Data-driven identification of functional network changes in Neurofeedback stroke rehabilitation: A clinical validation of network-based statistics

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***Abstract*—Functional connectivity (FC) analysis is crucial for understanding neuroplasticity in stroke rehabilitation. Neurofeedback (NF) training has shown promise in facilitating recovery, but its whole-brain effects remain poorly understood due to limitations in traditional FC analysis methods. Many studies rely on region-of-interest (ROI)-based approaches, which restrict analysis to predefined regions, or whole-brain mass univariate tests, which suffer from the multiple comparisons problem. In this study, we apply Network-Based Statistics (NBS), a graph-theoretic signal processing approach, to identify data-driven FC changes following NF-based stroke rehabilitation. Using fMRI data, we detected two significant network components: one within the somatomotor network, reflecting expected motor recovery processes, and another within the default mode network (DMN), highlighting broader neuroplasticity effects. Our findings validate NBS as a robust tool for unbiased, whole-brain connectivity analysis, offering new insights into the distributed impact of NF training in stroke rehabilitation.**

***Index Terms*—fMRI, Functional Connectivity, stroke rehabilitation, Network Based Statistics.**

I. Introduction

Functional Magnetic Resonance Imaging (fMRI) provides a powerful tool for studying brain function by analyzing patterns of functional connectivity (FC), which measure statistical dependencies between different brain regions. Understanding how FC evolves over time is crucial for assessing neuroplasticity, particularly in stroke rehabilitation, where recovery depends on the brain's ability to reorganize functional networks [1, 2]. Moreover, this process is not always beneficial—maladaptive plasticity can lead to inefficient connectivity patterns that may hinder recovery rather than support it [3]. Identifying both adaptive and maladaptive changes is therefore essential for refining rehabilitation strategies.

In this work, we focus on the study of neurofeedback (NF)-based stroke rehabilitation. NF is a promising intervention that enables patients to self-regulate brain activity, and while it has been shown to drive neuroplasticity [4], its effects on whole-brain connectivity remain poorly understood. Many studies assessing the effect of NF-based neurorehabilitation in stroke rely on region-of-interest (ROI)-based approaches, where specific brain areas are selected based on prior hypotheses about their role in recovery [5, 6]. While this method provides valuable insights, it may overlook broader network-wide plasticity, particularly in regions not explicitly targeted by rehabilitation. Whole-brain FC analysis offers a more comprehensive perspective by capturing connectivity changes across the entire brain. However, analyzing a large number of connections introduces statistical challenges, requiring correction for multiple comparisons, which can reduce sensitivity to meaningful effects—particularly when changes occur in distributed networks rather than in isolated regions.

To address these limitations, we employ Network-Based Statistics (NBS) [7], a graph-based technique that identifies whole-brain subnetworks exhibiting significant connectivity alterations. By clustering interconnected connections, NBS improves sensitivity to distributed connectivity changes while rigorously controlling for multiple comparisons at the network level. Importantly, NBS involves fewer methodological decisions compared to other whole-brain graph metrics, thus enhancing the reliability and interpretability of results. Given that NF-driven neuroplasticity likely induces subtle and distributed connectivity changes extending beyond predefined regions, NBS is ideally suited to provide an unbiased and statistically robust assessment of NF-induced neuroplasticity.

The primary contributions of this study are:

- Validation of NBS in a neurorehabilitation setting, demonstrating its feasibility for studying whole-brain functional reorganization.
- Demonstration that NBS can be effectively applied in exploratory clinical studies without requiring pre-selection of specific connections.
- Identification of two key network components affected by NF training providing insights into rehabilitation-induced neuroplasticity.

II. Related works

Analyzing FC in fMRI presents several challenges, particularly in the context of neurorehabilitation. Stroke induces widespread changes in brain networks [1], and effective rehabilitation strategies depend on our ability to accurately measure and interpret these changes. Various signal processing techniques have been developed for FC analysis, each with its own advantages and limitations. At the core of these approaches lies a trade-off between statistical power and spatial specificity. Ideally, we want to detect all connectivity changes induced by rehabilitation, but given the high dimensionality

of whole-brain FC data, where thousands of connections are analyzed simultaneously—controlling for false positives without missing meaningful effects is a major challenge.

