



The effect of adjunctive acupuncture on brain structural and functional connectivity in ischemic stroke patients

by
Pearly Joubert
JBRPEA001

Thesis

In fulfillment of the requirements for the degree:
MSc Med (full dissertation)

HUB6005W
Neuroscience (Physiology)

Supervisor
Jia Fan

Co-supervisors
Frances Robertson and Chunhong Zhang

September 2025

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

Declaration

I, Pearly Joubert, hereby declare that this thesis titled "The effect of adjunctive acupuncture on brain structural and functional connectivity in ischemic stroke patients" is my own work, except where due acknowledgment has been made. This work has not been submitted for any degree or examination at any other university or academic institution.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signed: _____

Signed by candidate

Date: October 12, 2025

Pearly Joubert

Acknowledgements

I would like to express my heartfelt appreciation to my supervisors, Jia Fan and Frances Robertson, for their invaluable support and guidance throughout my research journey. Joining the Data Science Team at the end of 2022, I had no prior experience in analyzing MRI data. However, the supportive and collaborative environment fostered by my supervisors and fellow students made the learning process immensely enjoyable.

I extend special thanks to Frances Robertson for her meticulous attention to detail and for challenging my thinking, which significantly enhanced my critical thinking skills. To Jia Fan, I am profoundly grateful for his encouragement to explore beyond my initial research scope, allowing me to propose a project that incorporated both DWI and rs-fMRI data. His trust in my independence and creative thinking laid a strong foundation for my upcoming PhD studies. Additionally, I deeply appreciate the numerous recommendation letters he wrote, which enabled me to participate in international programs that enriched my skills in scientific communication, mathematics, and multidisciplinary approaches to research. I am also grateful to Prof. Chunhong Zhang for generously sharing the data from China, without which this study would not have been possible.

Beyond technical growth, my experience assisting with patient scanning at the Cape Universities Body Imaging Centre (CUBIC) was invaluable. I am thankful to Mariaan Jafta and Petronella Samuels for teaching me MRI jargon and making the experience engaging. I also owe a great deal to Ndivhuwo Magondo, a former MSc student, for guiding me in writing scripts for tractography in DWI images, and to Soné Fouché, an MSc student, for her collaboration on rs-fMRI and voxelwise brain volume analysis. Their patience in addressing my many questions and fostering my curiosity was truly remarkable. I am grateful for meeting Dr. Marc Combrinck, whose wisdom and humility left a lasting impression.

To the international scientists I corresponded with, I extend my heartfelt thanks. Dr. Paul A. Taylor at the NIH provided detailed and insightful responses to my questions about 3dTrackID, which were immensely helpful. I am also grateful to Professor Jinhui Wang from the IBRR at South China Normal University for generously agreeing to meet virtually to assist with a GRETNA issue I encountered, and to my supervisor, Jia Fan, for facilitating communication by translating to English. Additionally, I thank Dr. Andrew Jahn, a neuroimaging consultant at the University of Michigan, for his prompt and helpful emails answering my questions about the CONN Toolbox.

I am grateful to the National Research Foundation (NRF) and the UCT Postgraduate Funding Office for their financial support for this research project.

I deeply appreciate my family's steadfast support throughout every stage of this journey. Finally, I thank the Genius who created me, Jesus, for granting me the strength to complete this research and for placing inspiring individuals in my life who have challenged, comforted, and loved me. Overall, this has been an amazingly rich experience, and as I close this chapter, I am excited and ready for the next. PhD, here I come!

Abstract

Introduction

Ischemic stroke, which makes up about 85% of all cases, disproportionately impacts individuals in low- and middle-income countries (LMICs), where access to multidisciplinary rehabilitation is limited. Consequently, many survivors turn to complementary therapies such as acupuncture, a traditional Chinese practice with a long history. Although acupuncture shows promise for motor recovery, its underlying neural mechanisms remain unclear.

Methods

This study examined the effects of adjunctive acupuncture on whole-brain structural connectivity (SC) and functional connectivity (FC) in nineteen right-handed male ischemic stroke patients from China. Participants were randomly assigned to True Acupuncture (TA; $n=5$), TA plus Physiotherapy (TA+PT; $n=6$), or Sham Acupuncture plus Physiotherapy (SA+PT; $n=8$). The Xing Nao Kai Qiao protocol (manual and electroacupuncture) was delivered over three weeks, with physiotherapy following the same regimen. Motor recovery was assessed using the Fugl-Meyer Assessment (FMA). Diffusion Weighted Imaging (DWI) and resting-state fMRI data were acquired pre- and post-treatment. For FC analysis, Fisher-Z-transformed correlations were calculated within a GLM framework, and the resulting significant FC findings guided the extraction of fractional anisotropy (FA) values from SC matrices. SC–FC coupling was calculated to evaluate structure–function relationships, and graph theory analyses assessed network-level changes pre- and post-treatment.

Results

In the TA group, FC significantly increased post-treatment compared to pre-treatment ($p < 0.05$), particularly in the thalamus, caudate, hippocampus, and vermis, but decreased in the left SMA coactivated with the rolandic operculum, Heschl's gyrus, and superior temporal gyrus. In the

TA+PT group, FC increased between the insula and cerebellar vermis (lobules I–II), but decreased between the amygdala and bilateral orbital superior frontal gyrus, as well as the left middle temporal pole, post-treatment compared to pre-treatment. The SA+PT group showed the opposite trend, with post-treatment increases in connectivity among the amygdala, prefrontal cortex, fusiform gyrus, and SMA. Between-group post-treatment comparisons revealed that the TA group exhibited a significant increase in functional connectivity (FC) between brain regions associated with motor function compared to the SA+PT group. A similar trend was observed for the TA+PT group relative to the SA+PT group; however, in this case, FC between non-motor brain networks significantly decreased compared to the SA+PT group. Furthermore, the TA+PT group showed a significant reduction in FC between higher-order cognitive brain regions compared to the TA group.

