Pamukkale Univ Muh Bilim Derg, XX(X), XX-XX, 20XX

Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi

Pamukkale University Journal of Engineering Sciences

Factorial designs in fMRI analysis: A comparative exploration of full and flexible factorial approaches

fMRI analizinde faktöriyel tasarimlar: Tam ve esnek faktöriyel yaklaşımların karşılaştırmalı bir araştırması

Cemre CANDEMİR[1*]

[1]International Computer Institute, Ege University, Izmir, Turkey.
cemre.candemir@ege.edu.tr

Received/Geliş Tarihi: 22.02.2024 Revision/Düzeltme Tarihi: 25.04.2024 doi: 10.5505/pajes.2024.11127
Accepted/Kabul Tarihi: 18.05.2024 Research Article/Araştırma Makalesi

Abstract

Understanding the intricacies of the human brain demands rigorous analysis of dynamic functional neuroimaging data like functional Magnetic Resonance Imaging (fMRI). This paper investigates the application of two powerful analytical approaches - full and flexible factorial analysis - for exploring brain activity in fMRI studies. First, the main principles of each method are given broadly, by highlighting their strengths and limitations. Then, design structures, adaptability, data complexity, flexibility, and factor effects are handled in this context. Utilizing theoretical and real-world fMRI scenarios, it is shown how full and factorial analyses provide the factor combinations in simple and complex designs. Drawing on these insights, the critical role of aligning the chosen approach with the specific research question and data structure of each fMRI study is emphasized. Researchers can use these statistical analyses to reveal the complex structure of brain activity by diverse experimental designs. By exhibiting the unique strengths and limitations of full and flexible factorial analysis, this paper aims for researchers to choose the right methodology for their research.

Keywords: Brain Imaging, Factorial Designs, fMRI Analysis, Full Factorial, Flexible Factorial

Öz

İnsan beyninin karmaşıklıklarının anlayabilmek, fonksiyonel Manyetik Rezonans Görüntüleme (fMRG) gibi dinamik fonksiyonel nörogörüntüleme verilerinin titiz bir analizini gerektirir. Bu makale, fMRI çalışmalarında beyin aktivitesini araştırmak için iki güçlü analitik yaklaşımın (tam ve esnek faktöriyel analiz) uygulanmasını araştırmaktadır. İlk olarak, her yöntemin temel ilkeleri, güçlü yönleri ve sınırlamaları vurgulanarak geniş bir şekilde verilmektedir. Daha sonra tasarım yapıları, uyarlanabilirlik, veri karmaşıklığı, esneklik ve faktör etkileri bu bağlamda ele alınmaktadır. Teorik ve gerçek dünyadaki fMRI senaryolarından yararlanılarak, tam ve faktöriyel analizlerin basit ve karmaşık tasarımlarda faktör kombinasyonlarını nasıl sağladığı gösterilmiştir. Bu içgörülerden yola çıkarak, seçilen yaklaşımın her fMRI çalışmasının spesifik araştırma sorusu ve veri yapısı ile uyumlu hale getirilmesinin kritik rolü vurgulanmaktadır. Araştırmacılar bu istatistiksel analizleri, çeşitli deneysel tasarımlarla beyin aktivitesinin karmaşık yapısını ortaya çıkarmak için kullanabilirler. Tam ve esnek faktöriyel analizin benzersiz güçlü yönlerini ve sınırlamalarını sergileyen bu makale, araştırmacıların araştırmaları için doğru metodolojiyi seçmelerini amaçlamaktadır.

Anahtar kelimeler: Beyin görüntüleme, faktöriyel tasarım, fMRG Analizi, Tam Faktöriyel, Esnek Faktöriyel

Düzeltilmemiş Sürüm - Uncorrected Version

1 Introduction

Functional Magnetic Resonance Imaging (fMRI) is a non-invasive brain imaging technology that detects brain activity by measuring changes in blood oxygenation levels. During the last decades, it stands at the forefront of neuroscience, offering valuable insights into the working and connection mechanisms of the human brain [1]. fMRI can determine which area of the brain is active during resting state (i.e. lying still) and/or performing specific functions, such as lifting your arm or even just thinking about something [2]. Researchers can use these areas to better understand, diagnose, monitor, and treat various conditions. Through the sensitivity of fMRI, the neural underpinnings of perception, cognition, emotion, and behavior could be understood with the implications for the fields of psychology, neuroscience, and medicine.

The base of the fMRI research lies in the recognition that the brain is a dynamic and adaptive organ, continuously responding to a multitude of internal and external stimuli. This complex interaction of neural processes demands the development of sophisticated experimental designs that can capture the subtleties of brain function [3]. Since fMRI BOLD (blood-oxygen-data-dependent) data is not an exact measure of neuronal activity, the experimental design becomes to play a crucial role in providing the required information from the fMRI studies [4]. It shapes the questions we ask, the answers we obtain, and the depth of our understanding. It affords the opportunity to statistically contrast the neuronal activity of related area with appropriate conditions. In this way, the experimentally designed fMRI scans allow us to dissect cognitive processes, decipher neural networks, and determine the neural signatures of mental states. It is the fundamental framework upon which the entire structure of fMRI analysis is built.

The base of the fMRI data analysis lies the General Linear Model (GLM) approach. The GLM leverages the principles of linear regression to model the relationship between experimental factors and the observed fMRI signal [5]. In the context of fMRI, this relationship is typically represented as a set of equations, where the fMRI signal at each voxel is expressed as a linear combination of experimental conditions, along with nuisance variables to account for sources of variability such as motion or physiological noise. Due to being a powerful methodology, it

*Corresponding author/Yazışılan Yazar

allows to estimate the contribution of different experimental conditions to the observed brain activity, assess the statistical significance of these conditions and generate contrasts to determine the areas of the brain where activation varies across the conditions. This modeling approach is the cornerstone for conducting hypothesis-driven research and answering specific questions about the neural basis of cognition [6].

On the other hand, factorial designs are a fundamental component of fMRI analysis. They provide a systematic context for investigating the effects of multiple independent variables (factors) and their interactions on neural responses [7]. In the context of fMRI, factors can represent experimental effects, such as different task conditions, stimuli, or subject characteristics. Factorial designs offer a structured way to explore how the brain responds to the combinations of these factors, ultimately helping researchers access the answer to the research question.

In a factorial design, factors are typically categorized into levels, and different combinations of factor levels are systematically presented to participants during the experimental fMRI task design. In this way, researchers can disentangle the unique and interactive effects of each factor during the analysis [8]. This approach is valuable for understanding not only the main effects of individual factors but also how they may interact, revealing the subtle nuances of neural activity and the potential for non-additive effects. Factorial designs enable researchers to address a wide range of research questions in fMRI, from exploring the modulatory influence of factors like age, gender, or clinical status on brain function to investigating how different sensory modalities or cognitive processes are neurally represented.

This study searches the power of factorial designs in fMRI analysis, focusing on two distinct yet interrelated methodologies: full factorial and flexible factorial approaches. Researchers often struggle to decide which method is appropriate for their problem. The concepts are often confused with each other. As the research methods become increasingly complex, it is critical to carefully consider which factorial approach is most suitable. Therefore, we aim to shed light on the deterministic role that these designs play in enlightening brain function. In this context, this study contributes to the field in several ways:

i) Methodological Advancement: The study advances methodological understanding in fMRI data analysis by systematically comparing the performance of different factorial analysis approaches. It provides insights into the strengths and limitations of each approach.

ii) Practical Guidance for Researchers: Exploring factorial approaches would guide researchers in selecting and conducting the most appropriate method in factorial designs for their specific research questions and data characteristics in fMRI analysis. Additionally, the study provides a comprehensive guide to their application in the Statistical Parametric Mapping (SPM) framework.

iii) Precision in Data Analysis: By improving the mathematical sensitivity of fMRI data analysis, the study enables researchers to extract more accurate and meaningful results from their fMRI data.

iv) Understanding in Neuroscientific Knowledge: Finally, -along with the above contributions - the study would help to enhance our understanding of brain function and cognition by enabling more accurate and reliable interpretations of fMRI data, leading to more robust scientific discoveries and insights.

