3
Methodology

This chapter describes the way the present research was designed and carried out. Initially, the choices made regarding the experimental task and method are explained. Thereafter, the method adopted is explained by giving details of the sample, the apparatus and stimuli used in the research, as well as the general design and procedure. Finally, the methods used for analyses of data are described.

3.1 METHODOLOGICAL CONSIDERATIONS

3.1.1 The change detection task

Several methods such as fMRI, TMS, EEG, magneto encephalography [MEG], positron emission tomography [PET], and single cell recordings can be used to investigate the physiological process of feature binding. At the behavioral level, researchers have generally used the change detection task. In this task, participants are presented two visual displays one after the other. The first display is the study display and the second display is the test display Participants decide and answer whether the two displays are 'same' or 'different'. Usually, the change involves adding a new stimulus, removing an earlier one, or a swap between two stimuli that have been already presented in the study display. Wheeler and Treisman [2002] introduced the swap detection task to particularly test feature binding. An example is shown in Figure 3.1.

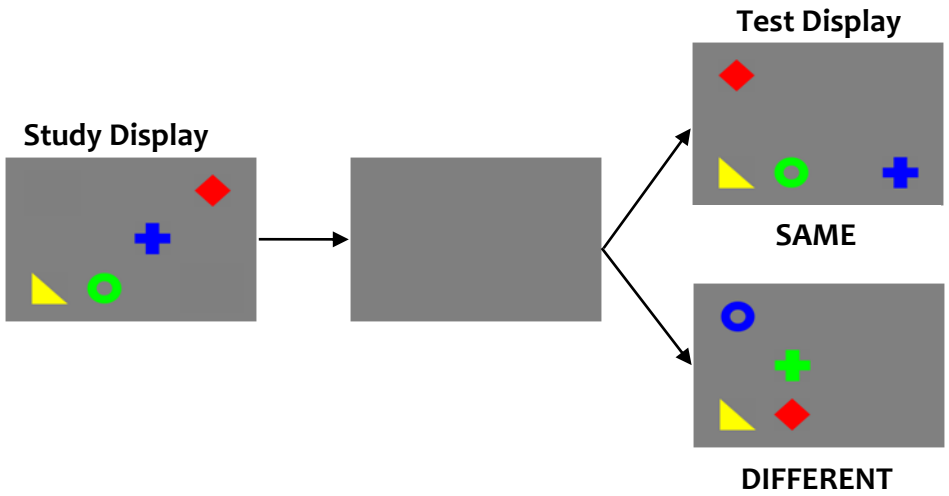

Figure 3.1: The swap detection task

The study display contains stimuli that are combinations of different colors and shapes to be remembered by the participant. The test display shows the same number of stimuli, which are in the same colors and shapes. When the combinations of shape and color are the same for all stimuli, the participant responds 'same', but if the combination of shape and color changes from the study to test display, the participant responds 'different'. As shown in Figure 3.1 with example stimuli, the initial/study display has a green plus and blue circle. If the test display shows all stimuli with exactly the same combinations of colors and shapes [upper panel], the participant needs to respond 'same'. But if the test display [lower panel] has a green circle and a blue plus, the participant needs to respond 'different'.

Success on this task cannot be achieved by remembering single features [only colors or only shapes], as all the features used in the study display are also present in the test display. To do the task efficaciously, one needs to remember the combination of the features for each stimulus and find out which combination has changed. Alvarez and Thompson [2009], calling it 'feature switch detection', held it to be an effective way of studying bindings, even though it seemingly underestimates the binding capacity in visual working memory.

There are, of course, other behavioral ways of assessing memory for feature bindings. Some researchers have used recall as a measure [e.g., Logie *et al.*, 2009] while others have preferred that the participant remember and reconstruct the exact color or orientation of a stimulus in an attempt to measure the precision of memory [Bays and Husain, 2008; Fougnie *et al.*, 2013]. Recently, Pilling *et al.* [2020] suggested that tasks in which participants are asked to report the difference are superior to the tasks in which they are asked to report sameness. They suggested that this is due to the bottom up, reflexive mechanism in VSTM that generates a mismatch signal during the comparison of visual input with information maintained in VSTM. The present researcher chose the change-detection task for its general popularity with researchers and because it easily lends itself to experimentation in the fMRI environment.