Across groups, FC and SC showed weak, non-significant decoupling post-treatment (TA: $r = -0.15$; TA+PT: $r = -0.07$; SA+PT: $r = -0.11$). FC metrics shifted toward a more random topology post-treatment ($0.74 < \text{Sigma} \leq 0.81$), though not significantly, while SC remained a regular network ($\text{Sigma} < 0.26$) throughout. L_p for FC significantly decreased post-treatment compared to pre-treatment in the SA+PT groups. In contrast, L_p for SC showed a non-significant increase in the SA+PT group. E_{loc} for FC remained largely unchanged post-treatment compared to pre-treatment, whereas SC E_{loc} significantly increased in the TA group. Notably, only FC measures correlated significantly with FMA scores, restricted to the SA+PT group. Although no between-group differences in FMA scores were observed, all groups showed significant within-group improvements post-treatment compared to pre-treatment ($p < 0.001$).

Discussion

Acupuncture combined with physiotherapy may enhance motor recovery by strengthening motor-related networks while suppressing non-motor networks that could hinder recovery. Both SC and FC showed disrupted segregation–integration balance, which acupuncture may help restore to-

ward a more efficient, random FC organization. FC thus holds potential as a biomarker for monitoring rehabilitation. Despite divergent neural patterns, all groups achieved significant FMA improvements, indicating convergent behavioral recovery.

Conclusion

Overall, these findings support integrating acupuncture into neurorehabilitation strategies, particularly in LMICs where access to conventional therapies is limited. However, the small sample size and absence of female participants constrain generalizability.

Keywords

- Adjunctive Acupuncture
- Ischemic Stroke
- Structural Connectivity
- Functional Connectivity
- Structural-Functional Connectivity Coupling
- Graph Theoretical Network Measurements
- Motor Recovery
- FMA

Research Outputs

Conference Presentations

1. Joubert, P., Robertson, F., Combrinck, M., Ma, X., Warton, F., Ntsinde, S., Lu, H., Song, Q., Chen, J., Langerak, N., Ferguson, G., Ren, X., Zhang, C., Meintjes, E., Fan, J. "The Effect of Adjunctive Acupuncture on the Structural and Functional Connectivity Coupling in Ischemic Stroke Patients." Poster presented at the 31st Organization for Human Brain Mapping (OHBM) Annual Meeting, Brisbane, Australia, June 2025.
2. Joubert, P., Fan, J., Robertson, F., Lu, H., Warton, F., Wang, Y., Er, S., Chen, J., Langerak, N., Combrinck, M., Ma, X., Ren, X., Meintjes, E., Zhang, C. (2024). "Effect of Adjunctive Acupuncture on the White Matter Tract Integrity in Ischemic Stroke Patients." Poster presented at the 30th Organization for Human Brain Mapping (OHBM) Annual Meeting, Seoul, Korea, June 2024.
3. Joubert, P., Fan, J., Lu, H., Warton, F., Wang, Y., Er, S., Robertson, F., Combrinck, M., Langerak, N., Ma, X., Ren, X., Meintjes, E., Zhang, C. (2023). "Potential Benefits of Acupuncture Treatment on White Matter Integrity in Ischemic Stroke Patients." Poster presented at the 29th Organization for Human Brain Mapping (OHBM) Annual Meeting, Montreal, Canada, July 2023.
4. Joubert, P., Fan, J., Lu, H., Warton, F., Wang, Y., Er, S., Robertson, F., Combrinck, M., Langerak, N., Ma, X., Ren, X., Meintjes, E., Zhang, C. (2023). "Acupuncture Improves White Matter Integrity Which Might Be Associated with Physical Performance." Poster presented at Deep Learning IndabaX, Cape Town, South Africa, July 2023.
5. Fan, J., Barden, F., Lu, H., Fouché, S., Joubert, P., Wang, Y., Warton, F., Robertson, F., Er, S., Chen, J., Langerak, N., Ren, X., Ma, X., Meintjes, E., Zhang, C., Combrinck, M. (2023). "Potential Benefits of Adjunctive Acupuncture Therapy in Unilateral Stroke Rehabilitation." Invited Oral Presentation at the 16th International Academic Symposium of Acupuncture and Moxibustion, Tianjin, China, November 2023. Oral Presentation.

List of Figures

1	Acupoint locations as interpreted in Traditional Chinese Medicine teaching, illustrating anatomical reference points relevant to both research applications and clinical acupuncture practice. ^[49]	34
2	Flow chart depicting the mechanism of reperfusion injury, outlining three stages, with the third stage comprising two distinct phases. ^[76]	38
3	Diagram illustrating diffusion tensors from various brain microstructures, highlighting their impact on isotropic (p and MD) and anisotropic (q and FA) tensor derivatives. The isotropic component, p, represents the average diffusion within a voxel, similar to Mean Diffusivity (MD), while the anisotropic component, q, reflects directional diffusion, akin to Fractional Anisotropy (FA). Note that the fourth image demonstrates how FA changes when white matter is damaged, such as in ischemic stroke. ^[119]	43
4	Diagram illustrating (a) the hemodynamic processes driving the blood oxygenation level-dependent (BOLD) signal, and (b) the BOLD hemodynamic response function. In part (a), the BOLD signal arises from changes in blood flow and oxygenation. Neural activity triggered by a stimulus increases oxygen demand, causing an initial dip in oxygenated blood as deoxyhemoglobin levels rise briefly. This is followed by an influx of oxygenated blood, reducing deoxyhemoglobin and enhancing the BOLD signal. Part (b) shows the BOLD response function, which captures the temporal dynamics of this signal, reflecting the initial dip and subsequent rise in signal intensity due to the interplay of oxygenated and deoxygenated blood. ^[129]	45
5	Network topologies. From left to right: Regular Network (high clustering coefficient C, long characteristic path length L), Small-World Network (high C, short L), and Random Network (low C, short L), illustrating distinct patterns of connectivity and efficiency in complex systems. ^[133]	50
6	Graph theory metrics used to characterize brain network organization in neuroimaging data. The metrics are divided into two categories: segregation and integration, reflecting distinct aspects of network connectivity and efficiency. Segregation is measured using clustering coefficient, local efficiency and modularity. Integration is measured by strength, and global efficiency. ^[149]	52