In the following sections, the nuances of full and flexible factorial designs will be handled in detail, elucidating their unique features, differences, and complementary strengths. Theoretical and real-world examples will explore, discuss the considerations that govern the choice between these approaches, and provide guidance on how to interpret and report their results. The results of the analysis will be exhibited, and will be provided a guide how to conduct of the methods by using SPM.

2 Related Work

Understanding the intricate neural processes underlying human cognition necessitates advanced analytical approaches in fMRI studies. Two predominant factorial analysis methods—full factorial and flexible factorial designs—have emerged as pivotal tools for operating the complexities of experimental paradigms and subject-specific variations within fMRI datasets. Beside these, group-level analysis in fMRI studies is an important component of neuroimaging research that allows researchers to draw meaningful conclusions about brain activity at the population level. While individual-level analysis focuses on the activation patterns of single participants, group-level analysis synthesizes data from multiple individuals to identify common neural responses and make inferences about the broader population. In this context, especially in group-level analyses, the factorial analysis becomes the key aspect of fMRI analyses. The studies in the application of full factorial analysis within the fMRI framework are based on systematically exploring the effects of multiple experimental factors on neural responses. Several state-of-art studies elegantly demonstrate the capacity of full factorial analysis to discern main effects and interaction effects, providing a robust foundation for subsequent research. In [9], the authors employee a full factorial design to investigate the multiple target and distractor processing effects of visual attention and stimulus modality on dorsal attention system and visual cortex. Similarly, frontoparietal activity is explored with recognition memory and visual detection tests by fMRI scanning [10]. Moreover, it can be used effectively in clinical research such as investigating the effects of gender on brain neuroimaging features of patients with anhedonia in major depressive disorder [11]. The neural basis of several different research can also be analyzed with full factorial such as social comparison between groups [12], learning the control behavior according to the social feedbacks in childhood and adolescence [13], and detection of behaviorally relevant deviant stimuli [14].

On the other hand, in response to the demand for greater adaptability in experimental designs, flexible factorial analysis has gained importance. A recent study by Torrecuso et al. showcased the utility of flexible factorial designs in accommodating subject-specific factors in investigations of motor functions in Parkinson disease [15]. Murray et al. investigates the mixed emotion states, i.e., coexisting of opposite emotions such as sadness and pleasure, during fMRI scan and analysis with the flexible factorial design [16]. Tomasino et al. examined the functional brain mechanisms involved in state/psychological imagery in individuals with anxiety disorders [17]. The researchers employed a flexible factorial approach to adaptively model variations in subject and control groups, revealing patterns of neural activity associated with behavioral and brain functional alterations during imagery.

Düzenlenmemiş Sürüm - Uncorrected Version

Full and flexible factorial analysis in the abovementioned fMRI studies showcases the application of these methodologies in response to the diversity of research questions. These studies stress the importance of selecting the most appropriate factorial design based on the complexity of experimental paradigms, the presence of individual differences, and the adaptability required for comprehensive neural investigations. Additionally, the comprehensive study by Antony is strongly recommended for readers are who interested in deeper information about the factorial designs with examples and case studies [18].

3 Basics of Factorial Desings

3.1 Full Factorial (FF) Analysis

Full factorial analysis in fMRI studies is a robust statistical method that holds particular importance in the exploration of neural activity and its relationship to various experimental factors. It can be applied in many problems in engineering such as optimization [19]. This method systematically to investigate the effects of multiple independent variables (factors) and their interactions on a dependent variable. In the context of fMRI studies, FF analysis is employed to examine how different experimental conditions, represented by various levels of factors, influence the neural activity. Thus, multiple factors can be simultaneously searched, providing a more comprehensive understanding of how different variables interact and influence brain functions.

In many fMRI studies, researchers tend to examine the effects of multiple experimental factors and conditions. FF analysis tests all possible interactions between these factors. For example, in a cognitive task fMRI study, suppose that how both the type of stimulus (i.e., visual or written) and task difficulty (i.e., easy or hard) impact brain activity would be investigated. In such a scenario, every level of one factor with every level of the other factors, i.e., visual/easy – visual/hard –written/easy – written/hard can be combined by FF analysis. In this way, it enables to assess the main effects of each factor and interactions.

FF can be designed for $k-$factors, with $n-$levels, yields n^k experimental conditions in total. In general, FF includes 2-levels with k factors. In such design, the total number of effects would be 2^k with k main effects, $\binom{k}{2}$ 2-factor interactions, $\binom{k}{3}$ 3-factor interactions, $\binom{k}{n}$, n factor ($n \leq k$) interactions and 1 k-factor interaction.

The basic structure of an FF design is $2\ x\ 2$ with $2-$ factors of 2 levels of each factor. Let's denote the factors as Factor A and Factor B, and their respective levels as A1, A2 for Factor A; B1, B2 for Factor B. The design result in $2^2 = 4$ unique conditions as shown in Table 1: *i)* A1B1 *ii)* A1B2 *iii)* A2B1 *iv)* A2B2. The design consists of 2-factor interaction with 2-main effects of factors A and B, and 1-interaction effect (AxB). The full model that contains all factor effects and interactions can be written as in Equation (1):

$$Y = \beta_0 + \beta_1 A_1 + \beta_2 A_2 + \beta_3 A_1 A_2 \qquad (1)$$

The main effects and interactions effects are as follows:

Table 1. The structure of a simple $2x2$ factorial design with 2-factors A and B, each has 2-levels.

	Factor B	
Factor A	*A1B1*	*A2B1*
	A1B2	*A2B2*

The main effect of Factor A measures the overall effect of changing levels of Factor A, while ignoring the levels of Factor B. It is calculated by averaging across all levels of Factor B in Equation (2). Similarly, main effect of Factor B measures the overall effect of changing levels of Factor A, while ignoring the levels of Factor A. It can be calculated by averaging across all levels of Factor A in Equation (3). On the other hand, the interaction of A and B determines whether the effect of one factor depends on the level of the other factor. Like given with Equation (4), it is calculated by comparing the differences in the main effects across the levels of the other factor. In other words, the interaction assesses whether the effect of one factor (A) depends on the level of the other factor (B). In the context of fMRI analysis, main factor of A compares the average activation between A1 and A2, collapsing across levels of Factor B. Similarly, main effect of Factor B compares the average activation between B1 and B2, while collapsing across levels of Factor A.

$$\text{Main Effect of A} = \frac{(A1B1+A1B2)}{2} - \frac{(A2B1+A2B2)}{2} \qquad (2)$$

$$\text{Main Effect of B} = \frac{(A1B1+A2B1)}{2} - \frac{(A1B2+A2B2)}{2} \qquad (3)$$

$$\text{Interaction Effect of } AxB = \frac{(A1B1-A1B2)}{2} - \frac{(A2B1-A2B2)}{2} \qquad (4)$$

In a full factorial design, the beta (β) coefficients represent the weights assigned to each condition in GLM. Here, conditions refer to the combination of each factor levels. Thus, they indicate the contribution to the predicted linear model.

In a $2\ x\ 2$ full factorial design, as abovementioned, there are 4-conditions: *A1B1*, *A1B2*, *A2B1* and *A2B2*. Let's denote these conditions as *C1*, *C2*, *C3* and *C4,* respectively. Thereby, the GLM can be expressed as given in Equation (5):

$$Y = \beta_0 + \beta_1 C_1 + \beta_2 C_2 + \beta_3 C_3 + \beta_4 C_4 + \epsilon \qquad (5)$$

In terms of fMRI analysis, Y corresponds to the observed fMRI signal, whereas the intercept term β_0 denotes the baseline activity. $\beta_1, \beta_2, \beta_3, \beta_4$ are the beta coefficients which represent the effect of conditions *C1, C2, C3* and *C4*, respectively. Finally, ϵ denotes the error term of the model.

