demand, more oxygenated hemoglobin appears in the venous capillaries. This shifts the relation between oxygenated and deoxygenated hemoglobin,[4] BOLD contrast relies on variations in dHb, which acts as an endogenous contrast agent. Therefore, changes in the local dHb concentration in the brain lead to changes in the signal intensity of magnetic resonance images (MRIs).[5] This effect is reflected in the fMRI BOLD contrast images. To comprehend, BOLD effect can be described as a positive contrast caused by relative decrease in the magnetic field inhomogeneities due to relative increase in the oxyHb level contributed by the blood flowing in the region of brain with increased activity. Compared to other imaging techniques,[6,7] fMRI is more powerful with relatively good spatial and temporal resolution.[8,9] Since fMRI is quite efficient in elucidating neural correlates associated with specific brain functions, the technique has got wide acceptance in functional brain mapping both clinically and in research setting.

fMRI Paradigm Designing Tools

A paradigm is a temporal allocation of stimuli to acquire BOLD responses from the subject. During a fMRI experiment, specific paradigms with stimuli or events are used to evoke hemodynamic response or brain activation.
in the subject. An entire fMRI experiment relies on precise and effective paradigm design. At present, there are many software packages available for designing fMRI paradigms. Each one of them has its own pros and cons. Here, we provide a comparative description of three prominent stimulus delivery packages – Cogent (Laboratory of Neurobiology, Wellcome Trust, London, UK.), E-Prime (Psychology Software Tools, Inc.; Pennsylvania, USA), and Presentation (Neurobehavioral Systems, Inc.; California, USA). At the end of this section, mention is also made of fMRI experimental design and the tool developed in-house at our institute for stimulus presentation.

Cogent
Cogent is an open-source Matlab toolbox for delivering experimental stimuli as well as to collect response from brain imaging equipments. Cogent program is available in two forms – Cogent Graphics and Cogent 2000.[10] Cogent Graphics is a graphical toolbox for Matlab which facilitates the generation of real-time graphical animations. Cogent 2000 is a software program which can effectively deliver different types of stimuli, synchronized with the scanner. Cogent is completely programmable and is based on Matlab commands.

Cogent program uses Matlab and Cogent 2000 commands sequentially, and therefore, it is essential that the user must be familiar with Matlab scripting as well as scripting in Cogent. The main drawback of cogent program is that when network connections are available, it gets a hold-up which results in a delayed stimulus delivery, especially in auditory stimulus, and creates timing inaccuracies. There are also issues in establishing serial and parallel port communication. Cogent runs in Windows platform together with Microsoft Application Programming Interface called DirectX and Matlab version 6.0 or above. The software is made by Laboratory of Neurobiology, University College London and can be downloaded freely from www.vislab.ucl.ac.uk.

E-prime
The name E-Prime stands for experimenter's prime. It is quiet easy to learn and use E-Prime suit. There are five programs (subdivisions) that constitute E-Prime; they are E-Studio, E-Run, E-DataAid, E-Merge, and E-recovery.[11,12] Among these, E-Studio is the major program and it aids in the creation of experiments. The structure of the paradigm is described in E-studio. A basic experiment is represented as frames, trials, and blocks. It is very user friendly and has drag and drop graphical interfaces for experimental design. E-Run is used to run an experiment. One can also use this module for experiment testing, i.e. the use of licence key is not essential for the working of E-Run since it can be used to test the same paradigm in different systems, different labs, and different users. E-DataAid program gives the recorded data in a tabular form. The user can modify this data and it can also be exported to other formats compatible with other data analysis packages. E-Merge utility facilitates group analysis of data and gives a single output file with results from multiple subjects. E-Recovery program is meant for data recovery. It can recover an aborted experiment text file and convert the same into a new data file.

