Multiverse Analysis in Functional Magnetic Resonance Imaging Across Cognitive Tasks

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Functional magnetic resonance imaging (fMRI) is a common method to measure neural activity in the brain in the field of cognitive neuroscience. As fMRI gains popularity in the research field, methodological concerns that of such technology arise, with reproducibility as one of the most recognized issues (Botvinik-Nezer & Wager, 2023; Niso et al., 2022). Unlike behavioral measures of human cognition, cognitive neuroscience techniques, specifically fMRI, require numerous preprocessing steps from raw data before getting to the data analysis stage. Kristanto et al. (2024) reviewed 220 papers that utilize graph-based fMRI and discovered 220 unique analysis pipelines. Sixty-one steps of analysis pipelines were identified, 17 of which has frequently varied parameters, yielding in 102 total probable parameters that may vary. One single research question could lead to various conclusions solely based on the analysis pipeline of a specific research team (Dafflon et al., 2022). Multiverse analysis attempts to answer the question: how do different choices of preprocessing and analysis affect the results of fMRI studies?

The issue of variability in fMRI analysis pipelines has been identified by multiple studies, as choices including software environment (Glatard et al., 2015), segmentation (Palumbo et al., 2019), motion correction (Oakes et al., 2005), and registration (Klein et al., 2009) might contribute to various findings. Most recently, Botvinik-Nezer et al. (2020) effectively and empirically demonstrated the issue. Seventy independent research teams were provided with the same fMRI raw dataset and nine hypotheses to test, as they were instructed to conduct the analysis in a conventional way in their laboratories. Results found that conclusions for the hypothesis test of five out of the nine hypotheses yielded large variation from 21.4% to 37.1%. There is low consistency in the nine hypothesis, as significant finding of one hypothesis was

discovered by 84.3% of the research teams, and the other three non-significant hypothesis reached 94.3% consensus. In fact, across all hypotheses, 20% teams reported a different conclusion from the majority of teams, indicating a level that almost falls midway between finding completely random results and completely consistency (i.e., the maximum possible variability is 50%). Several parameters in the preprocessing and analysis pipeline were discovered to result in significant findings at a higher chance. Size of the smoothing kernel, software package used, and method of multiple correction were all significant factors that could lead to variation in the analysis, as larger smoothing kernel (decreasing signal-to-noise ratio by taking the average value of nearby voxels), difference in software packages, and parametric methods often yield in higher probability of significant outcomes.

Notably, while various studies have indicated the effect of choices in fMRI analysis that tend to lead to specific outcomes (Botvinik-Nezer et al., 2020; Bowring et al., 2022; Li et al., 2021), Kiar et al. (2024) pointed out that there is no single stage in the pipeline that is solely responsible for accounting the differences; rather, the interaction complexity between steps also contributes to the variation. Such suggestion invites a conversation that discusses the higher-order mechanism of inter-pipeline variability. However, at the current stage, researchers' attempts continue to primarily involve parsing out the effect of step-specific differences.

The goal of this project is to investigate the variations of subject-level contrast maps obtained from different pipelines. The methodology of the project is an adaptation of Germani et al. (2025), where they conducted a fMRI analysis and processed the raw data with 24 unique pipelines in a motor task. Importantly, the present project focuses on a decision-making task (i.e., gambling) that represents higher cognitive function as well as how task-related differences might change between-pipeline variation results. A gambling task is a more cognitively abstract, with

more frontal lobe involvement than a motor task. Tasks involving decision making, learning and memory are more common in neuroimaging research. Abstract cognitive processes often lead to more distributed neural representations, specifically in the prefrontal cortex and limbic areas. Regions of interests such as the orbitoprefrontal or ventrofrontal areas usually noisier than the motor cortex. Prefrontal regions have more cortical folding (gyrification) than the primary and secondary motor cortices. Air-tissue boundaries (e.g., nasal cavity) that the prefrontal cortex is adjacent to might create magnetic susceptibility gradient, which degrades fMRI image quality (Yoo et al., 2020) and decreases signal-to-noise ratio. As such, abstract cognitive tasks such as the gambling task might be more susceptible to different results in a multiverse analysis.

This project aimed to answer the questions: How do different fMRI preprocessing and analysis pipelines change the results we obtain? What is the spatial distribution of such pipeline-driven differences? To what extent do such differences vary across cognitive tasks?

Methods

Tasks

The motor task was adapted from Buckner et al. (2011). Participants were presented with visual cues and instructed to tap their left or right fingers, squeeze their left or right toes, or move their tongue. There were 10 movements in a block, with each movement preceding by a 3-second cue and lasting for 12 seconds. There were 13 blocks in a run, with 2 tongue movements, 4 hand movements (2 right and 2 left), and 4 foot movements (2 right and 2 left).