The specific values of the β_s depend on the data and the results of the GLM analysis. The GLM estimation process determines the optimal values for these coefficients to best fit the observed fMRI data given the experimental conditions.

Interpreting the β coefficients involves assessing the direction and magnitude of the effects associated with each condition. Positive coefficients indicate an increase in fMRI signal compared to the baseline (intercept), while negative coefficients indicate a decrease.

To obtain the actual values of the β coefficients, it is need to perform the GLM analysis using software such as SPM, FSL, or other fMRI analysis tools. The software outputs the estimated β coefficients along with statistical measures that allow you to assess the significance of each effect. In this study, the SPM is used to conduct these analyses, which is detailed in Section 3.2.

Düzenlenmemiş Sürüm - Uncorrected Version

3.2 Flexible Factorial (F_xF) Analysis

As mentioned in detail in the previous section, the FF design allows the analysis of all possible combinations of interactions among every factor and level. However, this may not be the most suitable design in every case. Since they have a fixed structure, they may have some limitations when dealing with complex or dynamic experimental designs, in which are only interested in two or three-factor interactions. On the other hand, it may become impractical or inefficient when dealing with a large number of factors and/or levels, as the number of conditions increases exponentially. For example, a design with 5 factors at two levels results in $2^5 = 32$ combinations, while 10 factors results in $2^{10} = 1024$ combinations. Thus, its limited adaptability can be restrictive in circumstances such as time, resources, number of subjects, cost, etc. in high-factored scenarios.

Conversely, the flexible factorial (F_xF) analysis offers an advanced and adaptable statistical approach that can investigate the complex experimental design and interactions between factors in fMRI studies. This approach is particularly crucial for studies with varying and dynamic factors, making it an important tool in the exploration of neural activity and its relationships to diverse experimental conditions.

Unlike the fixed structure of FF analysis, F_xF analysis allows for the inclusion or exclusion of factors and levels needed, making it a versatile approach. Researchers can adapt the analysis to match the specific goals of their study, incorporating conceptually relevant factors and excluding those that are not. This adaptability ensures that the analysis aligns with the research questions and hypotheses. Moreover, in some cases, there may be factors that are not of primary interest but still need to be considered to control for their potential influence on the fMRI data. F_xF analysis allows for the inclusion of nuisance variables or covariates, which can help decrease undesired sources of variability.

Besides this flexibility, F_xF can offer to investigate individual subject variations, such as age, gender, education level, or clinical status which modulate neural responses. F_xF allows accommodating such individual subject variations by including the subject-specific factors or covariates in the analysis. This can admit for a nuanced understanding of how various factors interact and influence brain activity.

Like as FF, the underlying equations for F_xF also lie on GLM, expressed as $Y = X\beta + \epsilon$. Here, Y denotes the observed fMRI signal, and X is the design matrix, constructed based on the experimental conditions, i.e. C_is, including factors, levels and any subject-specific covariates. This matrix is flexible and can be adjusted to include or exclude factors and levels based on the experimental design. ϵ is the error term, accounting for unexplained variance or noise in the data. β is the parameter vector, representing the coefficients associated with each condition in the design matrix. These coefficients can be used to assess main effects, interaction effects, and other contrasts of interest, depending on the specific hypotheses being tested. Similar to FF analysis, the β_s can be obtained by using various fMRI analyzing tools.

For a design with 2-factors, Factors A and B, with two-levels each, the main effect and interaction effects would be the same with the equations (2) - (4). It can be said that F_xF is a special case of FF design. However, in complex experimental designs, the adaptability of F_xF offers enables the specification of different levels of factors for different subjects or conditions, so that it can provide a more precise characterization of brain responses. This method provides the flexibility needed to address diverse research questions and offers enhanced precision in characterizing neural responses under a wide array of conditions.

3.3 Key Differences

The FF and F_xF designs offer distinct advantages and limitations, with adaptability and applicability being key distinguishing features. While analyzing the fMRI data, it should be carefully considered the trade-offs between the factorial structures and adaptation ability of the methods. The choice between these methods should align with the specific research objectives, the complexity of the experimental design, and the level of adaptability required for the fMRI study. Hence, it could be helpful to exhibit the various fundamental differences to decide the most appropriate method as follows:

- Design Structure:
  - FF : Examines all possible combinations of levels from each included factor in a systematic and structured manner.
  - FxF : Allows for adaptability in including or excluding factors and levels based on the specific experimental design, providing greater flexibility.
- Adaptability:
  - FF : Less adaptable to changes in the experimental plan during the study, as it requires predefining all factors and levels.
  - FxF : Well-suited for dynamic experimental designs where factors or levels may need to be adjusted during the study.
- Complexity:
  - FF : Can become impractical or inefficient when dealing with a large number of factors or levels, as the number of conditions increases exponentially.
  - FxF : More manageable in handling complex designs involving a large number of factors, levels, or subject-specific variables.
- Efficiency:
  - FF : May be more efficient for studies with a relatively small number of factors and levels where a structured, systematic exploration is sufficient.
  - FxF : More efficient for studies with dynamic designs or a large number of factors, accommodating changes and specific considerations.
- Main and Interaction Effects:
  - FF : Main and interaction effects are systematically explored for all predefined combinations of factor levels.
  - FxF : Main and interaction effects are assessed based on the conditions included in the flexible design matrix, allowing for targeted exploration.
- Conducting Research:
  - FF : Often employed for hypothesis-driven research where specific combinations of factors are of primary interest.

Düzenlenmemiş Sürüm - Uncorrected Version

- FxF : Suited for both hypothesis-driven and exploratory research, offering adaptability for evolving research questions.

- Interpretability:
  - FF : Results are straightforward to interpret in terms of the predefined factors and levels.
  - FxF : Results may require careful consideration of the included factors and conditions, as they are adapted based on the specific study requirements.

On the other hand, both FF and F_xF can incorporate with subject-specific factors, such as age, gender, heart rate, etc. and both designs can be implemented in fMRI analysis tools, such as SPM for structured and/or adaptable varying experimental conditions and effects.

4 Experimental fMRI Designs for Factorial Analyses

In this section, we provide some fMRI experimental task design examples that can be analyzed with factorial analyses. First, some theoretical designs are presented for gaining the main idea of how the factorial analyses are applied to the fMRI problems. Then, in the second part of the section, the analyses are taken a step further by applying the methods to the real fMRI data.

4.1 Theoretical Scenarios

In this subsection, theoretically possible scenarios at various factors and levels were discussed. These scenarios were examined in terms of main effects and interaction effects. It should be noted that all given theoretical scenarios can also be implemented as real fMRI experiments.

- Scenario I : Visual Perception of Shapes

Suppose that researchers are interested in investigating how the perception of shapes is influenced by two factors: the type of shape (Factor A) and the color of the shape (Factor B). Factor A has two levels: circles and triangles, representing two different types of shapes. Factor B has two levels: red and blue, indicating the color of the shapes.

The study aims to examine the main effects of the type of shape and color as well as their interaction effect on brain activity during shape perception. A full factorial analysis design is ideal for this scenario because it allows researchers to systematically explore the influence of both factors and their interaction on neural responses. For this problem, the full factorial design would look:

Type of Shape (Factor A):	*Color of Shape (Factor B):*
Level 1: Circles (A1)	Level 1: Red (B1)
Level 2: Triangles (A2)	Level 2: Blue (B2)

Participants in the study would be presented with shapes that combine the levels of both factors (e.g, red circles and blue triangles) while undergoing fMRI scans. The data collected would then be analyzed using a full factorial design to investigate the effects of Factor A (type of shape), Factor B (color of shape), and their interaction on brain activity during shape perception. The full factorial design would involve all possible combinations of the levels of these two factors, resulting in four conditions: C1: Red Circle (A1B1), C2: Blue Circle (A1B2), C3: Red Triangle (A2B1), C4: Blue Triangle (A2B2). On the other hand, main and interaction effects would be as follows:

- *Main Effect of Shape Type (A):* Examines how the average neural activity differs between circles and triangles, collapsing across levels of color.
- *Main Effect of Color of the Shape (B):* Assesses how the average neural activity differs between red and blue shapes, collapsing across types of shapes.
- *Interaction Effect (A x B):* Investigates whether the influence of shape type on neural activity depends on the color of the shape, and vice versa.