A. ROI-based Approaches: reducing statistical complexity at the cost of flexibility

A widely used solution to mitigate the multiple comparisons problem is to focus on predefined regions of interest (ROIs) or specific connections [8, 9, 10], drastically reducing the number of statistical tests. Many studies in stroke rehabilitation adopt this approach by selecting for example motor-related brain areas [9] based on prior knowledge of stroke recovery mechanisms. While this hypothesis-driven strategy improves statistical power and ensures biological interpretability, it risks overlooking network-wide plasticity, particularly in areas that were not explicitly targeted but still contribute to functional reorganization.

B. Whole-brain edge-wise analysis: the multiple comparisons challenge

To capture connectivity changes beyond predefined ROIs, we could adopt whole-brain FC analysis, evaluating all pairwise connections between brain regions. This allows for a more exploratory, data-driven assessment of neuroplasticity. However, this comes at the cost of an extreme multiple comparisons problem: analyzing thousands of connections requires stringent statistical corrections (e.g., Bonferroni [11], False Discovery Rate (FDR)[12]), which often reduce sensitivity and may lead to false negatives. While this method maintains spatial specificity, its reliance on independent statistical tests fails to account for interdependencies between connections, which are critical in network-based brain function.

C. Global graph metrics: summarizing connectivity at the expense of specificity

Some studies bypass the multiple comparisons issue by summarizing whole-brain connectivity into single global values using graph-theoretic metrics such as modularity, global efficiency, or small-worldness [13, 14]. These approaches provide valuable insights into overall network organization and allow for a more computationally efficient analysis. However, they suffer from a loss of spatial specificity, as they do not identify which connections or subnetworks drive the observed effects, and may oversimplify complex neural dynamics, potentially masking clinically meaningful connectivity alterations. Moreover, variability in methodological choices, such as thresholding and network definitions, further raises concerns regarding their reliability and interpretability [15]. This makes them less suited for studies seeking to localize specific connectivity changes induced by stroke rehabilitation interventions.

D. NBS

To balance statistical power and spatial specificity, NBS [7] has been proposed as an alternative that detects network-level connectivity changes rather than testing individual connections separately. Instead of treating each edge as independent, NBS clusters interconnected edges that show significant FC changes, allowing for the identification of subnetworks rather than isolated connections. By using permutation testing, NBS controls for multiple comparisons at the network level, improving sensitivity to widespread neuroplasticity effects while maintaining statistical rigor. Moreover, NBS requires relatively few methodological decisions, reducing the potential for variability and increasing the robustness and replicability of its findings.

Originally introduced for detecting connectivity disruptions in cognitive and psychiatric disorders, NBS has shown promising results in identifying functionally relevant networks in neuroimaging studies [7, 16]. However, its application in stroke rehabilitation remains limited, despite its potential to uncover both adaptive and maladaptive plasticity.

III. Methodology

A. Dataset

This study is based on data from a randomized controlled trial conducted at Rennes University Hospital [6], designed to evaluate the effects of NF training in stroke rehabilitation. The trial included two groups: one that underwent NF training (n = 15) and a control group that did not (n = 15). In this paper, we focus specifically on the NF group.

Eligible participants were 18 to 80 years old, had a unilateral supratentorial stroke at least six months prior, and had an FMA-UE score between 22 and 53, indicating moderate motor impairment. Additionally, participants were required to have intact corticospinal tract integrity, assessed via fractional anisotropy estimated using Diffusion MRI. Exclusion criteria included MRI contraindications, severe vascular lesions, or prior neurological conditions.