- 7 **Locations of the six acupoints (green oval) used in the Xing Nao Kai Qiao needling method.** The acupoints PC6, GV26, SP6, and BL40 were manually stimulated, while electroacupuncture was applied to the GB34 and GB40 acupoints. Figure adapted from. Ma et al.^[110] 60
- 8 **Processing pipeline for functional connectivity analysis using the CONN toolbox for all three groups, before and after treatment.** Groups included: TA (n=5), TA+PT (n=6), and SA+PT (n=8). Resting-state fMRI data were preprocessed with QC to mitigate motion artifacts (e.g., blurring, ghosting). FC matrices captured time-invariant correlations between AAL116 regions, and are exported to GREYNA for graph-theory analysis, and integrated with structural connectivity (SC) data from the same AAL116 atlas parcellation to evaluate SC–FC coupling. 65
- 9 **Overview of Diffusion Tensor Imaging (DTI) processing pipeline across groups, before and after treatment.** The workflow for processing DWI data for all subjects in three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8). In short the pipeline shows DWI, T1, and T2 images were converted from DICOM to NIfTI format, quality control (QC) was performed to identify and address any artifacts in the imaging data, the AAL116 atlas was warped to each subject's native space for region-specific analysis, tractography was conducted to calculate fractional anisotropy (FA) values, mapping white matter tracts. Connectivity matrices were generated based on the tractography results, providing structural connectivity data. The connectivity matrices were used for downstream analyses, including graph theoretical calculations and assessment of coupling strengths, performed consistently across all subjects in the three different groups. 70
- 10 **Significant changes in brain functional connectivity post-treatment in the TA group, depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p-value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed at each node in the image. A color bar indicates the T-statistic values for the color-coding of brain regions. 85
- 11 **Significant changes in brain functional connectivity post-treatment in the TA+PT group, depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p-value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions. 86

12	Significant changes in brain functional connectivity post-treatment in the SA+PT group, depicted in two 3D superior view representations. (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.	87
13	Significantly decreased brain functional connectivity in the SA+PT group compared to the TA group, post-treatment. The 3D superior view illustrates the decreased (blue lines) functional connections between regions of interest (AAL116 atlas). Spheres represent brain regions color-coded according to their T-statistic values. Lines indicate significant pairwise connections (p -value < 0.05). A color bar denotes the T-statistic range for the color-coded nodes. The names of the involved brain regions are listed alongside the figure.	90
14	Significant changes in brain functional connectivity in the TA+PT group compared to the SA+PT group, post-treatment depicted in two 3D superior view representations. (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.	91
15	Significant changes in brain functional connectivity in the TA+PT group compared to the TA group, post-treatment depicted in two 3D superior view representations. (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.	93
16	Structural connectivity adjacency matrices for the TA group ($n=5$). (A) Before treatment, (B) After treatment. The indices in the matrices are color-coded based on FA values between brain regions.	95
17	Structural connectivity adjacency matrices for the TA+PT group ($n=6$). (A) Before treatment, (B) After treatment. The indices in the matrices are color-coded based on FA values between brain regions.	96

18	Structural connectivity adjacency matrices for the SA+PT group (n=8). (A) Before treatment, (B) After treatment. The indices in the matrices are color-coded based on FA values between brain regions.	96
19	Structural-Functional Connectivity Coupling (SC-FC Coupling) across three groups: (a) TA, (b) TA+PT, and (c) SA+PT. Scatter plots illustrate Pearson correlations between fractional anisotropy (FA) values and absolute Fisher Z-transformed values of correlated mean time series from 13 brain regions, assessed before (blue) and after (red) treatment interventions. Lines of best fit are shown, with corresponding r-values (Pearson correlation coefficients) and p-values indicated for each group and time point. A p-value of ≤ 0.05 was considered statistically significant.	98
20	Graph theoretical measures of functional connectivity networks before and after treatment across three groups, using weighted connections between the 116 brain regions where the weights represent the correlated mean time series values and represented by bar plots. Data are shown for three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8), with the keys indicating the specific group. Measures were calculated using the GREYNA Toolbox, and mean values were reported as the area under the curve (AUC). (A) Clustering Coefficient (Cp), (B) Characteristic Path Length (Lp), (C) Sigma, (D) Global Efficiency (Eg), and (E) Local Efficiency (Eloc). Mean $\pm$ SD and SEM are given as error bars. A p-value of ≤ 0.05 was considered statistically significant and is indicated with an asterisk (*), after adjusting for motion. Note: Significant baseline differences are not shown.	100
21	Graph theoretical measures of structural connectivity networks before and after treatment across three groups, using weighted connections between the 116 brain regions where the weights represent fractional anisotropy (FA) values and represented by bar plots. Data are shown for three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8), with the keys indicating the specific group. Measures were calculated using the GREYNA Toolbox, and mean values were reported as the area under the curve (AUC). (A) Clustering Coefficient (Cp), (B) Characteristic Path Length (Lp), (C) Sigma, (D) Global Efficiency (Eg), and (E) Local Efficiency (Eloc). Mean $\pm$ SD and SEM are given as error bars. A p-value of ≤ 0.05 was considered statistically significant and is indicated with an asterisk (*). "(+)" indicates significance before adjusting for motion (but not after). Note: Significant baseline differences are not shown.	106
22	Functional connectivity graph measure, Cp, correlated with FMA. Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the Clustering Coefficient (Cp), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant	114

- 23 **Functional connectivity graph measure, Lp, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the Characteristic path length (Lp), a graph theoretical measure of network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant 115
- 24 **Functional connectivity graph measure, Sigma, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Sigma, a graph theoretical measure of small-worldness, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant 116
- 25 **Functional connectivity graph measure, Eg, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Global efficiency (Eg), a graph theoretical measure of local network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant. 117
- 26 **Functional connectivity graph measure, Eloc, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Local Efficiency (Eloc), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant. 118
- 27 **Structural connectivity graph measure, Cp correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the Clustering Coefficient (Cp), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant. 119