This design is a classic example of a full factorial analysis because it systematically examines all combinations of the levels of two factors, allowing researchers to assess both main effects and interaction effects on neural responses in a controlled and structured manner. These effects provide insights into how each factor independently influences the neural activity (main effects) and whether there is an interaction, indicating that the combined influence of the factors is different from what would be expected based on their individual effects.

- Scenario II : Cognitive Processing

The experimental setup of this scenario is supposed to be based on investigating cognitive processing with two factors in an fMRI study: task type (Factor A) and difficulty levels (Factor B). Let Factor A has two levels, i.e., memory and attention and Factor B has three levels, i.e., easy, moderate, difficult. For scenario 2, the full factorial design would be as follows:

Type of Task (Factor A):	*Difficulty Level (Factor B):*
Level 1: Memory Encoding (A1)	Level 1: Easy (B1)
Level 2: Attention Control (A2)	Level 2: Moderate (B2)
	Level 3: Hard (B3)

The participants in the fMRI study would perform all task with all possible combinations of both factors, which resulting in six conditions: C1: Memory Encoding - Easy (A1B1), C2: Memory Encoding - Moderate (A1B2), C3: Memory Encoding - Difficult (A1B3), C4: Attentional Control - Easy (A2B1), C5: Attentional Control - Moderate (A2B2), C6: Attentional Control - Difficult (A2B3). For this design, the main effect of the interactions can be determined as

- *Main Effect of Task Type (A):* Examines how the average neural activity differs between memory encoding and attentional control, collapsing across difficulty levels.
- *Main Effect of Difficulty Level (B):* Assesses how the average neural activity differs between easy, moderate, and difficult tasks, collapsing across types of tasks.
- *Interaction Effect (A x B):* Investigates whether the influence of task type on neural activity depends on the difficulty level, and vice versa.

- Scenario III : Working Memory Task in F_xF Design

Consider that an fMRI study aims to investigate the working memory with a focus on task demands and participant age. In this study, how the working memory is influenced would be explored by two factors. Task Demand (Factor A) and Stimulus Type (Factor B). Both Factors A and B have two levels, i.e., low and high, and letters and numbers, respectively. Additionally, researches want to investigate the impact of the subjects' age, so an age group, young and older adults, is included as a subject-specific factor. For such scenario, the factorial design would be as follows:

Task Demand (Factor A)
Level 1: Low Demand (A1)
Level 2: High Demand (A2)

Stimulus Type (Factor B):
Level 1: Letters (B1)
Level 2: Numbers (B2)

Age Group (Subject-Specific Factor): Young Adults (S1) / Older Adults(S2)

Here, low and high demand refer to different levels of cognitive load or difficulty associated with the task. Tasks categorized as low demand typically involve simpler and less mentally taxing cognitive processes. Participants may be asked to remember a small set of easily distinguishable stimuli or perform a straightforward operation on the presented information. On the other hand, tasks categorized as high demand are more complex and mentally challenging. They often require participants to process and manipulate a larger set of stimuli or engage in more intricate cognitive operations. For example, in such task with letters and numbers, participants might be asked to remember a short sequence of letters or numbers in low-demand sequences, whereas they might be asked to perform mental manipulations on a longer sequence of mixed letters and numbers, requiring more cognitive effort to maintain and manipulate the information in working memory in high-demand sequences.

In the analysis of this scenario, the F_xF design allows researchers to adapt the design matrix based on the specific experimental conditions, including or excluding factors and levels. This design could involve 8-conditions like: C1: LowDemand-Letters-Young (A1B1S1), C2: LowDemand-Numbers-Young (A1B2S1), C3: HighDemand-Letters-Young (A2B1S1), C4: HighDemand-Numbers-Young (A2B2S1), C5: LowDemand-Letters-Older (A1B1S2), C6: LowDemand-Numbers-Older (A1B2S2), C7: HighDemand-Letters-Older (A2B1S2), C8: HighDemand-Numbers-Older (A2B2S2). The main effects and interactions can be defined as:

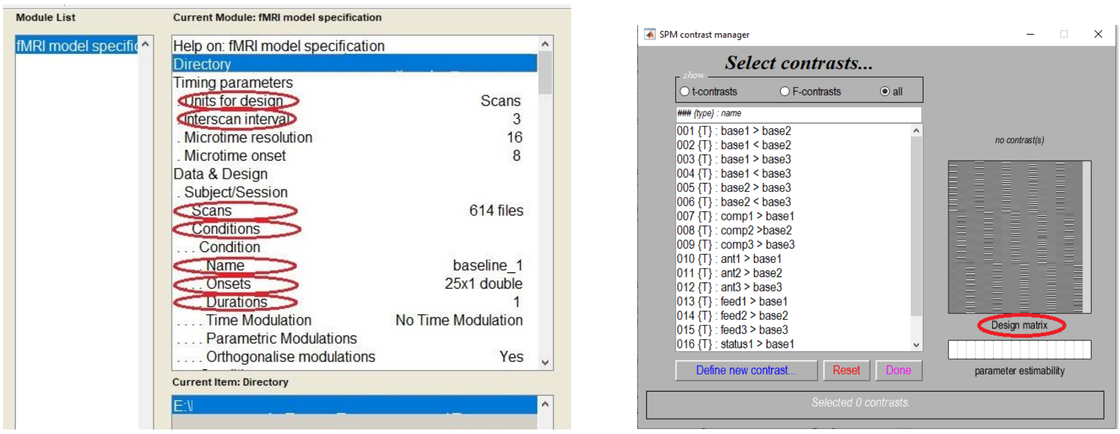
- *Main Effect of Task Demand (A):* Examines how the average neural activity differs between low and high task demand, collapsing across stimulus type and age group.
- *Main Effect of Stimulus Type (B):* Assesses how the average neural activity differs between letters and numbers, collapsing across task demand and age group.
- *Main Effect of Age Group (Subject-Specific Factor):* Explores how the average neural activity differs between young and older adults, collapsing across task demand and stimulus type.
- *Interaction Effect (A x B):* Investigates whether the influence of task demand on neural activity depends on stimulus type, and vice versa.
- *Interaction Effect (A x S):* Examines whether the influence of task demand on neural activity depends on participant age, and vice versa.
- *Interaction Effect (B x S):* Assesses whether the influence of stimulus type on neural activity depends on participant age, and vice versa.
- *Interaction Effect (A x B x S):* Explores the three-way interaction, considering how the influence of task demand on neural activity depends on the interaction between stimulus type and participant age.

This flexible factorial design accommodates subject-specific factors and provides a comprehensive exploration of the effects of different factors and their interactions on neural activity during a working memory task.