3.1.2 Set size of stimuli

The stimuli to be used in the present research were defined by combinations of only two features, color and shape. Thus, the task for the participant was to remember the binding between color and shape. It was decided to use only four stimuli as the memoranda on each trial. Set size has a significant effect on performance as visual working memory has a limited capacity, and as Oberauer [2019] has demonstrated, working memory capacity limits memory for bindings. The consensus regarding the capacity of visual working memory is not more than four items [Cowan, 2005, 2010; Luck and Vogel, 1997; Wheeler and Treisman, 2002]. A larger number of stimuli might create unnecessary cognitive congestion, while a lesser number of stimuli [such as two or three] would render the task trivial, and probably yield ceiling effects.

3.1.3 Articulatory suppression

To get a clean assessment of visual working memory, it is important that the participants do not encode the stimuli verbally. Many researchers use a verbal task such as repeatedly saying a single item aloud to ensure that the visual stimuli are not encoded linguistically [e.g., Allen *et al.*, 2009; Jaswal and Logie, 2011; Zhang and Luck, 2008]. Since all the stimuli used in the present research are known geometrical shapes and familiar colors, presumably they can be encoded verbally as well. Thus, articulatory suppression was used, and participants had to repeat the word "the" continuously throughout the trial from fixation until after the response was given. A review of several studies using articulatory suppression by Baddeley [1986] indicated that even repeating a single word is enough to preclude verbal coding of visual stimuli, with more complex operations, not being necessarily more effective. In fact, complex manipulations may demand attentional resources, thus going beyond what is required.

3.1.4 Studying activation in the brain using fMRI

The technique of fMRI was preferred over other methods due to the good spatial resolution and non-invasive nature of the method. Since the aim was to investigate the brain areas involved in processing different modes of presentation of stimuli, fMRI best fit the criteria of a relatively low cost method, accessible to the researcher in India, and completely safe for normal human population. It detects activity based on oxygen consumption by a particular brain region due to a particular task. Other investigation techniques [including EEG, MEG, PET, TMS] for a cognitive process do not combine the fine spatial resolution and specificity of fMRI with a non- invasive nature.

The main shortcoming of fMRI is that it is measuring only a surrogate signal, which is presumably correlated with the actual physiological changes in the brain. Unlike methods, which use a direct brain signal [single cell recordings] or stimulation [TMS], it cannot establish causal relationships. Another demerit is that it reflects the mass neuronal activity, i.e., it shows activity from a region, not from a specific cell. The neuroscience literature suggests the existence of a variety of neurons differing in function. Further, these multiple types of neurons reside in any one given region. Thus, the specificity of fMRI is questionable in comparison to other more precise techniques. Finally, the fMRI signal does not have a good temporal resolution. A well-structured review of advantages and shortcomings of fMRI is by Logothetis [2008].

A greater concern is the controversy regarding the criteria adopted in earlier fMRI research [Brown and Behrmann, 2017; Eklund *et al.*, 2016, 2017; but see Bansal and Peterson, 2018]. With the view that the debate is still on, and that is not prudent to throw out the baby with the bathwater, the present researcher adopted the fMRI method, but used ways of analyses [conjunction null analyses and ROI analyses] and criteria that were stringent enough to reduce the risk of false positives.

An important choice made for the fMRI experiment was between a block design and an event related design. In event related designs, experimental trials pertaining to different experimental conditions are presented in a random sequence to the participant. Event related designs are good for measuring cognitive efficiency and differences in particular neural diseases or conditions. In the block design, all trials within a block are of the same kind, i.e., they pertain to exactly the same experimental condition. Since a BOLD signal takes approximately 4-6 seconds to achieve its peak, so for a small duration task it is preferable to have a block of experimental trials together to get clear results. The present researcher preferred the block design for the fMRI experiment, because each trial in the experiment lasted for very little time.

3.2 METHOD

3.2.1 Sample

In all, five behavioral experiments and one fMRI experiment were carried out in the present research. In each of the experiments, data was collected from 18 different participants. Thus, there were a total of 108 participants. Informed consent was taken from every participant in accordance with the Declaration of Helsinki. An honorarium of a hundred Indian Rupees [INR 100] was given to every participant. Although they were informed about the task, and indeed, given practice on it, all participants were naïve to the purpose and hypotheses of the research. All participants of the first five experiments were male undergraduate students at the Indian Institute of Technology Jodhpur in the age range of 17-21 years. No females figured in this group as the institute had an overwhelming majority of male students in this age group. No participant reported having a history of any kind of neural/psychological disorder and all reported normal or corrected to normal visual acuity.