28	Structural connectivity graph measure, Lp correlated with FMA. Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the characteristic path length (Lp), a graph theoretical measure of network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant.	120
29	Structural connectivity graph measure, Sigma, correlated with FMA. Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Sigma, a graph theoretical measure of small-worldness, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant.	121
30	Structural connectivity graph measure, Eg, correlated with FMA. Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Global efficiency (Eg), a graph theoretical measure of network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant	122
31	Structural connectivity graph measure, Eloc, correlated with FMA. Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and local efficiency (Eloc), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant.	123
32	Line graphs illustrating the mean Fugl-Meyer Assessment (FMA) scores for three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8), pre-treatment and post-treatment. Conditions are distinguished by a color-coded key. Each bar represents the group mean FMA score, with error bars indicating the standard error of the mean (SEM). A p-value ≤ 0.05 is considered statistically significant.	125
33	Basic information for set-up in the CONN Toolbox.	178

34	3D surface projections of the AAL116 atlas in CONN toolbox using the random activation colormap. (A) Anterior, (B) Superior, (C) Left, and (D) Right views showing the spatial distribution of 116 brain regions. The color bar indicates region-specific colors, with names listed in Appendix A (Table 22) in the same order as the table and color scheme (starting from region 1). "L" indicates the left hemisphere, and "R" indicates the right hemisphere.	181
35	Montage of structural scans (T1) for all 19 subjects before and after treatment	182
36	Montage of rs-fMRI scans for all 19 subjects before and after treatment	182
37	Inspection of T1 registration to MNI space.	183
38	Making sure the rs-fMRI data loaded correctly.	184
39	Inspection of rs-fMRI to T1 space	185
40	Inspection of rs-fMRI to MNI space	186
41	Inspection of anatomical overlay	187
42	Inspection of MNI overlay	187
43	CONN toolbox set-up for condition 1 (MRI data before treatment intervention).	187
44	CONN toolbox set-up for condition 2 (MRI data after treatment intervention).	188
45	First-level covariates	188
46	Quality Control (QC) pipeline used in the CONN toolbox. ^[218]	189
47	Quality assurance plot of functional connectivity distribution across sessions. This figure displays the distribution of functional connectivity values (r) for all subjects in Session 1 (before) and Session 2 (after), across all treatment groups. The plot compares the distribution before and after denoising. Post-denoising, the functional connectivity values exhibit a closer alignment to a Gaussian distribution, indicating improved data quality.	190
48	Motion estimates in subject 5 of the TA group post-treatment.	193
49	Visualization of the diffusion data after basic preprocessing to spot intensity outliers, signal dropouts, motion artifacts, or slices with unusually high/low intensity.	195
50	QC overlay of FA (fractional anisotropy) and MD (mean diffusivity) maps after running fat_proc_dwi_to_dt command to check that tensor-derived maps look anatomically plausible.	196
51	A snippet of the SC_FA_Winsor input file used above for the SC-FC Coupling analysis.	213

52	A snippet of the FC_Zvalues_Winsor input file used above for the SC-FC Coupling analysis.	214
53	Pre-treatment sigma graph measure that violated the assumption of ANCOVA: Homogeneity of slopes	226
54	Pre-treatment linearity check for Sigma.	226
55	Post-treatment Lp that violated the Homogeneity of Slopes assumption of ANCOVA.	227
56	Post-treatment Eg that violated the Homogeneity of Slopes assumption of ANCOVA.	228
57	Post-treatment Sigma that violated the normality assumption of ANCOVA.	229
58	Pre-treatment Lp that violated the homogeneity of slopes assumption of ANCOVA.	230
59	Pre-treatment Lp that violated the linearity assumption of ANCOVA.	230

List of Tables

1	Treatment design for ischemic stroke patients. Treatment regimen for patients with moderate basal ganglia infarcts and unilateral limb dysfunction following recruitment. The regimen was administered over 4 weeks, starting at Week 0 and continuing through Week 3. <i>TA=True Acupuncture, SA=Sham Acupuncture, PT=Physiotherapy, PE=Physical Examination, MRI=Magnetic Resonance Imaging</i>	61
2	Experimental design for first-level functional connectivity analysis in the CONN toolbox. Pre-post setup with Session 1 (before) and Session 2 (after) data across three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8).	66
3	Structural and functional demographic data across ischemic stroke study groups. This table presents between-group and within-group differences for structural and functional data across three groups: TA (n=5), TA+PT (n=6), SA+PT (n=8). It includes variables such as brain volume, age, Fugl-Meyer Assessment (FMA), and motion, with mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group. Statistical metrics include p-values, Eta-squared (η^2), F-statistics for between-group comparisons, and absolute Cohen's d and T-statistics for within-group comparisons (before vs. after treatment). Note: Framewise displacement is a motion variable for rs-fMRI, and the Euclidean norm, a motion variable for DWI data. A p-value of ≤ 0.05 , was considered statistically significant.	81
4	Group comparisons of hemispheric infarcts. This table presents comparisons between groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8) based on the number of subjects with left and right hemispheric infarcts. The Fisher's Exact test was used for each comparison (TA vs TA+PT, TA vs SA+PT, TA+PT vs SA+PT). The table includes the count of subjects with left and right infarcts, along with statistical metrics: p-value, Odds Ratio, and 95% Confidence Interval. A p-value of ≤ 0.05 was considered statistically significant.	83