The five-week NF training program consisted of five bimodal EEG-fMRI NF sessions, nine unimodal EEG-NF sessions, and one motor imagery (MI) fMRI session both before and after NF training. During NF training, participants modulated their brain activity in real time, with feedback computed based on activity in the supplementary motor area (SMA) and the primary motor cortex (M1). For this study, we specifically analyze fMRI data from the pre- and post-training MI sessions to assess NF-induced changes in functional connectivity.

B. Data preprocessing

Anatomical preprocessing was performed using fMRIPrep 23.0.0 [17], a widely used pipeline for fMRI data. The T1-weighted image underwent bias field correction with N4BiasFieldCorrection [18] and was then skull-stripped using the ANTs Brain Extraction workflow. Brain tissue segmentation into cerebrospinal fluid (CSF), white matter, and gray matter was performed with FAST (FSL 6.0.5.1) [19], while the brain surface was reconstructed using FreeSurfer 7.3.2 [20]. Spatial normalization was applied using ANTs to align the data to MNI152NLin2009cAsym space [21], with a cost function masking approach to prevent lesion-induced warping [22].

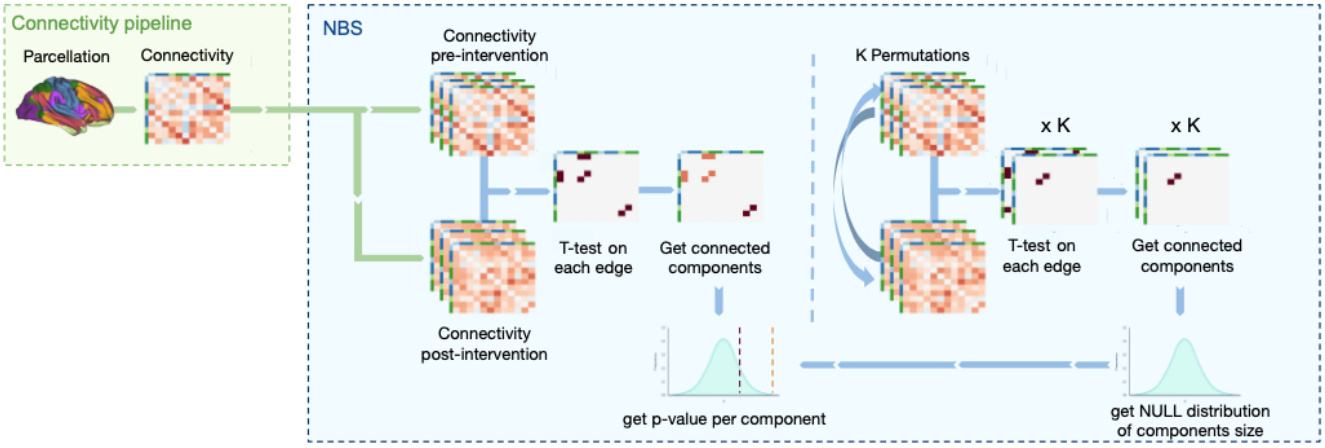

Fig. 1. Methodological pipeline for identifying functional connectivity changes using NBS. In the preprocessing pipeline illustrated in green, the brain is parcellated using the Schaefer atlas, and functional connectivity matrices are computed for both pre- and post-intervention data. A paired t-test is then applied to each connection in the upper triangular part of the symmetric connectivity matrices, generating a test statistic for each edge. An initial threshold of $p \leq 0.001$ is applied, creating a binary adjacency matrix where each connection is assigned 1 (significantly different pre- vs. post-intervention) or 0 (not significant). Connected components are then identified using graph-theoretical algorithms, grouping interconnected suprathreshold edges into subnetworks. To assess statistical significance, K permutations are performed by randomly shuffling subject labels and recomputing the test statistics and component structures. This generates a null distribution of component sizes, against which the observed components are compared. Components with corrected $p \leq 0.05$ are considered statistically significant

Functional MRI preprocessing was conducted using fMRIPrep followed by fMRIStroke, a dedicated pipeline for stroke-related fMRI processing [23]. Motion correction was performed using MCFLIRT (FSL) [24], and slice timing correction was applied using AFNI 3dTshift [25]. The functional images were co-registered to the T1-weighted reference using boundary-based registration (bbregister, FreeSurfer) [26] and then spatially normalized to MNI152NLin2009cAsym space with ANTs.