The major advantage of using E-Prime is that it provides an easy-to-use environment for experimental design, data collection, and analysis of cognitive experiments. Most of the interfaces in E-Prime Graphical User Interface (GUI) have “drag and drop” option, which makes it very user friendly. Compared to other stimulus delivery programs, it is quite fast and easy to use. A user can generate programs without a deep understanding about the tool. One needs to know only what is required to be done in their own paradigm/task. E-Prime is a commercial software package. One requires a license key to activate and use it. In order to run E-Prime, one requires a Windows OS (XP/vista/7) and DirectX video card. It is developed by Psychology Software Tools, Inc., Pennsylvania, USA. The download option and more information can be obtained from www.pstnet.com.

Presentation
Presentation (Neurobehavioral Systems, Inc., California, USA) is a software application for handling psychological and neurobehavioral experiments. Presentation tool is comparatively more precise and accurate; it tracks stimulus response, reaction time, and different performance measures with sub-millisecond temporal accuracy.[13] Presentation facilitates effective control of parallel and serial port, which enables the communication to and from fMRI system and other devices. To enable synchronization with the scanner, presentation has an fMRI mode which facilitates reception of pulses from the scanner and also enables start of stimulus sequence on specific pulses.

Presentation experiments are subdivided into units called Scenarios. Scenarios are sequence of actions that presentation performs without interruption. They are specified using text description, and the main text file is called Scenario file. Programmability enables effective creation, manipulation, delivery, and control of stimuli in different neurobehavioral experiments. This tool has its own built-in programming language and is subdivided into (i) Scenario Description Language (SDL) and (ii) Program Control Language (PCL). To simplify the concept of programming language, we can assume the Presentation paradigm to be our favourite cuisine. To prepare it, we need to know the ingredients and the method of preparation. Here, SDL refers to the ingredients (the stimuli which need to be included in the paradigm). PCL can be related to the method of preparation (describes the order/sequence in which each stimulus has to appear).

Presentation program is more accurate and powerful among other programs.[14] It has precise timing accuracy (less than
Unlike a neurobehavioral experiment, here, the processing of data is done in real time with the inline BOLD. The results obtained from the inline BOLD had significant correlation with the results from Statistical Parametric Mapping (SPM) image processing software. Both the results maintained a degree of similarity in indicating the region as well as the amount of BOLD activation. Here, variation was made only in specifying the threshold values, i.e., the inline bold required comparatively higher threshold than that of SPM. The promising point is that the inline bold system is less time consuming, which is a demanding aspect in clinical procedures.

The research setting requires more sophisticated paradigms and processing techniques and probably combinations of different brain mapping techniques. Here, the processing and data analysis may be time consuming. The experiments need to have time-bound and response-dependent stimulus presentation. Accurate logging of timings corresponding to stimulus as well as response may be essential for data processing. Research investigations also require advanced image processing tools.

**fMRI - Experimental Design and Setup**

The fMRI experiments mainly fall under two design categories: (i) block design and (ii) event-related design. The block designs use interleaved and equitemporal blocks of activity and rest to elucidate the actual response. In an event-related design, the trials are presented randomly and a specific cognitive event is in focus. It is often used to establish neurobehavioral tasks, since it provides a subjective response to the stimuli being presented.

The fMRI procedure is being utilized for clinical examinations as well as for research investigations. In clinical practice, fMRI mainly aids in surgical planning, for analyzing the localization of language, sensory and motor function, and to follow-up functional rehabilitation after neurological disorders. Unlike a neurobehavioral experiment laboratory, in a hospital setup, we need to have routinely accessible fMRI setting and the delivery of results needs to be quick. Modern scanners have inbuilt real-time processing option. The real points to be looked into are paradigm presentation and scanner synchronization. Laborious setup procedures and time-consuming processing techniques may be difficult in a clinical setting. Therefore, clinical procedures require custom-designed fMRI setting. Our institute has developed an in-house fMRI setting for carrying out clinical investigation. The visual and auditory paradigms are programmed with the help of Visual Basic and presented in coordination with the scanner trigger. This is achieved by engineering a synchronization box that tracks the scanner pulses. The whole setup includes a stimulus presenting computer, a synchronization box, a projector (kept in the console room), an earphone for auditory stimulus delivery, and a screen (in the scanner room) to project visual stimuli. The cost of making such a system is quite low compared to the cost quoted by the vendors. The processing of data is done in real time with the inline BOLD. The results obtained from the inline BOLD had significant correlation with the results from Statistical Parametric Mapping (SPM) image processing software. Both the results maintained a degree of similarity in indicating the region as well as the amount of BOLD activation. Here, variation was made only in specifying the threshold values, i.e., the inline bold required comparatively higher threshold than that of SPM. The promising point is that the inline bold system is less time consuming, which is a demanding aspect in clinical procedures.