4.2 Real fMRI Experiment with SPM Analysis

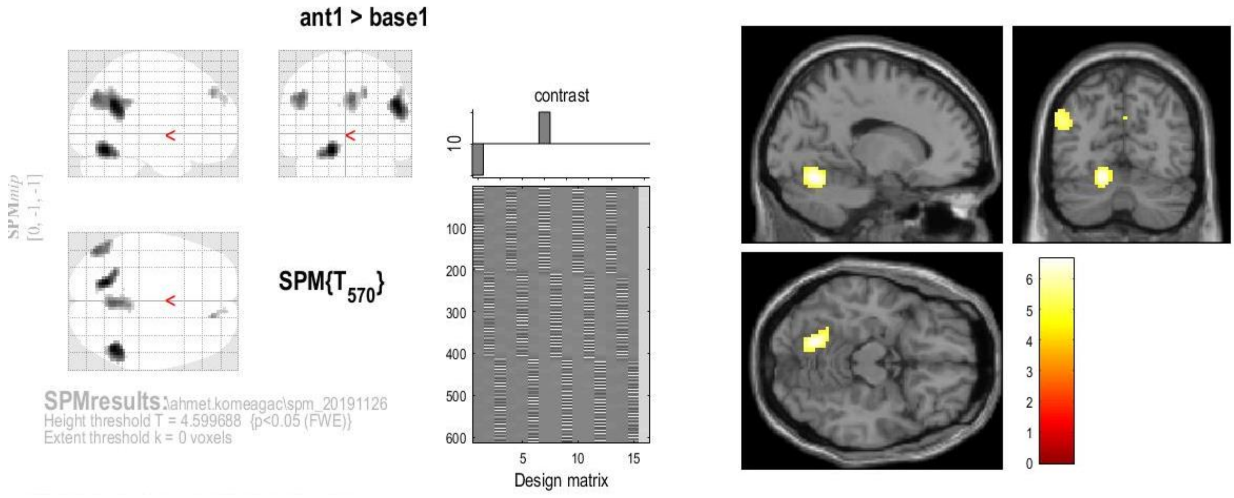
In the context of neuroimaging, factorial analyses are often used to assess the interaction effects between two or more factors as explained in the previous subsection in detail. Thus, researchers can investigate how different experimental conditions or factors influence brain activity in combination. In this subsection, a general guide on how to perform factorial analysis in SPM on a real fMRI experimental task is presented.

SPM is a widely used software package and a general statistical framework for the analysis of brain imaging data [20]. SPM is designed for the analysis of brain images to identify areas of the brain that show significant changes in activity or connectivity in response to the experimental conditions. It is commonly used in the study of cognitive processes, perception, and various neurological and psychiatric disorders. Besides offering a preprocessing pipeline consisting of several steps for raw neuroimaging data, the core of SPM is to involve statistical modeling of brain activity across different experimental conditions or groups. It uses the general linear model (GLM) to analyze the data voxel by voxel [5]. It also employs statistical inference techniques to identify regions of the brain that show significant differences in activity. This often involves t-tests (one and two samples), factorial analysis, and correcting for multiple comparisons to reduce the likelihood of false positives. The results are typically displayed as statistical parametric



(a) (b)

Figure 1. Designing the first-level analysis in SPM (a) fMRI Model Specification (b) Contrast manager



(a) (b)

Figure 2. fMRI first-level analysis results (a)Statistical maps, design matrix and contrast vectors (b) Active regions on canonical brain

maps showing activated or deactivated brain regions in an individual subject and/or subject groups.

Before performing factorial analysis in SPM, researchers should be ensured that their data has been preprocessed appropriately. This typically includes realignment, slice timing, coregistration, normalization, and smoothing steps. After that, the first-level analysis should be performed according to the experimental task for each subject. During the first-level analysis, model specifications, i.e., conditions, onsets (timing of each stimuli), duration, interscan interval (TR) of the task should be defined correctly as shown in Fig. 1a. After it is carefully completed and the model is estimated, researchers can specify contrasts of interest to test hypotheses about the effects of experimental manipulations using the contrast manager window (Fig. 1b). Once the contrasts are determined and applied, the statistical inference can be done with the estimated parameters. This process helps determine which brain regions show activity changes that are unlikely to be due to chance, such as given in Fig. 2a. Furthermore, the active areas could be visualized by overlaying on anatomical images for easier interpretation, given in Fig. 2b.

When these stages in the first-level analysis are completed for all participants, the statistical maps are generated for each subject. These maps will be used for further second-level analysis, which is also known as group analysis. Factorial analyses and t-tests are performed during the second-level analysis, so that the hypothesis can be generalized to the population.

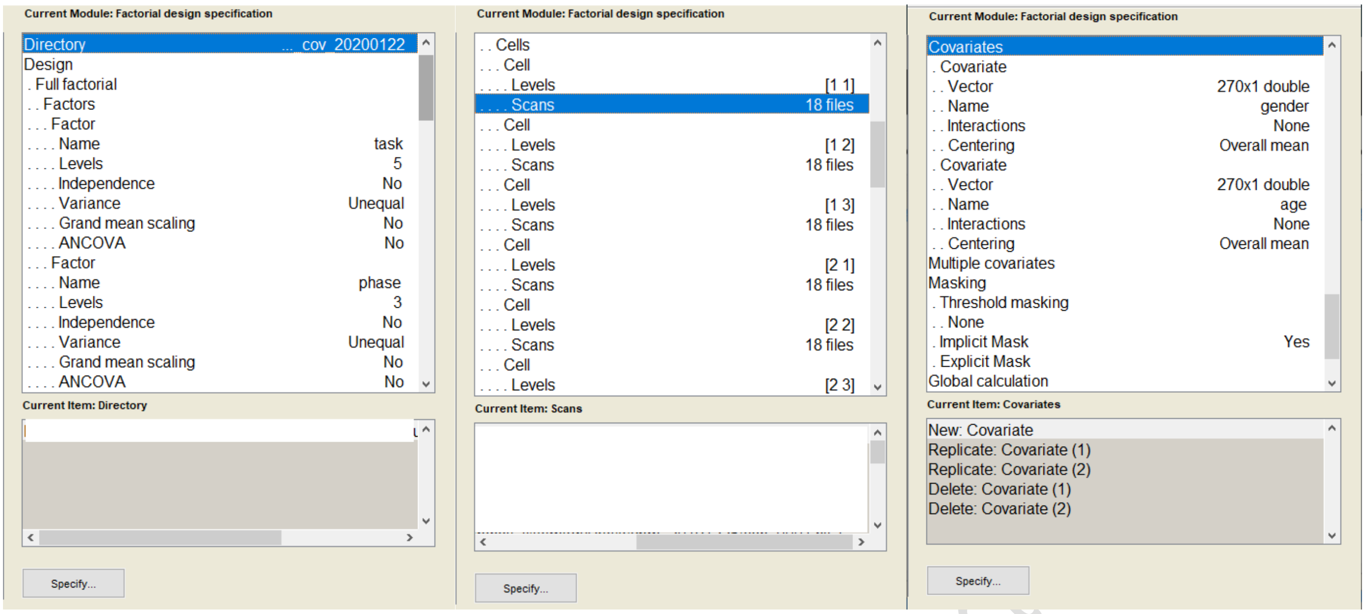
In second-level analysis, the crucial point is to decide the appropriation of the analysis between the FF and F_xF, which are powerful tools for analyzing data in experimental research. While they share some similarities, they also have key differences in their application and flexibility as given in detail before. FF analyzes the effects of all possible combinations of multiple factors on a dependent variable. However, it may be complex and grueling for studies with many factors or levels. Time, resources, or budget may limit the carrying out conducting a FF analysis [18]. Also, since it requires a large amount of data to achieve sufficient statistical power, it may not be suitable for situations where interactions are not of primary interest.

On the other hand, F_xF provides greater flexibility in analyzing data from mixed-effects designs, where some factors have fixed levels (e.g., drug vs. placebo) and others vary across participants (e.g., age, gender). So that, it allows modeling random effects for individual participants alongside fixed effects for experimental factors.

In our previous study, an fMRI protocol was designed and conducted consisting of 5 conditions for consecutive 3 stages [21]. In this fMRI task, we demonstrated a guessing game in which the subjects competing against a rival scored by a jury. The jury was the friends of the subjects, and it was told that the jury would score the subject and his rival on each trial of the game. Indeed, the scoring was prearranged before the fMRI scan and the game was established on 3-phases: high-support phase (HSP), fair phase (FP) and ostracism phase (OP). The subject had significantly more points in 80% of trials in the first third of the game (i.e., in HSP); in the second third, the subjects had more points in 48% of the trials (i.e., in FP). During the last third of the game, subjects received only 20% of the trials (ostracism phase, OP). The participants felt the support of their friends in HSP, while lost their support in OP. The participants explicitly said that they were excluded by their friends from the jury and felt frustrated about the results after the game. The game consisted of 5-conditions in each trial: baseline, computation, anticipation, feedback and status.