Denoising was performed using a combination of standard and stroke-specific confound regression. Physiological and motion-related confounds were removed using CompCor [27], with nuisance regressors including global signal, motion parameters, tissue signals (white matter and cerebro spinal fluid), and anatomical CompCor components. Additional stroke-specific confounds were computed with fMRIStroke, incorporating lesion-specific tissue regressors, Independent Component Analysis (ICA)-based artifact removal excluding components overlapping with lesion masks [28], and hemodynamic lag estimation to make sure participants do not have excessive delays exceeding one second in the affected hemisphere [29].

C. Functional connectivity

FC was computed using Nilearn [30]. The Schaefer 400-region atlas was used for brain parcellation, but due to a reduced field of view (FOV) in the fMRI acquisition, only 200 regions were included in the analysis. The regional time series were extracted by averaging the BOLD signal within each parcel. Pairwise Pearson correlation was computed for all region pairs, and Ledoit-Wolf shrinkage [31, 32] was applied to improve covariance estimation. The resulting 200 × 200 functional connectivity matrix represented whole-brain connectivity patterns for each subject.

D. NBS

NBS was used to identify significant changes in functional connectivity pre- and post-rehabilitation while controlling for multiple comparisons (figure 1). A paired two-sided t-test was applied to each connection, with an initial threshold of $p \leq 0.001$ to identify significant connectivity changes. Connected components were then formed, and their significance was assessed using 5,000 permutations, with a threshold of $p \leq 0.05$ for significance. The analysis was performed using the Brain Connectivity Toolbox implementation of NBS [33].

IV. Results and Discussion

A. NBS identified two key networks

NBS identified two significant components showing changes in functional connectivity following NF training. The first component primarily involved 42 regions of interest (ROIs) and 48 connections mainly within the somatomotor network, as defined by the Yeo 7-networks [34] parcellation (Figure 2.A), with a significance level of p = 0.0006. The second component consisted of 18 ROIs and 23 connections, localized within the default mode network (DMN) (Figure 2.B), with a significance level of p = 0.003.

Both components exhibited a decrease in connectivity strength following intervention, indicating a reduction in network interactions post-training. This suggests that NF-based rehabilitation induced widespread modifications in whole-brain functional connectivity, affecting both motor-related and non-motor networks.