The gambling task was adapted from Delgado et al. (2000). Participants were instructed to guess the number on a card to win or lose money. They were told that the card number ranges from 1-9, and their task is to indicate if the number is less than 5. Participants were immediately

given feedback after each trial as the correct number is shown, which included positive feedback (reward; "$1" with green arrow), negative feedback (loss; "-$0.50 with red arrow), and neutral feedback (number 5). There were two runs of the task. Within each run, there were 2 mostly reward blocks (6 reward trials paired with either 1 loss trial and 1 neutral trial, 2 loss trials, or 2 neutral trials) and 2 mostly loss blocks (6 loss trials paired with either 1 loss trial and 1 neutral trial, 2 reward trials, or 2 neutral trials). Each block was consisted of six trials. Each trial starts with a question mark where participants were able to make a response (1500 ms) and is followed by 1000 ms feedback.

Data and Code Availability

This project analyzes the gambling task of the HCP Young Adult S1200 release (Van Essen et al., 2013). Datalad (Halchenko et al., 2021) was used to obtain the raw imaging data from the Human Connectome Project. While the dataset consisted of 1080 participants, this project included analysis for four subjects in the motor task and three subjects in the gambling task.

The preprocessing and subject-level analysis of the projects were adapted from code published by Germani et al. (2025). The Github repository of the published multiverse analysis paper (https://github.com/elodiegermani/hcp_multi_pipelines) was cloned and the Docker (Merkel, 2014) container that the program runs upon was pulled. The folder structure of the raw fMRI images, as downloaded from the HCP dataset, was altered to match with the expected structure of the existing code. The Python notebooks used in this project are available at: https://github.com/mwzhang851/hcp_multi_pipelines/tree/debug_docker/src.

Project Workflow

The workflow of the project involved three primary steps: preprocessing, subject-level analysis, and between-subjects analysis. Preprocessing and subject-level analysis were run on Nipype (Gorgolewski et al., 2011) using FSL (FMRIB Software Library, RRID: SCR_002823; Jenkinson et al., 2012). All analysis were run on Python notebooks.

The preprocessing step was completed first, where a prewritten python script describing the workflow in FSL was call called and run in a Python notebook. This step involved within-scan volume alignment (aligning volumes between time points to adjust for movement), structural image coregistration (aligning functional images with preprocessed, high-resolution structural image of each participant), and standard space coregistration (aligning each subject's scan to the MNI152NLin6Sym standard space, allowing for between-subject comparisons).

Subject-level analysis involved fitting the neural signal of each voxel into a general linear model with the form: *BOLD ~ task-related convolved signal + motion regressors + derivatives of the hemodynamic response function*. BOLD stands for blood oxygen level dependent, which is an indirect measure of change in neural responses in neuroimaging research. BOLD is used as a proxy for the strength of neural activity in fMRI research, as it is assumed that larger neural activity requires more oxygen in the associated brain area. The regressor of interest was the "task-related convolved signal," which was calculated by convolving the 0 and 1 values that represent task-related signal presentation (e.g., when a stimulus appears) with the hemodynamic response function kernel. The gambling task included five contrasts: reward block, loss block, reward trials, loss trials, and neutral trials. The motor task included five contrasts: movement of left hand, right hand, left foot, right foot, and tongue. All contrasts were computed by *condition minus baseline*. The subject-level analysis model was fit five times for both the motor and

gambling task, one for each contrast. Upon fitting the model, a t-map is constructed using the t-statistics associated with the task-related convolved signal regressor (t_{L1task}) for each contrast. Sixty (12 pipelines * 5 contrasts) t-maps (91 * 109 * 91 voxels, in MNI dimension) were generated for each participant per task.

Lastly, between-subject analysis was carried out, where a mixed-effect linear model is used to fit the data. The model was $t_{L1task} \sim FWHM + motion\ regressors + HRF\ derivative + (1 \mid subject)$. FWHM included two levels (5mm, 8mm), which represented the size of the smoothing kernel during the preprocessing step. Motion regressors had three levels (0, 6, 24), which represented the number of motion regressors included in the subject-level analysis. Zero means no inclusion of any motion regressors, six means the inclusion of head translation and rotation motion regressors in all x, y, z directions, and twenty-four means the inclusion of all the above six motion regressors plus the six squares of each motion regressor, as well as the twelve derivatives of all motion regressors and their squares. HRF derivative had two levels (0, 1), which represented the inclusion of hemodynamic response function derivatives in the subject-level analysis. HRF derivatives captures the lingering effects of the BOLD signal, such as slight time shifts. All three factors were treated as continuous rather than categorical.