In the fMRI experiment, 18 participants [10 females and 8 males] in the age range 18 - 30 years participated from the Institute of Nuclear Medicine and Allied Sciences, Delhi. They reported no psychiatric or neurological problems. All of them were screened for depression using the 21-item Beck Depression Inventory II [Beck *et al.*, 1996]. Four people were rejected for a score higher than 18 on the scale. All participants had normal or corrected to normal visual acuity. They were given instructions and extensive practice on the task outside the scanner.

3.2.2 Stimuli and apparatus

The experiments were designed in E-Prime 2.0 software [Psychology Software Tools, 2008] and the specifications of stimuli for all the trials were generated by a script in MATLAB [MathWorks, 2014]. The experiment was presented on a laptop computer.

The four stimuli presented in each trial were random combinations of four shapes and four colors. The four different shapes were plus, triangle, doughnut, and diamond. The four different colors were red, green, blue, and yellow. All the stimuli were of the same size, being 2.5 cm × 2.5 cm in area with the pixel distribution being 110×110.

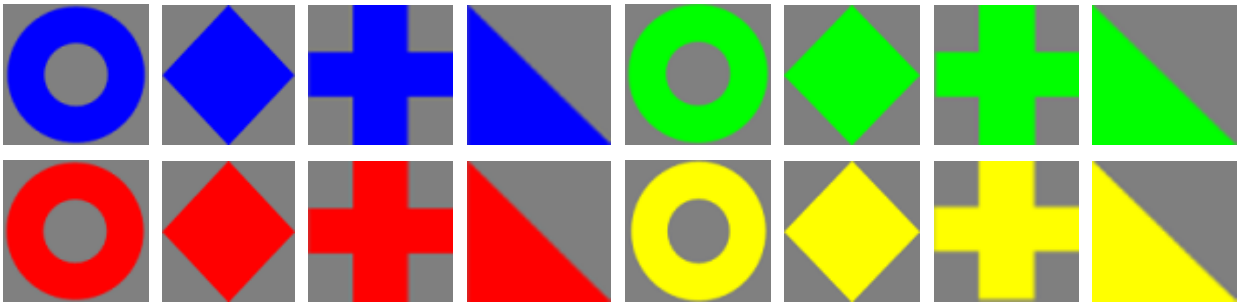

Figure 3.2: Stimuli made up of four colors and four shapes used in the study

The stimuli were presented in a 3×4 imaginary grid in foveal vision [i.e., two degrees to left and right from the fovea] in each trial. Stimuli were displayed on a 50% gray background on a 14-inch display of a laptop computer with resolution set at 1366 × 768. The participants viewed the display from an unconstrained distance of 70 cm and could move their eyes freely.

Behavioral responses were collected through the keyboard. The keys for the responses "same" and "different" were the equidistant "c" and "m" keys on the keyboard. For the fMRI experiment, the display was shown using the Nordic visual system and the response was taken with the left hand button press used for "same" and the right hand button press for "different" using the Nordic Sync box connected to the computer through a serial port. Response keys were not counterbalanced in the behavioral experiments or the fMRI experiment to avoid spatial conflict, and also because accuracy of response rather than response latency was anyway the dependent variable.

3.2.3 General design and procedure

All the experiments were designed as 2×2 factorial experiments with repeated measures on both independent variables, viz., mode of presentation and locations. The two levels of each of the two independent variables were fully crossed to obtain four experimental conditions. With 96 trials in each experimental condition, there were 384 trials in each experiment. The participants also practiced all experimental conditions in 96 trials.

Each trial began with a fixation screen, followed by the study display. In the study display four stimuli having distinct colors and shapes were shown at different locations in each

trial. The display contained all stimuli together in the simultaneous presentation condition. In the sequential presentation mode, the stimuli appeared one after the other. The participants were required to remember the association of color and shape for each stimulus, and upon the presentation of the test display comprising all the stimuli together, they had to detect whether the latter contained stimuli which were the 'same' or 'different' as compared with the study stimuli. Thus, the essential task of the participant was to remember the binding between colors and shapes. In the unchanged locations condition, all stimuli in the test display were presented at the same locations as the study display. In the random locations condition, the locations of the stimuli in the test display were random [with replacement] with respect to the study display.

Blocks of experimental conditions were counterbalanced across participants. Trials within the blocks were presented in a random order. Whenever, the binding change occurred, any two stimuli swapped colors [or shapes] in comparison to how they occurred in the study display. Changes in binding happened on 50% trials in each experimental block. In the behavioral experiments, responses were collected using the keyboard with equidistant keys c and m for "same" and "different" responses respectively. In the fMRI scanner responses were collected through the Nordic response grip.