5	Significantly increased and decreased functional connections between brain regions of interest (ROIs) were identified using a paired t-test on Fisher-transformed bivariate correlation coefficients derived from a general linear model (GLM), post-treatment compared to pre-treatment in the TA Group. The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p-uncorrected (p-unadj) and p-FDR (False Discovery Rate-corrected) values. A p-value < 0.05 was considered statistically significant.	85
6	Significantly increased and decreased functional connections between brain regions of interest (ROIs) were identified using a paired t-test on Fisher-transformed bivariate correlation coefficients derived from a general linear model (GLM), post-treatment compared to pre-treatment in the TA+PT Group. The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p-uncorrected (p-unadj) and p-FDR (False Discovery Rate-corrected) values. A p-value < 0.05 was considered statistically significant.	86
7	Significantly increased and decreased functional connections between brain regions of interest (ROIs) were identified using a paired t-test on Fisher-transformed bivariate correlation coefficients derived from a general linear model (GLM), post-treatment compared to pre-treatment in the SA+PT Group. The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p-uncorrected (p-unadj) and p-FDR (False Discovery Rate-corrected) values. A p-value < 0.05 was considered statistically significant.	88
8	Significantly decreased functional connections between the SA+PT and TA groups, post-treatment, identified using a two-sample t-test on Fisher-transformed correlation coefficients derived from a GLM. The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p-uncorrected (p-unadj) and p-FDR (False Discovery Rate-corrected) values. A p-value < 0.05 was considered statistically significant.	90
9	Significantly increased and decreased functional connections in the TA+PT group compared to the SA+PT group, post-treatment, identified using two-sample t-test on Fisher-transformed correlation coefficients derived from a general linear model (GLM). The table lists the brain region pairs (AAL116 atlas), their corresponding T-statistics, uncorrected (p-unc), and FDR-corrected (p-FDR) p-values. A p-value < 0.05 was considered statistically significant.	92
10	Significantly increased and decreased functional connections in the TA+PT group compared to the TA group, post-treatment, identified using two-sample t-tests on Fisher-transformed correlation coefficients derived from a general linear model (GLM). The table lists the brain region pairs (AAL116 atlas), their corresponding T-statistics, uncorrected (p-unc), and FDR-corrected (p-FDR) p-values. A p-value < 0.05 was considered statistically significant. . . .	94

11	Within-group analysis of the functional connectivity graph measures. Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.	101
12	Pre-treatment between-group analysis for functional connectivity graph measures. Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.	102
13	Post-treatment, between-group analysis for functional connectivity graph measures. Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.	102
14	Pre-treatment, pairwise comparisons for Lp functional graph measure for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). The Adj. mean order corresponds to the order in the comparison column. T-statistic with degrees of freedom, p-unc (p-value before correcting for multiple comparisons), p-Sidak (p-value after correcting for multiple comparisons), and the effect sizes: absolute Cohen's d and Hedge's g are given. A p-value of ≤ 0.05 was considered statistically significant.	103
15	Within-group analysis of the structural connectivity graph measures for the TA (n=5), TA+PT (n=6) and SA+PT (n=8) groups. Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.	107
16	Pre-treatment, between-group analysis for structural connectivity graph measures. Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.	108

17	Post-treatment, between-group analysis for structural connectivity graph measures. Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.	108
18	Pre-treatment pairwise comparisons for Cp, Lp, Eg, and Eloc structural graph measures across groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8). The Adj. mean order corresponds to the order in the comparison column. T-statistic with degrees of freedom, p-unc (p-value before correcting for multiple comparisons), p-Sidak (p-value after correcting for multiple comparisons), and the effect sizes: absolute Cohen's d and Hedge's g are given. A p-value of ≤ 0.05 was considered statistically significant.	109
19	Post-treatment pairwise comparisons for the Lp structural graph measure for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). The Adj. mean order corresponds to the order in the comparison column. T-statistic with degrees of freedom, p-unc (p-value before correcting for multiple comparisons), p-Sidak (p-value after correcting for multiple comparisons), and the effect sizes: absolute Cohen's d and Hedge's g are given. A p-value of ≤ 0.05 was considered statistically significant.	109
20	Pearson correlations between FMA and functional graph measures. Shown are correlation coefficients (r), p-values, and 95% confidence intervals (CI) for each group: TA (n=10), TA+PT (n=12), and SA+PT (n=16), with the pre-treatment and post-treatment data pooled together. A p-value of ≤ 0.05 was considered statistically significant.	112
21	Pearson correlations between FMA and structural graph measures. Shown are correlation coefficients (r), p-values, and 95% confidence intervals (CI) for each group: TA (n=10), TA+PT (n=12), and SA+PT (n=16), with the pre-treatment and post-treatment data pooled together. A p-value of ≤ 0.05 was considered statistically significant.	113
22	Within-group analysis of the FMA scores in the 3 groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8). The mean $\pm$ SD and SEM are given. The p-value, effect size, absolute (Cohen's d), and T-statistic are shown. A p-value ≤ 0.05 was considered statistically significant.	124
23	Within-group analysis of the FMA scores after winsorization in the 3 groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8). The mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) are given. The p-value, effect size, absolute (Cohen's d), and T-statistic are shown. A p-value ≤ 0.05 was considered statistically significant.	124
24	Between-group analysis of the FMA scores in the 3 groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8). The mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) are given. The p-value, effect size (Eta-squared, η^2), and F-statistic are shown. A p-value ≤ 0.05 was considered statistically significant.	125

25	AAL116 brain regions and their coordinates. This table lists the 116 brain regions defined by the Automated Anatomical Labeling (AAL116) atlas, used for structural and functional connectivity analysis. Columns include: (1) Region ID , the abbreviated identifier for the brain region; (2) Region Name , the full anatomical name; (3) X , (4) Y , and (5) Z , the MNI coordinates of the region's center of mass. Data are ordered as per the AAL116 atlas specification and are used for mapping structural and functional connectivity measures, such as Fractional Anisotropy (FA), in neuroimaging studies.	178
26	Hypotheses and statistical tests for between-subject effects in functional connectivity analysis using the CONN toolbox. The analysis evaluates differences across conditions (before vs. after) for each treatment group: TA (n=5), TA+PT (n=6), SA+PT (n=8). Each hypothesis is represented by contrast vectors, where 1s indicate the variables of interest being tested, 0s denote covariates adjusted for (e.g., age, motion), and negative signs (e.g., -1) specify the direction of the comparison.	191
27	Side of ischemic infarct. This table presents data for subjects across three groups: TA (n=5), TA+PT (n=6), SA+PT (n=8), including group assignment, subject ID, hemispheric infarct side, and a variable value. The groups represent different treatment or assessment conditions, with the number of subjects indicated (e.g., TA, n=5). The hemispheric infarct side indicates the affected brain hemisphere, and the variable column represents a coded value (0 = Unknown, 1 = Right, 2 = Left).	203