The Harvard Oxford atlas (RRID:SCR_001476), was applied to categorize all voxels into 48 regions of interest (ROIs) in a three-dimensional space. In total, there were 48 (ROIs) * 5 (contrasts) = 240 models. FDR correction for multiple comparison (48 comparisons) was then implemented with an alpha level of 0.05. Forty-eight t-statistics were obtained for each pipeline variation factor. To visualize the results, only ROIs with significant corrected p-values were plotted back on the brain, with the color representing the direction of the effect and the shade of the color indicating the value of the t-statistic of the specific pipeline variation factor.

Results

Note that for each pipeline variation factor (FWHM, motion regressors, HRF derivatives), between-subject analysis yielded in five t-maps, one for each contrast. As such, results reported below were overall effects. For instance, if four out of the five maps showed a negative effect of increased FWHM kernel size on the task-relevant t-statistic, it was reported as an overall negative effect.

Effect of Pipeline Variation on Results

There was an overall significant fixed effect for all FWHM, motion regressors, and HRF derivatives on the t-statistics of the task-relevant regressor in the subject-level analysis. FWHM had the largest effect on results, followed by motion regressors and HRF derivatives (Figure 1). There was an overall negative effect of the task-relevant regressor t-statistics in subject-level analysis with increased kernel size and inclusion of HRF derivatives. The direction of such effects for motor regressors differed across tasks (Figure 2).

Figure 1

Effect of FWHM (left) and HRF Derivatives (right) Variation on the Loss Event Contrast of the Gambling Task

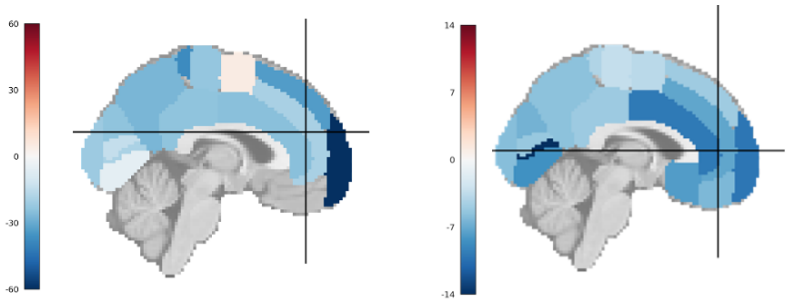
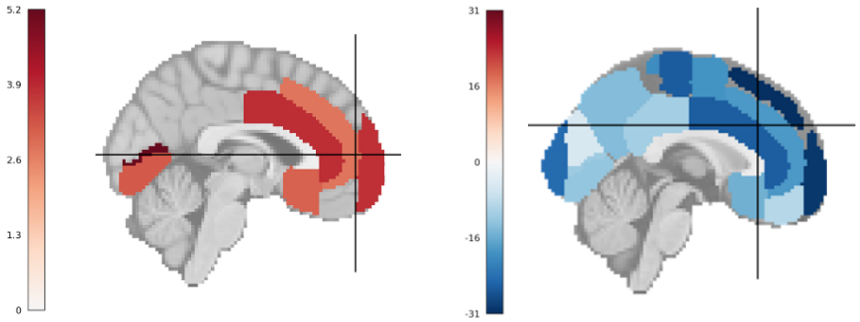


Figure 2

Differential Effects of Motor Regressors Variation on the Gambling Task (Loss Event Contrast; left) and the Motor Task (Right Foot Contrast; right)



Effect Pipeline Variation on Results in Different Regions of Interests

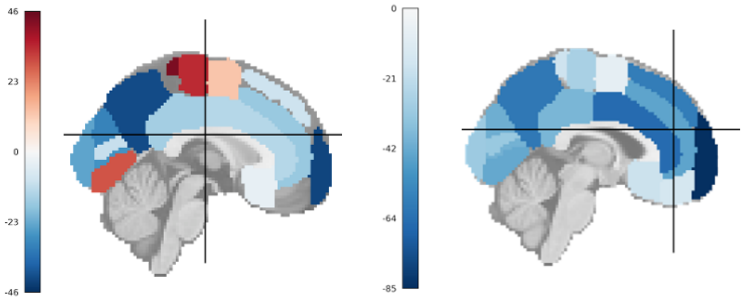
For both the gambling task and motor task, the highest levels of significant between-pipeline variations on results were clustered around the frontal regions. The primary motor cortex also yielded in large effects of between-pipeline variations for some contrasts in the motor task (see right image of Figure 2).

Effect of Pipeline Variation on Results in Different Cognitive Tasks

Motion regressors had an overall larger effect on results of the motor task than the gambling task, despite reaching levels of significance for both tasks. FWHM had an overall larger effect on results of the gambling task, specifically amplified in the frontal areas (Figure 3). While more motion regressors led to overall smaller t-values in the motor task, the direction was reversed in the gambling task (see Figure 1).