Every experiment was completed in a single session. In each experimental session, after obtaining written consent as per the Declaration of Helsinki, participants were given a detailed description of the study presented on the computer monitor. They were told that they could pause and rest any time between the trials, simply by not initiating the trial, by not pressing the key to move to the study display from fixation. They were also informed that change would occur only on 50% trials. Participants were requested to aim for maximum possible accuracy, with no regard to speed of response. For every experiment, a practice session was conducted to be assured that the participant had fully understood the task. For the fMRI experiment, practice was carried out outside the scanner.

The changes to this general procedure are noted in the subsequent chapters giving the details of each experiment.

3.2.4 Experiment using fMRI

An experiment was conducted in the fMRI environment to study the brain areas activated in the four experimental conditions, resulting from completely crossing simultaneous and sequential presentation with unchanged and random locations,. The task was presented in two blocks for each of the four experimental conditions, interleaved within baseline blocks. The four experimental conditions repeated twice, and thus produced eight experimental and nine baseline blocks in a single session. Thus, the full factorial design was used in the task and for analyses. Instructions and practice were given to the participant outside the scanner. Some changes to the design and procedure were made due to the constraints imposed by the fMRI environment. These and other details appear in Chapter 6, which reports the fMRI experiment.

3.2.5 Analyses of behavioral data

For all experiments, accuracy of response was the dependent variable. The raw scores were converted into d prime scores for each individual participant. A response of 'different' on the trials where a change occurred was taken as a hit and a response of 'different' when there was actually no change in the two displays was considered a false alarm. For the purpose of further calculations, proportions of hits as 1 and false alarms as 0 were converted respectively to .9999 and .0001. In the analysis, d prime scores are used as measures of accuracy, as d prime is a pure measure of sensitivity, irrespective of bias. Beta was calculated as a measure of bias.

Analysis of variance [*ANOVA*] was used to assess the effect of experimental manipulations, as the sample elements were random and independent, and drawn from a

normally distributed population. Since all experiments were designed using a full factorial repeated measures design, a 2 × 2 repeated measures *ANOVA* was carried out for every experiment with mode of presentation [simultaneous vs. sequential] and locations [unchanged vs. random] as the independent variables and change detection accuracy as the dependent variable. To explore the serial position effects in the sequential presentation conditions, a 2 × 4 repeated measures *ANOVA* [location × swaps] was carried out. The swaps selected for this analysis were between stimuli at serial positions 1 and 4 [showing the joint effect of primacy as well as recency], 1 and 2 [showing only primacy effect], 2 and 3 [items in the middle positions], and 3 and 4 [showing only the recency effect]. Where sphericity was violated as indicated by a significant Mauchly's test, F values with Greenhouse-Geisser correction applied to the degrees of freedom are reported in this thesis. *Partial* η^2 is reported as a measure of effect size as it allows an easy comparison of effect sizes across experiments [Cohen, 1973, Levine and Hullett, 2002].

Apart from reporting conclusions based on null hypothesis significance testing using the frequentist approach, the results are augmented by using Bayesian *ANOVA*. The Bayesian approach has the merit that it specifies and competitively tests the null and the alternative hypotheses. In the frequentist approach, inferences about differences are made on the basis of the lack of evidence for the null hypothesis, whereas Bayesian methods provide the relative evidence in favor of the null or alternative hypotheses. The reported Bayes factors represent relative evidence between the two hypotheses [BF_{10} for the alternate hypothesis and BF_{01} for the null hypothesis] indicating which hypothesis is more prominent for the available data. Bayesian *ANOVA* were computed using Bayesian repeated measure *ANOVA* facility in JASP 0.9.2.0 [JASP Team, 2018].

All important and significant results of statistical analyses are reported in the text of this thesis. The associated tables are in the annexure. However, the tables for fMRI results, giving the activated areas and their coordinates resulting from conjunction null analyses are reported in the text, as they represent the major outcome of analyses to be deliberated on in the discussion.

The mean performance in each of the experimental conditions is shown in graphs. These graphs clearly depict the interactions [or lack thereof]. In all the graphs showing these major results, the x-axis shows the mode of presentation, and the y-axis shows the d prime scores. The graph lines show the two location conditions, viz., unchanged and random locations. In the graphs comparing swaps in the sequential condition, the swaps are represented on the x-axis. The error bars in all graphs represent ±1 standard error of the respective means.