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**fMRI Data Analysis Tools**

**Basic concepts of fMRI data analysis**

The principal aim of fMRI data analysis is to determine cortical brain regions, where the signal changes occur during the presentation of a passive, sensory, motor, or cognitive task (paradigm) designed to specific brain functions. Three main stages are involved in the analysis of the data from any fMRI experiment. They are as follows: (a) the preprocessing steps which can be applied to the data to improve the detection of activation events; these include image registration to correct for subject movement during the experiment, normalization of subject head to a standard stereotactic template (e.g., Talairach template, International Consortium for Brain Mapping (ICBM) template, Montreal...
Neurological Institute (MNI) template], and smoothing to improve the signal to noise ratio; (b) performing statistical analysis which determines the voxels in the image that show a response to the stimulus; and (c) displaying the activation images and probability values which give the statistical confidence. In a typical fMRI experiment, whole‑brain functional images are acquired every 2‑3 s resulting in several images (time series data), which include roughly about 100,000 voxels. This data needs to be further processed in a statistically meaningful way. A general issue in fMRI data analysis is to understand the relationship between the neurobiological hypothesis and the statistical models adopted to test that hypothesis.[25]

fMRI data analysis can be done by two ways: (a) offline processing using a popular Matlab‑based software package called SPM or similar software packages (Brain Voyager, AFNI, FSL, etc.) working on the same principle (General Linear Modeling) and (b) real‑time fMRI (Rt‑fMRI) data analysis which can be done by using the module provided by MRI vendor. Using Rt‑fMRI, the imaging data can be analyzed, and displayed more interactively, as it is acquired from the scanner with a latency of only a few seconds. The major advantages of this method are immediate confirmation of the experimental results from simple block design paradigms, real‑time monitoring of “resting‑state fMRI,” and tracking of the subject head movement and giving instant feedback to the subject, enabling clinicians to investigate the dynamic nature of the human brain. Most of the MRI vendors have an Rt‑fMRI analysis tool which can be used in busy clinical settings for eloquent cortex mapping and language lateralization. Earlier studies have shown that the Rt‑fMRI post‑processing gives similar results as offline processing for simple block design paradigms in the clinical setting.[25]

In this review, we discuss the fMRI data processing using SPM and BrainVoyager, the two techniques that we use in our practice. The modules used for basic data analysis of brain images in BrainVoyager are similar to those of SPM: preprocessing, co‑registration, spatial normalization, and statistical analysis.

### Statistical Parametric Mapping

The SPM tool was originally developed for the statistical analysis of functional neuroimaging data from Positron Emission Tomography (PET) by Karl Friston,[27,28] along with conceptual and technical help from John Ashburner[29] (Functional Imaging Laboratory, Wellcome Trust Institute, London, UK). This software was made available to the emerging functional imaging community in 1991.[30] SPM'94 was the first major version of the SPM software. The revised versions are SPM'95, SPM'96, SPM'99, SPM2, SPM5 and SPM8, which are exactly based on SPM’94 and represent the ongoing technical improvements and theoretical advances.

SPM is a voxel‑based technique which makes inference about regionally specific responses of cortical brain areas to experimental tasks (paradigms). In order to observe the response of a particular brain region, the data must conform to a standard anatomical space.[31] Usually a univariate approach is followed in which the parametric map is computed by examining every voxel location across all images, meaning that a statistical value (e.g. $t$‑value) is calculated for every voxel using a statistical approach called “General Linear Model (GLM).”