In such fMRI task, where Factor A represents the five levels (i.e., baseline, computation, anticipation, feedback, and status) and Factor B has three levels (high, medium, low), the interaction effects and main effects are defined as follows:

- *Main Effect of Factor A (Baseline, Computation, Anticipation, Feedback, Status):* Represents the average effect of changing the levels of Factor A (baseline, computation, anticipation, feedback, status) while ignoring the levels of Factor B (high, medium, low).
- *Main Effect of Factor B (High, Medium, Low):* Represents the average effect of changing the levels of Factor B (high,



(a) (b) (c)

Figure 3. Full Factorial design in SPM (a) Designing the factors (b) Designing the levels and scans (c) Inserting the covariates

medium, low) while ignoring the levels of Factor A (baseline, computation, anticipation, feedback, status).

- *Interaction Effects (A x B):* Since Factor A has five levels and Factor B has three levels, there would be a set of interaction effects corresponding to the combination of each level of Factor A with each level of Factor B. Each interaction effect assesses whether the effect of changing the levels of Factor A depends on the level of Factor B and vice versa.

In total, there would be 5×3=15 conditions in this full factorial design as given in Table 2, and each condition would contribute to the calculation of main and interaction effects. The exact values of these effects would be obtained through statistical analysis, such as ANOVA, to determine their significance.

In the SPM, first the factors and its levels should be defined first as given in Fig. 3a. After that, the cell parameters, i.e., levels and scans, should be set up as shown in Fig. 3b in the second level analysis. Here, the levels refer to the label of the cells given in Table 2.

Table 2. The total conditions in the 5x3 full factorial design with 2-factors A and B.

B (phase) / A (task)	1-HSP (high)	2-FP (medium)	3-OP (low)
1-Baseline	A1B1 con1	A1B2 con2	A1B3 con3
2-Computation	A2B1 con4	A2B2 con5	A2B3 con6
3-Anticipation	A3B1 con7	A3B2 con8	A3B3 con9
4-Feedback	A4B1 con10	A4B2 con11	A4B3 con12
5-Status	A5B1 con13	A5B2 con14	A5B3 con15

It should be noted that scans are defined with the images (con images) for each subject and these images given in Fig. 3b must be already computed in the model specification step during the first level analysis. Furthermore, if it is desired it is possible to add subject-specific covariates such as age and gender into the analysis as shown in Fig. 3c. In this case, the covariates should be included as a $mx1$ vector for each cell, $(nxc)x1$ in total. Here, n denotes the subject number and c denotes the cell number. After that, the effect of each factor is analyzed by the contrast vectors. Correct definition of the contrast vectors is crucial impact on the results. In this FF analysis, the complete list of contrast vectors will be as given in Table 3. Here, Factor A refers to the levels of the fMRI task conditions, whereas Factor B refers to the phases of the game.

However, in most cases the researchers seek for relation in certain conditions, commonly two-factor interactions, in the analysis [18]. Thus, it may not be suitable for situations where interactions are not of primary interest. Moreover, it requires a large amount of data to achieve sufficient statistical power [22].

In this situation, conducting F_xF analysis would be more suitable for the research question, so that researchers can focus on specific aspects of the design space.

In our previous study [21], according to the our research hypothesis, we focused on feedback effect of the fMRI task and seek how the brain activities change along with the different phases of the game. Thus, instead of conducting a full factorial analysis, a flexible factorial analysis is ideal since we have a specific hypothesis about factor effects.

As given in Table 4, the design was built on the factors task (A) and phase (B). The task has two-levels, baseline and feedback, whereas the phase has three-levels, HSP, FP and OP. All participants have been included to the FxF analysis. In FxF design in SPM, the parameters should be defined correctly as follows given in Fig. 4. In the FxF design, since the subjects are not related to each other, their independence should mark as 'yes', on the contrary, since the task and phase factors are

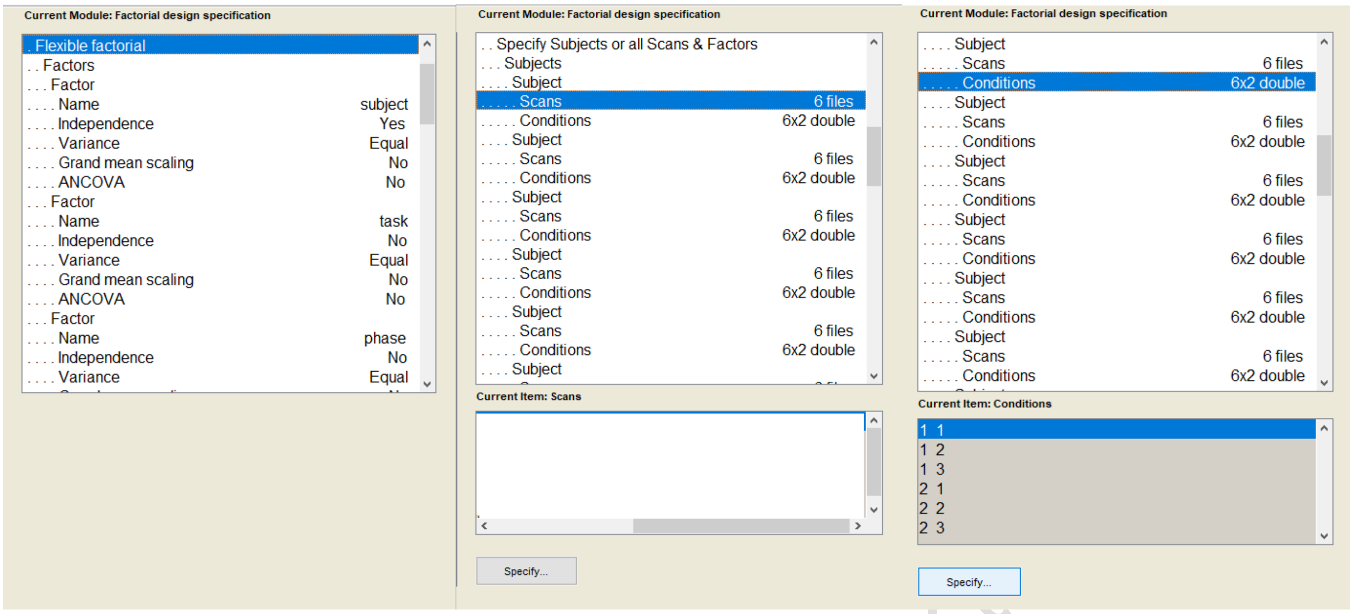


Figure 4. Flexible Factorial design in SPM (a) Designing the factors (b) Specifying the scans and factors (c) Inserting the conditions

Table 3. The total contrast vectors in the $5x3$ full factorial design with factors A and B.

	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3	A4B1	A4B2	A4B3	A5B1	A5B2	A5B3
Average effect of condition	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Main effect of Factor A	1	1	1	-1	-1	-1	0	0	0	0	0	0	0	0	0
	0	0	0	1	1	1	-1	-1	-1	0	0	0	0	0	0
	0	0	0	0	0	0	1	1	1	-1	-1	-1	0	0	0
	0	0	0	0	0	0	0	0	0	1	1	1	-1	-1	-1
Main Effect of Factor B	1	-1	0	1	-1	0	1	-1	0	1	-1	0	1	-1	0
	0	1	-1	0	1	-1	0	1	-1	0	1	-1	0	1	-1
Interaction, Factor A x B	1	-1	0	-1	1	0	0	0	0	0	0	0	0	0	0
	0	1	-1	0	-1	1	0	0	0	0	0	0	0	0	0
	0	0	0	1	-1	0	-1	1	0	0	0	0	0	0	0
	0	0	0	0	1	-1	0	-1	1	0	0	0	0	0	0
	0	0	0	0	0	0	1	-1	0	-1	1	0	0	0	0
	0	0	0	0	0	0	0	1	-1	0	-1	1	0	0	0
	0	0	0	0	0	0	0	0	0	1	-1	0	-1	1	0
	0	0	0	0	0	0	0	0	0	0	1	-1	0	-1	1

continuous and related during the task, they should mark as 'no' as shown in Fig. 4a. In Fig. 4b, the design specification should be defined for each subject. Since we have $2x3$ FxF design, it should be entered related 6 con_* images, which are specified in the design of first level analysis and obtained after it. Here, it should be paid attention that the images must be in the order of the FxF design, like in Table 4. The conditions are defined as mxn matrix, $6x2$ in this case, as shown in Fig. 4c. This is the way of associate the factors and related con_* images. Here, another important point is the scan and condition pairs should be repeated as the number of subjects, 18 in our case. For the final analyses, the complete list of the contrast vectors for the given FxF design would be as follows as given in Table 5.