Nomenclature

AAL116	Automated Anatomical Labeling 116 (refers to the AAL atlas with 116 brain regions)
ACC	Anterior Cingulate Cortex
ACPC	Anterior Commissure-Posterior Commissure
AFNI	Analysis of Functional NeuroImages
AMOBES	Active Mobility Very Early After Stroke
AP	Anterior Posterior
BBB	Blood-Brain Barrier
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BOLD	Blood-Oxygen-Level-Dependent
CEN	Central Executive Network
CNS	Central Nervous System
CompCor	Component-based Noise Correction
CSF	Cerebrospinal Fluid
CST	Corticospinal Tract
DALYs	Disability-Adjusted Life Years
DICOM	Digital Imaging and Communications in Medicine
DMN	Default Mode Network
DTI	Diffusion Tensor Imaging
DWI	Diffusion-Weighted Imaging
EA	Electroacupuncture
ECS	Endocannabinoid System
FA	Fractional Anisotropy
FC	Functional Connectivity
FMA	Fugl-Meyer Assessment
FSL	FMRIB Software Library
FWHM	Full Width at Half Maximum

GBD	Global Burden of Disease
GLM	General Linear Model
GRETNA	Graph-theoretical Network Analysis Toolkit
LMIC	Low- and Middle-Income Countries
LPNN	Limbic-Paralimbic Neural Network
LTP	Long-Term Potentiation
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MoCA	Montreal Cognitive Assessment
MPRAGE	Magnetization-Prepared Rapid Gradient Echo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NGF	Nerve Growth Factor
NIFTI	Neuroimaging Informatics Technology Initiative
NIHSS	National Institutes of Health Stroke Scale
NMDA	N-Methyl-D-Aspartate
Nogo-A	Neuron Growth Inhibitor-A
OHBM	Organization for Human Brain Mapping
PA	Posterior Anterior
PM	Premotor Cortex
PSD-95	Postsynaptic Density Protein 95
ReHo	Regional Homogeneity
RNA	Ribonucleic Acid
ROI	Region of Interest
rs-fMRI	resting-state Functional Magnetic Resonance Imaging
SFG	Superior Frontal Gyrus
SMA	Supplementary Motor Area
SN	Salience Network
SPM	Statistical Parametric Mapping
TCM	Traditional Chinese Medicine

TORTIOSE	Tolerably Obsessive Registration and Tensor Optimization Indolent Software Ensemble
VECTOR	Very Early Constraint-Induced Movement during Stroke Rehabilitation
WHO	World Health organization
WM	White Matter
XNKQ	Xing Nao Kai Qiao (a TCM acupuncture technique for stroke recovery)

Conventions and Terminology used in this Thesis

In this thesis, the term **structural connections** refers to the physical anatomical links between brain regions, as derived from diffusion-based tractography. By contrast, **functional connections** denote statistical associations between regional mean time series obtained from resting-state fMRI; these correlations reflect temporal synchrony of activity rather than direct physical pathways.

Additionally, a note on nomenclature: when referring to brain regions by their labels in the AAL116 atlas, the spelling “Cerebelum” (with a single “l”) was retained to reflect the atlas notation. However, when discussing the neuroanatomy in general text, the conventional spelling “cerebellum” (with a double “l”) was used.

Contents

1	Introduction	29
1.1	Stroke: A Cause of Global Mortality	29
1.2	Perspective from Low-and-Middle-Income Countries (LMICs)	30
2	Acupuncture	31
2.1	Stroke Rehabilitation in China	31
2.2	Acupuncture: An Overview	31
2.2.1	True and Sham Acupuncture	32
2.2.2	Acupoints	33
2.2.3	Xing Nao Kai Qiao	34
2.2.4	Adjunctive Acupuncture	35
2.2.5	Timepoint of Intervention	36
2.2.6	Blood Brain Barrier	37
2.2.7	Duration of Intervention	37
2.2.8	Acupuncture Modality	38
2.2.9	Acupuncture and Physiological Changes	39
3	Neuroplasticity and Structural Changes	40
3.1	White Matter	40
3.2	Ischemic Core versus Penumbra	41
3.3	DTI and Tractography	42
3.3.1	Tractography	43
4	Neuroplasticity and Functional Changes	44
4.1	Functional Magnetic Resonance Imaging (fMRI)	44
5	Graph Theory	48
5.1	Classification of Networks	49
5.2	Topology	49
5.2.1	Small-world Network	50
5.2.2	Network Topology in Stroke Patients	50
5.3	Graph-Theory-Based Measures	51
5.3.1	Effect of Acupuncture on Network Topology	53
5.4	Structural and Functional Connectivity	53
5.4.1	Structural Connectivity	53
5.4.2	Functional Connectivity	54
5.4.3	Structural-Functional Connectivity Coupling	54
6	Problem statement	56
6.1	Hypotheses	56
6.2	Aim and Objectives	56
6.2.1	Aim	56
6.2.2	Objectives	57
7	Methodology	58

7.1 Study-cohort	58
7.1.1 Inclusion Criteria	58
7.1.2 Exclusion Criteria	58
7.2 Treatment Intervention: Adjunctive Acupuncture	59
7.3 Study Design	60
7.4 Physical Examination	62
7.4.1 Fugl-Meyer Assessment (FMA)	62
7.5 Image and Data Acquisition	63
7.6 Functional Connectivity	63
7.6.1 Neuroimage Pre-processing: rs-fMRI data	63
7.7 Structural Connectivity	67
7.7.1 Neuroimage Pre-processing: DWI data	67
7.7.2 Tractography	69
7.8 Graph-Theory-Based Analysis for Structural and Functional Networks	70
7.9 Statistical analysis	72
7.9.1 Demographics	72
7.9.2 Functional Connectivity Analysis	72
7.9.3 Structural Connectivity Analysis	73
7.9.4 Structural-Functional Connectivity Coupling	74
7.9.5 Graph Theoretical Measurements	75
7.9.6 Correcting for Multiple Comparisons	77
7.9.7 Pearson Correlations of FMA with Graph Measures	77
8 Results	79
8.1 Functional Connectivity	83
8.1.1 Within-group differences	83
8.1.2 Between-group differences	88
8.2 Structural Connectivity	94
8.3 Structural-Functional Connectivity Coupling	96
8.4 Functional Connectivity Graph Theoretical Measurements	98
8.5 Structural Connectivity Graph Theoretical Measurements	103
8.6 Correlations of Structural and Functional Graph Measures with FMA	110
9 Discussion	126
9.1 Functional Connectivity: Within-Group Differences	126
9.1.1 True Acupuncture (TA): Increased Functional Connections	127
9.1.2 True Acupuncture (TA): Decreased Functional Connections	128
9.1.3 True Acupuncture (TA) Combined with Physiotherapy	130
9.1.4 Sham Acupuncture (SA) Combined with Physiotherapy	132
9.1.5 Physiotherapy	134
9.2 Functional Connectivity: Between-Group Differences	135
9.3 Structural Connectivity	137
9.4 Structural-Functional Connectivity Coupling	138
9.5 Functional Graph Measures	139
9.6 Structural Graph Measures	141
9.7 Correlation Between FMA and Graph Measures	143
9.7.1 Fugl-Meyer Assessment (FMA) Changes	144