#### Steps involved in SPM

**Preprocessing of fMRI data - preparing data for analysis**

The essential preprocessing steps are: (a) realignment, (b) co‑registration, (c) normalization, and (d) smoothing.

**Realignment**

During the scanning session, subjects may move inside the scanner. Even small head movements can cause movement artifacts, which may add up to the residual variance and reduce sensitivity. Data may be lost if sudden movements occur during a single volume and it may be correlated with the task performed. So, movement‑related variance components...
in fMRI present one of the most serious confounds of analysis. A rigid body registration with six parameters, three translations (X, Y, Z), and three rotations (pitch, roll, and yaw) are used for realignment in SPM. It minimizes the squared sum of differences between each successive scan and reference scan (usually the first or the average of all scans in the time series) and resamples the data.

Co-registration - within-subject registration
By means of co-registration between two modalities [a structural image (e.g. T2-weighted) to a functional image series, (echo planar imaging)], one can overlay functional activations onto an individual’s own anatomy and it is also possible to overlay group-level functional activations onto an average structural scan. Co-registration gives a better spatial image for further use in normalization step, as warps derived from the higher-resolution structural image can be applied to the functional image. This is again a rigid body transformation, but the registration cannot be simply performed by minimizing the residual sum of squares due to different imaging modalities. The 12 parameters affine transformation [3 translations, 3 rotations (rigid-body), 3 shears, and 3 zooms] step registers the structural image and the first image of the functional image series to template images. These transformations are constrained in such a way that only the parameters that describe the rigid body transformation are allowed to differ. Next the images are segmented using tissue probability maps of gray matter, white matter, and cerebrospinal fluid. At last, the image partitions can be simultaneously co-registered to produce the final solution.

Normalization - between-subject registration
In order to average the signal across different subjects, it is important to warp brain images into roughly the same stereotactic space. The advantage of spatially normalized images is that areas of functional brain activation can be reported within this standard space, in SPM94–SPM99, according to their spatial coordinates. The SPM2 uses MNI template, an average of 152 brain images, and hence is more representative of the population, as compared to the Talairach and Tournoux atlas. Later versions of SPM use ICBM template. The normalization step not only considers the six rigid-body transformations, but also considers three shears and three zooms to match the individual subject’s images to the template. A nonlinear transformation is also required for accurate normalization which would correct gross differences in head shapes that cannot be accounted for by the affine transformation. The normalization step need not be done while doing a single-patient fMRI analysis in clinical setting. The technique is used more often while analyzing group data in a research setting.

Smoothing
Functional images needs to be smoothed prior to the statistical analysis, especially in group-level analysis, so that corresponding sites of activation from the different brains are superimposed. Smoothing is generally done by convoluting the data with a Gaussian kernel and it potentially increases the signal-to-noise ratio according to the matched filter theorem. Since hemodynamic response functions (HRFs) are modeled to have the shape of a Gaussian filter, we need to use a Gaussian kernel of size at least twice the voxel size [Full Width Half Maximum (FWHM) of about 6 or 8 mm] for smoothing the functional images. During smoothing, the intensity value within each voxel is replaced with a weighted average that incorporates the intensity values of the adjacent voxels, which corrects the inter-subject variability between individuals after normalization. Smoothing allows the application of Gaussian random field theory to make inference at statistical analysis stage.

Statistical analysis - using GLM
The GLM is used to specify the different conditions/blocks in the form of a design matrix, which defines the experimental task and the nature of hypothesis to be tested. It provides a framework that allows us to make refined statistical inferences after taking into account (1) the (preprocessed) 3D MRI images, (2) BOLD time series, (3) user-defined experimental conditions, (4) HRF, and (4) technical/noise corrections.