Table 4. The total conditions in the $2x3$ flexible factorial design with 2-factors A and B

B (phase) / A (task)	1-HSP (high)	2-FP (medium)	3-OP (low)
1-Baseline	A1B1 con1	A1B2 con2	A1B3 con3
2-Feedback	A2B1 con10	A2B2 con11	A2B3 con12

Düzenlenmemiş Sürüm - Uncorrected

Table 5. The total contrast vectors in the $2x3$ flexible factorial design with factors A and B.

	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3
Average effect of condition	1	1	1	1	1	1
Main effect of Factor A	1	1	1	-1	-1	-1
Main Effect of Factor B	1	-1	0	1	-1	0
	0	1	-1	0	1	-1
Interaction, Factor A x B	1	-1	0	-1	1	0
	0	1	-1	0	-1	1

5 Results

The results of F_xF analysis were examined in several ways. First, the result of the main effect of factor A is given in Fig. 5a. The analysis revealed a significant main effect of task on brain activation. The statistical results were obtained with $p < 0.05$ value with family-wise error (FWE)-corrected. The cluster level was thresholded with $k = 20$ voxels. In conjunction with the statistical analyses, brain activation maps were generated to visualize the spatial distribution of effects. Moreover, consistent with the statistical results, these brain maps were visualized by using the single-subject canonical map in SPM. A similar way was followed for the results of main effect of factor B. The statistical maps were obtained with $p < 0.05$ FEW-corrected. The cluster level was chosen as $k = 20$ to eliminate the smaller voxel clusters. The results were visualized with canonical maps to clearly see the activation areas on the brain as given in Fig. 5b.

On the other hand, the F_xF analysis results were comprehensively examined to illuminate the nuanced effects of different experimental conditions. Fig. 6a illustrates the brain activation patterns associated with the interaction effect of factor A x B. This analysis shows the interaction effect, reflecting the interplay between factors A and B on brain activation. The statistical findings reached significance with $p < 0.05$ after FWE correction, and the since the clusters are relatively small, the cluster level was set to $k = 0$ voxels. Visualizing the spatial distribution of effects, brain activation maps were generated and overlaid on the single-subject canonical map, providing insights into the specific regions influenced by this interaction. Similarly, Fig. 6b showcases the outcomes of the interaction effect of factor B1 x B3 (high x low). The statistical maps, acquired again with $p < 0.05$ FWE-correction and a cluster level threshold of $k = 20$, revealed the impact of levels B1 and B3 on brain activation. These results were further elucidated using canonical maps to enhance the clarity of activation areas. The approach mirrors the methodology employed for the main effects, ensuring a comprehensive and visually informative representation of the interaction effects in the context of the experimental design. Since the interpretation of the results in a neuroscientific manner beyond the context of the paper, these results can be reached in our previous study [20].

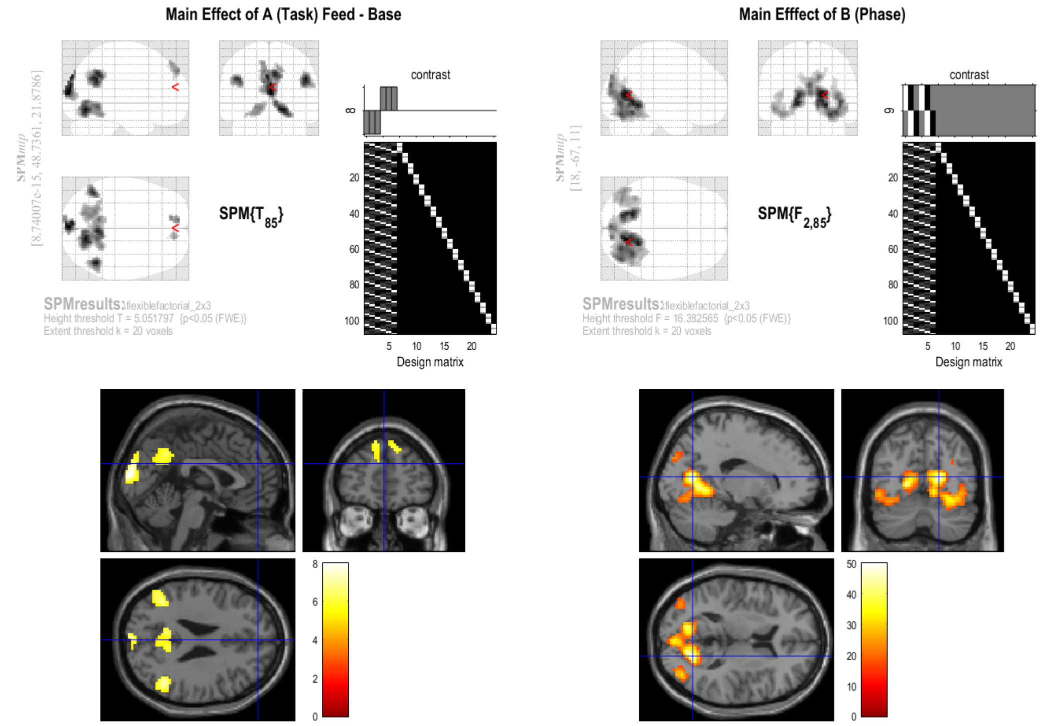
In summary, the choice between FF and F_xF designs should be based on the specific demands of the research questions and the complexity of the experimental design. If the study involves straightforward questions and a limited number of factors with few levels, a full factorial design is typically sufficient. On the other hand, if the research questions are complex, dynamic, or require more adaptability, flexible factorial designs offer the versatility needed to accommodate these demands. It's important to choose the design that best aligns with the study's objectives and the nature of the data.

6 Conclusions

This study effectively utilized both full and flexible factorial analyses to enlighten the complex neural responses under different experimental conditions. First, the designs of full and flexible factorial analyses mentioned widely, and then the key differences and advantages of each analysis were exhibited in comprehensive manner. In the following sections, we delved into the theoretical and practical application of these approaches in fMRI scenarios. In the light of the findings, it can be said that the utilization of full factorial designs allows for a systematic exploration of all possible combinations of factors and levels, providing a comprehensive understanding of main and interaction effects. On the other hand, the flexible factorial design introduces a nuanced approach, allowing for tailored investigations into specific interactions and main effects of interest. The adaptability of the flexible design offers the way of customizing the analysis to address specific research questions, emphasizing precision and efficiency in the exploration of complex experimental designs.