10 Conclusion	147
11 Limitations and Recommendations	149
12 Appendices	178
12.1 Appendix A: FC Processing Steps and QC	178
12.1.1 AAL116 Atlas	178
12.2 Appendix B: Scripts for Processing DWI Data.	192
12.2.1 dcm2nii Command: Converting DICOM Images to NifTI Images.	192
12.2.2 Motion Parameter Extraction from DWI Data.	192
12.2.3 Motion Estimates in DWI Data	192
12.2.4 Linux Code to Import Data to TORTOISE.	193
12.2.5 Script to Run Auto ACPC Detect.	194
12.2.6 DIFF_PREP Command in TORTOISE Software.	194
12.2.7 Grad FlipTest and fat_proc_dwi_to_dt Command to Calculate the Diffusion Tensor Parameters.	197
12.2.8 Inverse Warping of AAL116 Atlas from MNI to eac Subject's Native Space.	198
12.2.9 3dTrackID to Determine the FA Values Between the 116 Brain Regions Spec- ified using the AAL116 Atlas.	198
12.2.10 Extracting FA Matrices from .grid Files (Output After Running 3dTrackID.)	199
12.3 Appendix C: Scripts for Statistical Analysis.	200
12.3.1 Covariate Comparison for Structural Connectivity Data.	200
12.3.2 Covariate Comparison for Functional Connectivity Data.	201
12.3.3 Infarct Side	203
12.3.4 Fisher's Exact Test to Compare the Distribution of a Binary Variable ("Side") Across the Three Treatment Groups.	203
12.3.5 Matlab Code to Convert Fisher Z-values to r-values in the 116 by 116 Func- tional Connectivity Matrices	205
12.3.6 Converting r-value Back to Z-values	206
12.3.7 SC Adjacency Matrices of 13 Brain Connections Identified using the CONN Toolbox.	207
12.3.8 Calculating SC-FC Coupling: Pearson Correlation Between the Mean FA and Correlated Mean Time Series.	211
12.3.9 Graph measures: Adjusting for the Motion Covariate when Comparing FC Graph Measures using a Repeated Measures ANCOVA (within-group differ- ences) and one-way ANCOVA (between-group differences).	214
12.3.10 Comparing Motion Covariate Before and After Treatment in the DWI Data.	216
12.3.11 Adjusting for Motion in the SC Graph Measures using Repeated Measures ANCOVA for Within-group Comparisons, with the Mean Motion as a Covariate.	219
12.3.12 Adjusting for Motion in the SC Graph Measures Using One-way ANCOVA for Comparing Pre- and Post-treatment, Between Groups.	220
12.3.13 Statistical Analysis of SC Graph Measures that Violated Assumptions of ANCOVA	226
12.3.14 Statistical Analysis of FC Graph Measures that Violated Assumptions of ANCOVA	227
12.3.15 Outlier Detection to use for Winsorizing Graph Theoretical Data	231
12.3.16 Python Script used for Calculating the Pearson Correlation Between FMA and the Different Graph Theory Measures. Note: The Same Script was used for Structural and Functional Graph Measures. Only the Input File Changed.	231
12.3.17 Create FMA Line Graphs for Three Different Groups.	233

Chapter 1

Introduction

1.1 Stroke: A Cause of Global Mortality

Globally, stroke ranks as the second most common cause of death and the third most frequent cause of disability.^[1] The 2016 Global Burden of Diseases (GBD), Injuries, and Risk Factors Study found that neurological disorders were the primary cause of disability-adjusted life years (DALYs). Among these disorders, stroke emerged as the primary contributor to this burden.^[2]

Stroke is a cerebrovascular disease distinguished by either ischemic or hemorrhagic events, involving the obstruction or rupture of blood vessels, respectively.^[3] Constituting approximately 85% of all strokes, ischemic strokes, the most common form,^[4] are highly diverse, consisting of multiple subtypes: large-artery atherosclerosis, cardioembolism, small-artery occlusion, and cryptogenic stroke.^[5] It is predicted that in 2030 the global burden of ischemic cases will be more than 4 million due to population growth, aging, and rising prevalence of risk factors.^[6] The risk factors for ischemic stroke can be grouped into three categories: non-modifiable (age, sex), semi-modifiable (hypertension and diabetes mellitus), and modifiable (smoking, BMI, and alcohol consumption).^[7] Notably, hypertension and diabetes mellitus stand out as the most critical risk factors across all subtypes of ischemic stroke. Furthermore, hypertension is the primary driver of cardiovascular disease in Africa. Cardiovascular diseases constitute the most substantial contributor to the burden of non-communicable diseases on the continent, responsible for nearly 40% of deaths and approximately 23 million DALYs.^[8] Therefore, African countries have an added burden due to the rise of non-communicable diseases superimposed on communicable diseases.^[9]