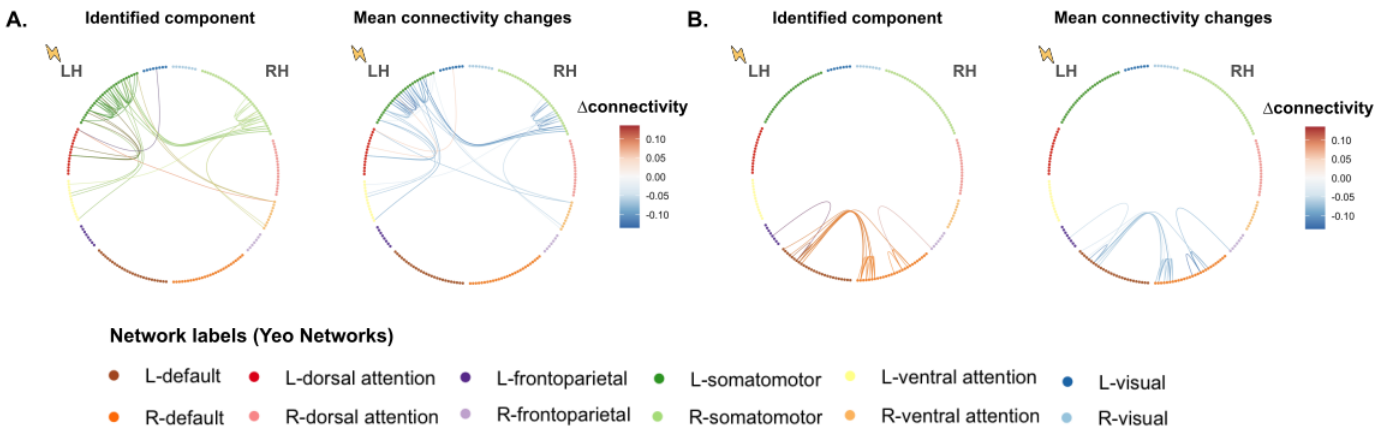

Fig. 2. Significant network components identified using NBS. Lightning bolt represents lesion side, LH: Left Hemisphere, RH: Right Hemisphere (A) First significant component. (B) Second significant component. *Left panels*: Circular connectivity graph displaying the detected subnetwork, with edge colors indicating the corresponding Yeo 7-network affiliation. *Right panels*: Circular connectivity graph of the same subnetwork, where edge colors represent the magnitude of mean connectivity changes (pre- vs post-intervention).

B. NBS identifies functionally relevant networks and meaningful changes

The fact that NBS detected well-established functional networks—the somatomotor network and the DMN—validates its effectiveness in this setting. These networks are commonly recognized in functional neuroimaging, confirming that the identified FC changes are biologically meaningful rather than statistical artifacts.

The reduction in motor network connectivity may reflect a refinement in network efficiency, potentially reducing the compensatory hyperconnectivity often observed in early recovery. Similarly, while the DMN is usually less active during tasks [35], stroke can lead to aberrant activation [36], potentially disrupting cognitive-motor processes. Its post-rehabilitation decrease may therefore indicate a restoration of typical brain dynamics, allowing for more efficient engagement of motor networks during motor imagery. While further work is needed to fully interpret these changes, the alignment of detected networks with established neurophysiological systems reinforces the applicability of NBS for unbiased whole-brain FC analysis.

C. NBS reveals connectivity changes beyond targeted regions

The results show that NF-induced changes extended beyond predefined motor regions, affecting large-scale brain networks. This underscores the need for whole-brain connectivity analysis and the advantages of data-driven approaches over hypothesis-driven methods that may overlook network-wide effects. These findings validate NBS as an effective tool for detecting distributed neuroplasticity in neurorehabilitation.

D. Limitations and perspectives

While this study demonstrates the effectiveness of NBS in identifying neurofeedback-induced changes in stroke rehabilitation, several limitations should be acknowledged. First, sample size is a critical factor in fMRI studies, as neuroplasticity varies significantly across individuals. Although NBS controls for multiple comparisons at the network level, a larger cohort would improve statistical power and allow for more robust generalization of the findings. Future studies should incorporate larger and more diverse stroke populations to account for inter-individual variability in recovery patterns. Second, while NBS detects significant connectivity changes, it does not directly assess their functional relevance. A deeper integration of clinical outcome measures, such as motor function scores or behavioral improvements, would help establish a clearer link between network reorganization and functional recovery. Longitudinal studies tracking both connectivity changes and clinical progress over extended periods could provide more insight into the long-term effects of NF training. Despite these limitations, this study highlights NBS as a valuable tool for unbiased, data-driven detection of functional network reorganization. Expanding on these findings with larger, and control-based studies will further refine our understanding of NF-induced plasticity and optimize its clinical application in stroke rehabilitation.

V. Conclusion

This study applied NBS to analyze whole-brain FC changes following NF training in stroke rehabilitation. NBS identified two significant network components: the somatomotor network, associated with motor execution, and the DMN, involved in higher-order cognitive and motor processes. The observed decrease in connectivity strength suggests a refinement of network interactions, supporting the idea that stroke recovery involves network optimization rather than global connectivity increases.

These findings highlight the value of NBS as a tool for detecting data-driven, network-wide FC changes beyond predefined regions. Unlike traditional ROI-based approaches, NBS enables exploratory whole-brain connectivity analysis while controlling for multiple comparisons at the network level, making it well-suited for studying distributed neuroplasticity.

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