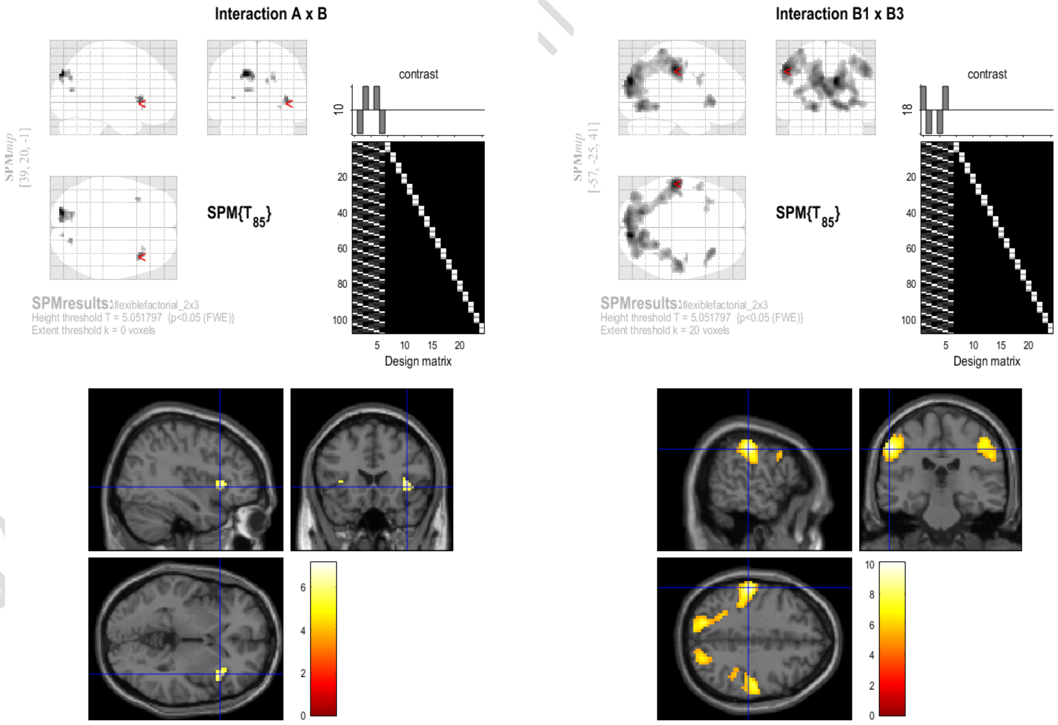
The comparison between the two methodologies shed light on their respective strengths and limitations. While the full factorial analysis offered an exhaustive examination of the entire design space, it may become impractical for studies with a large number of factors and levels. The flexible factorial approach, by contrast, emerged as a powerful tool for optimizing experimental designs, particularly when focused inquiries guide the analysis. In summary, the choice between full factorial and flexible factorial analyses depends on the nature of the study, the complexity of the experimental design, and the need for adaptability in accommodating changing conditions or subject-specific variables.

In conclusion, the integration of full and flexible factorial analyses in fMRI research offers a robust framework for uncovering the complexities of neural responses to experimental manipulations. The use of these methodologies contributes not only to the advancement of neuroimaging methodologies but also to the deeper comprehension of cognitive and perceptual processes. As the research methods become increasingly complex, it is vital to carefully consider whether a full or flexible factorial approach is most appropriate. This will ensure that our analytical methods are well-suited to the intricacies of the cognitive phenomena being studied, ultimately leading to more impactful and robust findings.



(a) (b)

Figure 5. FxF results (a) Main effect of Factor A (task) (b) Main Effect of Factor B (phase)



(a) (b)

Figure 6. FxF interaction results (a) Interaction effect of Factor A x B (b) Interaction Effect of Factor B1 x B3

7 Acknowledgement

The author thanks to all SoCAT research team for data acquisition and Prof. Dr. Ali Saffet Gönül for his encourage and supervision.

8 Author Contribution Statement

The author has contributions on the conceptualization, research, data analyses, and writing the manuscript text.

9 Conflict of Interest

No conflict of interest was declared by the author. No funding was received for conducting this study. There is no need to obtain ethics committee permission for the paper.

10 References

[1] Bandettini PA, "Twenty years of functional MRI: The science and the stories." *NeuroImage*, 62(2), 575–588, 2012. doi: 10.1016/j.neuroimage.2012.04.026.

[2] Logothetis NK, "What we can do and what we cannot do with fMRI." Nature, 453(7197), 869 -878, 2008. doi: 10.1038/nature06976, (2008).

[3] Bandettini PA, Birn RM, Donahue KM, "Functional MRI: Background, methodology, limits, and implementation." *Handbook of psychophysiology*, 2nd ed, New York, US: Cambridge University Press, 978–1014, 2000.

[4] Ogawa S. "Finding the BOLD effect in brain images." *NeuroImage*, 62(2), 608–609, 2012. doi: 10.1016/j.neuroimage.2012.01.091.

[5] Ashburner J, Friston KJ, Penny W. "The General Linear Model." *Human Brain Function*, 2nd ed., Elsevier, 2004.doi: 10.1016/B978-0-12-264841-0.X5000-8

[6] Monti MM, "Statistical Analysis of fMRI Time-Series: A Critical Review of the GLM Approach." *Frontiers in Human Neuroscience*, 5, 2011.

[7] Poldrack RA, Mumford JA, Nichols, TE, *Handbook of Functional MRI Data Analysis*. Cambridge: Cambridge University Press, 2011.

[8] Friston K, Price C. "Modules and Brain Mapping." *Cognitive Neuropsychology*, 28, 41–50, 2011.

[9] Ischebeck A, Hiebel H, Miller J, Höfler M, Gilchrist ID, Körner C. "Target processing in overt serial visual search involves the dorsal attention network: A fixation-based event-related fMRI study." *Neuropsychologia*, 153, 107763, 2021.

[10] Layher E. et al., "Widespread frontoparietal fMRI activity is greatly affected by changes in criterion placement, not discriminability, during recognition memory and visual detection tests." *NeuroImage*, 279, 120307, 2023.

[11] Wang X. et al., "The sex differences in anhedonia in major depressive disorder: A resting-state fMRI study." Journal of Affective Disorders, 340, 555–566, 2023.

[12] Fang Z, Wen H, Zhou Y, Gao X. "Comparisons are Odious? The neural basis of in-group and out-group social comparison among game players: An fMRI study." *Behavioral Brain Research*, 458, 114735, 2024.

[13] Dobbelaar S, Achterberg M, Van Duijvenvoorde ACK, Van IJzendoorn MH, Crone EA. "Developmental patterns and individual differences in responding to social feedback: A longitudinal fMRI study from childhood to adolescence." *Developmental Cognitive Neuroscience*, 62, 101264, 2023.

[14] Mazancieux A, Mauconduit F, Amadon A, Willem de Gee J, Donner TH, Meyniel F. "Brainstem fMRI signaling of surprise across different types of deviant stimuli." *Cell Representation*, 42(11), 113405, 2023.

[15] Torrecuso R. et al., "Improving fMRI in Parkinson's disease by accounting for brain region-specific activity patterns." *NeuroImage Clinical*, 38, 103396, 2023.

[16] Murray R.J, Kreibig SD, Pehrs C, Vuilleumier P, Gross JJ, Samson AC. "Mixed emotions to social situations: An fMRI investigation." *NeuroImage*, 271, 119973, 2023.

[17] Tomasino B. et al., "The mental simulation of state/psychological stimuli in anxiety disorders: A 3T fMRI study." *Journal of Affective Disorders*, 345, 435–442, 2024.

[18] Antony J. "Design of experiments for engineers and scientists" 3rd edition. Amsterdam, Elsevier, 2023. ISBN: 978-0-443-15173-6.

[19] Güneş E, Cihan MT. "COD and Color Removal from Wastewaters: Optimization of Fenton Process," *Pamukkale University Journal of Engineering Science*, 21(6), 239–247, 2015. doi: 10.5505/pajes.2014.37928.

[20] Ashburner J. "SPM: A history." *NeuroImage*, 62(2), 791–800, 2011. doi: 10.1016/j.neuroimage.2011.10.025.

[21] Ozkul B, Candemir C, Oguz K, Eroglu-Koc S, Kizilates-Evin G, Ugurlu O, Erdogan Y, Mull DD, Eker MC, Kitis O, Gonul AS, "Gradual Loss of Social Group Support during Competition Activates Anterior TPJ and Insula but Deactivates Default Mode Network". *Brain Sci.* **2023**, *13*, 1509.

[22] Candemir C, "A Practical Estimation of the Required Sample Size in fMRI Studies" Mugla Journal of Science and Technology, vol. 9, no. 2, pp. 56–63, 2023,

Düzeltilmemiş Sürüm - Uncorrected Version