3.2.6 Analyses of fMRI data

All the structural and functional images obtained from the scanner were analyzed through Statistical Parametric Mapping Version 12 [SPM 12] community toolbox [Friston *et al.*, 1994, 1995, 1996] in MATLAB 2014a on Windows 7 platform. The whole time series were analyzed in a series of steps described in the following sections.

Data Preprocessing

The data were analyzed with SPM 12 [Ashburner *et al.*, 2020, Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/] running on MATLAB [MathWorks, 2014]. Figure 3.3 depicts the steps used for preprocessing the raw data. The raw data is obtained in two categories. The first category comprises a set of structural images, representing the anatomical details for that participant for a particular session. These are T1 weighted images, T1 being the timing of radio frequency pulse sequence, which highlights fat issues. The second category comprises a set of functional images, which represent the functional changes [BOLD responses] over a period for a participant/session. These are T2* weighted images, T2 referring to the timing of radio frequency pulse sequence, which highlights fat and water.

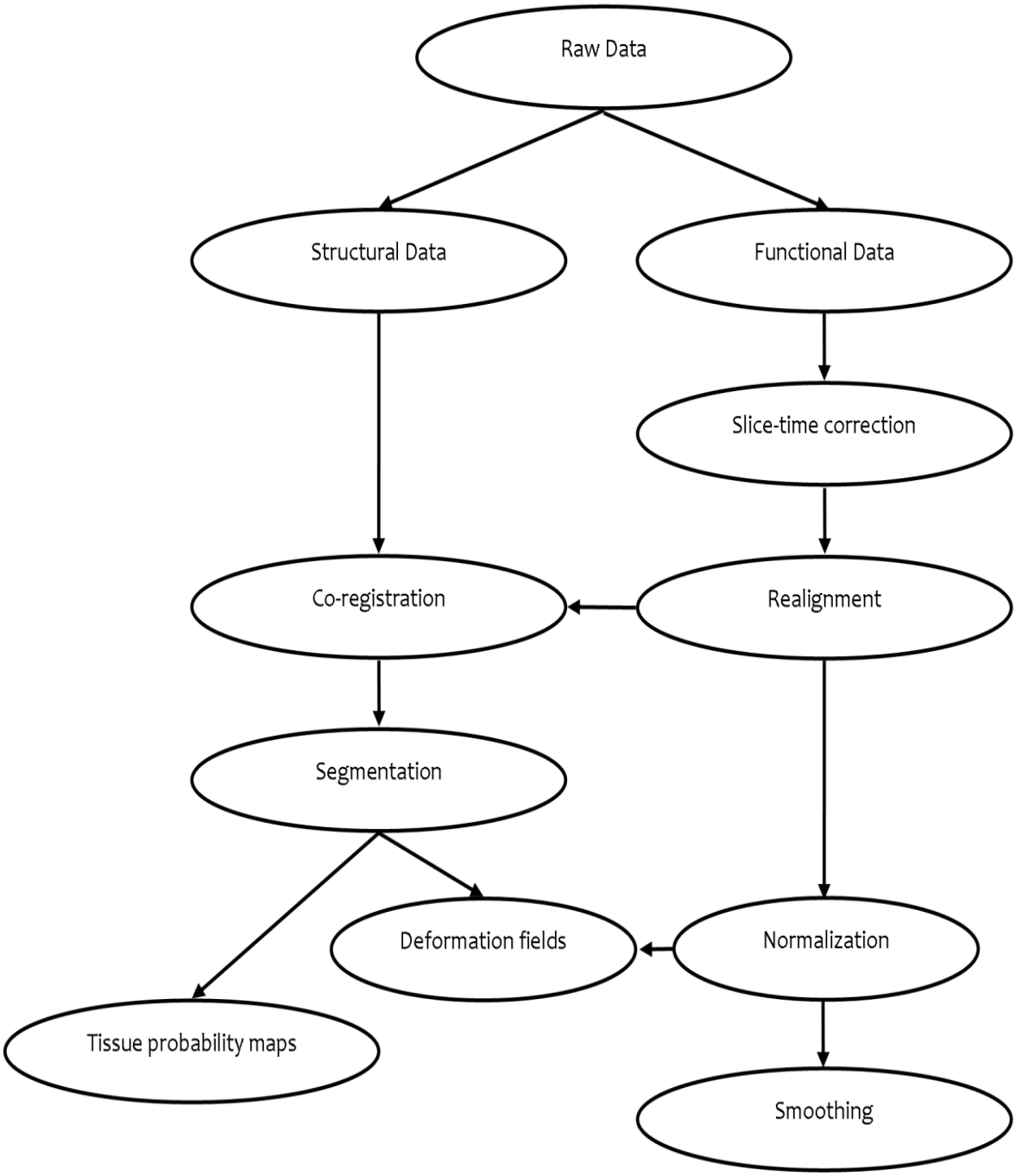

Figure 3.3: **Steps in pre-processing of the raw fMRI data**

The functional data needs to be slice-time corrected. Essentially, all the 2D images [called slices] are combined to create a 3D image [called volume]. It is often assumed that all slices, which are stacked to create a volume, are taken at a particular time point. But, in reality, the slices are taken sequentially. Thus, slice-time correction estimates and interpolates the slices between adjacent slices, using Fourier transformation.

The second step, realignment, is carried out on a complete functional time series to assess and remove motion artifacts created by head movements. The realigned volumes are used later in the stage of normalization. Realignment also generates an average functional image for each participant. This is used in the next step called co-registration, in which the single structural image and the average functional image are matched to correct the structural image. Thereafter,

segmentation is performed on the structural data to create tissue probability maps to filter out white matter, gray matter, cerebrospinal fluid, etc. Additionally, segmentation creates a deformation field file for every participant. This is used in the next step, normalization, along with the realigned functional volumes, to aid inter-participant averaging and to precisely delineate the functional anatomy of each participant. Lastly, smoothing is done by applying a Gaussian kernel over the whole volume of functional time series data to improve the signal to noise ratio. The details of each step can be found in an easily accessible format in the SPM manual [Ashburner *et al.*, 2020; http://www.fil.ion.ucl.ac.uk/spm/doc/manual.pdf]. The final smoothed functional volumes are ready to be processed further through statistical analyses.