The basic idea behind GLM is that the observed data (y) is equal to a weighted combination of several model factors (x) plus an additive error term (ε). It is a model (i.e. an equation: \( Y = X \cdot \beta + \epsilon \)), where Y is the BOLD signal at various time points at a single voxel (observed data), X represents several components which describe the observed data, i.e. the BOLD time series for a particular voxel (design matrix), \( \beta \) denotes the contribution of each component of the design matrix to the value of Y (parameter estimates), and \( \epsilon \) is the difference between the observed data, Y, and that predicted by the model, \( X \beta \) (error). The design matrix has one row for each scan and one column for each effect one has built into the experiment. These are referred to as explanatory variables, covariates, or regressors. Each column of the design matrix corresponds to experimental conditions of interest (the hypothesis under test) and a set of columns corresponds to model with effects of no interest. In this stage, the groups designated for the images are specified. This stage represents modeling the data to observe neurophysiologic responses, confounds, and error [Figure 3].

Inference and interpretation of results
The neuronal activity in response to an experimental task is obtained by specifying linear contrasts (T or F contrast). Cognitive subtraction logic is applied to predict brain activity scales in a linear fashion. The conditions of interest (active condition) are given a positive value, such as 1, and conditions (baseline condition) that are to be subtracted from these conditions of interest take on a negative value, such as -1. The end result is a statistical parametric map. The functional activations (blobs) obtained can be further
overlaid or rendered onto the high-resolution structural image of the subject in order to accurately locate the neural activity. Since SPM follows a univariate approach, each voxel should be separately analyzed. So, for a statistical threshold of \( P < 0.05 \), 5% of the voxels would show activation by chance alone (false activation – type I error), which means a multiple comparison correction is required. Bonferroni correction is the traditional way of doing this. However, due to involvement of a huge number of voxels, direct implementation would severely reduce the estimated number of degrees of freedom. Hence, to the extent that the image data approximate a random Gaussian field, correction for multiple comparison need to be only made for number of voxels that can be resolved independently (resells or resolution elements). The multiple comparisons correction is controlled for family-wise error (FWE) rate. This assumption of random Gaussian field is assured by applying a Gaussian smoothing filter in the preprocessing stages. A serious limitation of correcting for multiple comparisons is that the number of false negatives (type II error) is greatly increased. Further approach is to define the false discovery rate (FDR), that controls for about 5% \( (P < 0.05) \) of false positive activation. The FWE method controls for a 5% chance of a single false positive. To correct for multiple comparisons, some alternative approaches have been also used: (i) using a strict uncorrected threshold (e.g. \( P < 0.001 \)), (ii) using an inference over the cluster size, (iii) small volume corrections in regions where a prior hypothesis exists, and (iv) a region of interest (ROI) analysis in which the average signal for all voxels in an anatomical or functional ROI is used, thereby decreasing the number of multiple comparison voxel space to the number of ROIs [Figure 4].

**Brain voyager**

BrainVoyager QX (Brain Innovation, Maastricht, The Netherlands) is a software package for the analysis and visualization of MRI data sets. It supports on all major computer platforms including Windows XP/Vista/7, Linux, and Mac OS X. It is a multimodal analysis tool used for fMRI, diffusion tensor imaging (DTI), electroencephalogram (EEG), magnetoencephalography (MEG), and transcranial magnetic stimulation (TMS) data. BrainVoyager QX has been completely programmed in C++ and provides easy-to-use interactive GUI for all platforms. Automatic brain segmentation, surface reconstruction, cortex inflation and flattening can also be done by using this software. BrainVoyager also supports advanced data analysis of fMRI brain images, including Multi-Voxel Pattern Analysis (MVPA), Independent Component Analysis (ICA), and Probabilistic Maps. Hypothesis-driven statistical data analysis is an important tool to identify activations in brain regions exhibiting increased or decreased responses in specific experimental conditions as compared to others. OpenGL (Open Graphics Library), the most widely used cross-language for 2D and 3D graphics rendering, is implemented in BrainVoyager software for rendering 3D computer graphics (surface module). BrainVoyager is a commercial software and requires a Hardware against Software Piracy (HASP; a USB dongle that protects software vendors from unauthorized use or distribution of their software) key for a single computer or network dongle for many computers that works as a floating license. The steps for processing functional data include the following:

**Creation of functional MR project**

Functional MR (fMR) project is created by loading the raw functional data and converting it into FMR data format. fMR is the 2D visualization of functional data. BrainVoyager supports various types of data formats including DICOM, ANALYZE, and PHILIPS_REC. MR scanner names the DICOM files in a complex way. Before creating FMR project, BrainVoyager renames the DICOM files using header information. This will avoid the problems during importing and further analysis of the data.