Figure 3

Effects of FWHM in the Right Foot Contrast of the Motor Task (left) and the Win Event Contrast of the Gambling Task (right)



Discussion

This project was an attempt of multiverse analysis in fMRI research, specifically investigating how pipeline variations among the smoothing kernel size, the number of motion regressors, and the inclusion of HRF derivations affect results in the motor task and gambling task. All three factors of pipeline variations yielded in significant effects in results, with FWHM variation resulting in the largest effects. Effects of pipeline variations overall were the largest in the frontal areas as well as the primary motor cortex for the motor task. Critically, pipeline variations had differential effects in the motor task and the gambling task. Between-pipeline result differences caused by FWHM size were amplified in the frontal lobe for the gambling task, but motion regressors had a larger impact on results of the motor task. These findings aligned with the hypothesis that tasks involving higher-order cognition (e.g., gambling) might be more susceptible to specific factors of pipeline variations. In this case, effects in the frontal pole of the gambling task were enlarged due to larger kernel sizes (Figure 3), which might be explained by the lower signal-to-noise ratio in the prefrontal cortex and more frontal lobe involvement during the gambling task.

While the effect of between-pipeline variations was significant enough to alter the t-statistics obtained from the subject-level analysis, it is important to recognize that such differences is a result of not only stemming from the variation of single step, but the combinations of various steps and how they interact (Kiar et al, 2024). The goal was not to find one optimal pipeline such that all future analysis would adopt. On the contrary, by examining how pipeline variation might contribute to different results, we might be one step closer to reproducible neuroscience. That is, results that survive pipeline variations are more likely to be those that inform us about the neural representations of cognition.

The immediate next step of this project is to complete analysis on more participants. In the long run, the project of multiverse fMRI analysis has multiple aspects to expand upon. Firstly, analysis of subcortical areas would provide new insights to the findings we obtained, as only the cortical areas of the Harvard Oxford atlas were included. Secondly, this project investigated pipeline variations in a general linear model fMRI analysis. Pipeline variations of multivariate fMRI analysis (e.g., representational similarity analysis, multi-voxel pattern analysis) and functional connectivity differ from the traditional GLM approach, and modern fMRI research is gradually pivoting to methods beyond the GLM. Thirdly, there exists a gap between pipeline variations affect *results* versus *conclusions*. This project only attempted to answer how pipeline differences affect *results*. As the between-subjects analysis used all t-statistics from the subject-level analysis, those that wouldn't yield significant results were also included in analysis.

The ceiling of multiverse analysis is yet to be discovered. Since the groundbreaking paper that formally identified the fMRI pipeline variation issue (Botvinik-Nezer et al., 2020), research teams have extended it to electroencephalography (EEG; Trübutschek et al., 2024). fMRIPrep

(Esteban et al., 2018) is also becoming a prevalent tool for reproducible preprocessing. Eventually, building platforms that provide researchers the opportunity to run multiverse analysis might lead us closer to the goal of achieving reproducible neuroscience.

Ethical Considerations

There are mainly two aspects of ethical consideration involved in the project. Firstly, the project uses open-access fMRI dataset, which contains information about the participants that is publically available to any researcher. Secondly, the project engages with the broader issue of reproducible neuroscience, a domain in which data collection requires substantial financial investment.

Regarding the use of open-access data, concerns about participant privacy have been well-addressed within the neuroimaging community. Structural MRI scans contain identifiable facial features, but modern datasets undergo a "defacing" procedure in which all non-brain anatomical information is removed. In addition, these datasets do not retain any personally identifying information beyond basic demographic variables such as age and gender. The Human Connectome Project specifically acquired informed consent from all participants. In the consent form, it is stated to the participants that a scanning session involves minimal risk, as the technology is non-invasive. Prescreening also excludes participants who indicated they might not be suitable (e.g., claustrophobic) or interested in participating in the study. Participants are also financially compensated for their time and participation.

The topic of reproducible neuroscience has been widely discussed in the field for several decades. Specifically, it is uncommon that replication studies in cognitive neuroscience fail to reproduce similar results from the original study. The paradox of investing more financial and

intellectual input to fund new studies and generating potential unreliable conclusions from these studies is controversial. Several attempts have been made to address the reproducibility crisis. The Open Science Framework is a pre-registration platform that requires researcher to outline their hypothesis, methods, and analysis plan prior to conducting the study, preventing data-driven analysis and post-hoc interpretations. Another common approach in cognitive neuroscience is ensuring pre-processed data and statistical analysis code are publicly available. This project targets open-access neuroscience from steps prior to statistical analysis: fMRI research involves a multi-step preprocessing procedure prior to data analysis, which involve numerous parameters that researchers must choose. By examining the parameters that may lead to different results and conclusions, this project highlights the importance of scrutinizing each step of preprocessing in fMRI research. The preprocessing step is often overlooked in the field, as labs tend to repeatedly adopt established yet lab-specific procedures.

Statement of Gratitude

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