Conjunction null analyses

Conjunction null analyses were carried out to find the common areas activated in the different levels of each independent variable. Conjunction analysis was originally proposed in the work of Price and Friston [1997], and enunciated for fMRI studies with many participants by Friston *et al.* [1999]. After criticism by Nichols *et al.* [2005], the reply by Friston *et al.* [2005] clearly articulates the changes adopted in SPM. The conjunction null analysis is a conservative method to estimate effects that are present in all conditions of interest, and, contrary to global conjunction analyses, limits the risk of observing false positives [activated regions driven mainly by one condition]. Conjunction null analyses demonstrate the context-invariant nature of regional activation responses. The interactions, if any, are ignored.

In the present research, there are four experimental conditions: simultaneous presentation with unchanged locations [SIMU], simultaneous presentation with random locations [SIMR], sequential presentation with unchanged locations [SEQU], and sequential presentation with random locations [SEQR]. In the fMRI experiment, four contrasts were obtained by subtracting activation in the baseline condition [Base] from activation in these four conditions. Thereafter, conjunction null analyses was performed on five conjunctions:

1. Common areas recruited with simultaneous presentation for unchanged and random locations [[SIMU-Base] ∩ [SIMR-Base]]
2. Common areas recruited with sequential presentation for unchanged and random locations [[SEQU-Base] ∩ [SEQR-Base]]
3. Common areas recruited with unchanged locations across simultaneous and sequential presentation [[SIMU-Base] ∩ [SEQU-Base]]
4. Common areas recruited with random locations across simultaneous and sequential presentation [[SIMR-Base] ∩ [SEQR-Base]]
5. Common areas recruited in all four conditions [[SIMU-Base] ∩ [SIMR-Base] ∩ [SEQU-Base] ∩ [SEQR-Base]]

Inferences were drawn after applying the correction for family-wise error at the voxel level with $p<.01$ and $k>20$.

Repeated measures ANOVA in specific regions of interest

The ROIs were defined on the basis of the previous visual feature binding studies. All the ROIs were built as a sphere of 8 mm radius around the defined coordinates obtained from these studies. The significant ROIs were further explored using Marsbar toolbox designed for SPM [Brett *et al.*, 2002]. To obtain the contrast files, the baseline was subtracted from the experimental condition. Mean parameters were extracted from every contrast file at the participant level. These obtained mean parameters were analyzed at the group level by carrying out repeated measures *ANOVA* to study the interactions.

The next three chapters describe the rationale, expectations, procedure, results, and discussion of each experiment. These are followed by a general discussion, and then the summary of the thesis is provided.