**Preprocessing of FMR project**

Preprocessing is used to improve the image quality by suppressing undesired distortions or enhancing some image features made to be more suitable for further processing of the images.

Preprocessing of functional data includes several steps like mean intensity adjustment, slice scan time correction, 3D motion correction, spatial smoothing, and temporal filtering. This software provides a single window for all these preprocessing options and can run preprocessing steps at once.

**Creation of stimulation protocol**

Stimulation protocol allows defining the conditions (block or event-related design) used for the presentation of stimuli. BrainVoyager saves this data as protocol (PRT) file. The same protocol file can be used for different FMR projects, if the same conditions are used for different subjects.
Statistical analysis of functional data
The statistical analysis test is performed to determine which voxels in the brain are significantly activated by a certain type of stimulus. BrainVoyager software provides options for single subject and multi-subject statistical analysis. This software uses GLM for single subject analysis specifying statistical models. It is obtained by adding several explanatory variables known as predictors, which give precise activations. GLM analysis is a univariate method performed independently on each voxel time course and beta values are estimated for each voxel. Multi-subject data is analyzed by using fixed-effects group analysis and random-effects group analysis. BrainVoyager also supports multivariate approaches like ICA and MVPA. The MVPA tools include multivariate searchlight mapping and support vector machine. The end result of the statistical analysis is a statistical map that shows which voxels are significantly activated given a specified statistical threshold. The thresholded statistical map can be overlayed directly to the functional (FMR) data, co-registered anatomical [volumetric MR (VMR)] data, or surface module for visualization.

Anatomical Data Processing
The steps may include the following:

Creation of VMR projects
Anatomical data is loaded into BrainVoyager and converted into BrainVoyager-supported VMR data format. VMR is the 3D graphical representation of anatomical data. This data may exhibit inhomogeneous intensity and can be removed by using intensity inhomogeneous correction tool in BrainVoyager software.

Iso-voxel
Before FMR–VMR alignment, resolution of VMR data set is needed to match with the resolution of the functional data. In order to do this, VMR data set is iso-voxel to a resolution of 1 × 1 × 1 mm.

Talairach transformation
For multi-subject analysis, data set is transformed into a standard space. BrainVoyager provides two options for Talairach transformation: manual and automatic.
Co-registration of Functional and Anatomical Data

Co-registration allows alignment of functional and anatomical data for the purpose of overlaying the brain activity precisely. Co-registration has been divided into two steps:

- Initial alignment (IA)
- Fine-turning alignment (FA)

The IA brings the functional and anatomical data into same orientation. Head movements are rectified by using FA. FMR data is set as the “source” and VMR data is set as the “target.” FMR data is fixed and the VMR data is scaled, translated, and rotated with respect to FMR data.

Turbo-brain voyager

Turbo-BrainVoyager (TBV; Brain Innovation, Maastricht, The Netherlands) is a software package for the real-time analysis and visualization of fMRI data sets. TBV performs data preprocessing in real time, including 3D motion correction and spatial smoothing. It visualizes the data in multi-slice or single-slice functional data view and anatomical volume view. ROIs can easily be defined in TBV by dragging the mouse pointer around the activated cluster in functional data and also shows ROI time course window for the corresponding ROI. TBV is not a replacement for BrainVoyager Q. TBV does not have some functionality like segmentation, Talairach transformation, between-subject statistical data analysis, etc., TBV runs on Microsoft Windows 98/NT/2000/XP, Linux, and Mac OS X. TBV is a commercial software and requires a HASP USB dongle.

Conclusion

There are many choices available to a research group for analysis of fMRI data. Discussion in the current article was exclusive to the softwares and techniques familiar to the authors. We have provided sufficient information about different fMRI post processing tools that are of particular interest in cognitive and clinical neuroscience research.

References