1.2 Perspective from Low-and-Middle-Income Countries (LMICs)

The contrasts between high-income countries and LMICs are staggering, with the incidence of stroke decreasing in the former and increasing in the latter.^[10] 86% of all strokes occur in LMICs, and this figure is expected to rise.^[11] Strikingly, ischemic stroke patients in LMICs are younger and have a poorer prognosis compared to high-income countries.^[12;13] In South Africa, stroke incidence has notably shifted to a younger population, with an average age of 54.3 years.^[14] This is further shown by a study in Tanzania, where 123 out of the 369 patients were less than 45 years of age.^[15] Furthermore, a study in Pakistan found that 30% of stroke patients were under 45 years old.^[16] Consequently, this places added pressure on the healthcare system^[17] as this working-age population contributes to the economy and requires longer post-stroke rehabilitation services for full integration into society.^[18]

In developed countries, stroke rehabilitation encompasses a multidisciplinary approach, which includes physiotherapy, speech therapy, and occupational therapy.^[18] Stroke rehabilitation is important for improving the patient's quality of life, alleviate the consequences of functional limitations, and address potential psychological problems.^[19] However, due to the absence of accessible, quality, and multidisciplinary stroke rehabilitation services in LMICs, particularly in rural areas, stroke recovery is impeded.^[20] This is seen in Nigeria, where stroke rehabilitation is carried out by the general medical wards, instead of a multidisciplinary team.^[21]

Consequently, many stroke patients seek complementary and alternative interventions due to the delay and high cost associated with standard rehabilitation care.^[22] Acupuncture, a practice rooted in traditional Chinese medicine, has been used in China for 2,000 to 3,000 years and represents one such intervention.^[23–25]

Chapter 2

Acupuncture

2.1 Stroke Rehabilitation in China

China faces the highest global stroke burden, with approximately 2 million new cases annually.^[26] About 43% of stroke patients in China do not undergo any rehabilitation, with less than 12% undergoing rehabilitation in the first week of stroke onset.^[27] This is due to the underdevelopment of standardized stroke rehabilitation, insurance systems that do not fully cover rehabilitation,^[28] insufficient caregivers, and low medical resources.^[29] Furthermore, standard rehabilitation encompassing multidisciplinary teams of nurses, physiotherapists, occupational therapists, and social workers, is not as established in China as in the Western World, where it routinely forms part of the stroke recovery pathway.^[30] Although this is alarming, it provides opportunities to determine the potential benefit of rehabilitation compared to no intervention (control), which is considered unethical in much of the world. Indeed, a meta-analysis study showed that rehabilitation was able to significantly reduce the burden of disability in stroke patients compared to no intervention.^[30]

2.2 Acupuncture: An Overview

Acupuncture is the mechanical, electrical, or other physical stimulation of acupoints by inserting needles into the skin.^[23] 'Deqi' (Chinese for the "arrival of vital energy" or "qi arrival") is the needling sensation experienced by both the patient and the acupuncturist^[31] and according to Traditional Chinese medicine (TCM) it is regarded as a critical factor that influences acupuncture outcomes.^[32–34] The sensations include a combination of heaviness, numbness, and soreness for the patient and heaviness and tightness for the acupuncturist.^[35] Multiple studies have shown bet-

ter therapeutic outcomes when Deqi is experienced compared to when it is not.^[36–38] In particular, it was shown that acupuncture, in which Deqi was experienced, resulted in reduced spasticity after stroke compared to the group that did not experience Deqi.^[39] Interestingly, acupuncture where Deqi was experienced was associated with a spatial pattern that extended farther from the stimulus origin compared to tactile stimulation.^[35] In contrast, another study found that patients in the sham acupuncture group (n=20) had similar Deqi responses compared to the true acupuncture group (n=20).^[40]

2.2.1 True and Sham Acupuncture

True acupuncture is the needling performed at acupoints with physiological effects, whereas sham acupuncture methods are used as a control. Proper sham acupuncture methods should be indistinguishable from true acupuncture and physiologically inert.^[41] However, there is a lack of evidence for this; no consensus exists on which sham acupuncture method to use, and reporting quality remains suboptimal.^[42] This is seen in clinical trials where the sham methods involve either minimal or non-penetrating acupuncture that results in heterogeneous outcomes across studies.^[41] Another method that is considered sham acupuncture is stimulation at non-acupoints. A 2022 systematic review of 51 sham acupuncture trials revealed no notable differences between true and sham acupuncture in assessing over 30 biomarkers.^[43] This could be explained by the cross-contamination which occurs due to variability in point localization identified by acupuncturists.^[44] This means that if the point localization is off by 1-2 centimeters, it might overlap with non-acupoints. Some research indicates that the clinical benefits of acupuncture stem from patients' expectations of a positive outcome and the interactions they have with their practitioners.^[45–48] Therefore, evaluating the efficacy of acupuncture concerning both acupoints and non-acupoints continues to present a challenge.

2.2.2 Acupoints

Acupoints serve as the anatomical sites where acupuncture needles are introduced, and in TCM these points are believed to be linked by a system of meridians that extend lengthwise throughout the body.^[49] The meridian theory, a core concept in TCM, describes an intricate network that facilitates the flow of Qi and blood.^[50] Despite numerous efforts and thousands of years of practice, the anatomical location of acupoints and the acupoint selection for a specific disease remains controversial.^[51]

Clinical selection of acupoints revolves around three principles: Firstly, selecting local acupoints near the symptomatic area. Secondly, choosing distant acupoints along the meridian from the symptom's location and thirdly, opting for distant acupoints based on symptom differentiation, irrespective of symptom location.^[52] Using forward and reverse inference, it was shown that some acupoints are common among all diseases, while other acupoints are more specific to certain diseases or disorders.^[52] The most common acupoints used for ischemic stroke are Baihui (GV20), Zusanli (ST36), Quchi(LI11), Shuigou (GV26), Dazhui (GV14) and Hegu (LI4), which have been shown to activate different molecular pathways involved in stroke recovery.^[53]

A study examining acupuncture at the Baihui (GV20), Fengchi (GB20, bilateral), Xuanzhong (GB39, bilateral), Quchi (LI11, bilateral), Hegu (LI4, bilateral), Zusanli (ST36, bilateral), and Sanyinjiao (SP6, bilateral) acupoints found no association between enhanced white matter and motor recovery.^[54] Interestingly, in a study where standard rehabilitation was used without acupuncture, patients showed a positive correlation between white matter (WM) improvement and motor recovery.^[55]

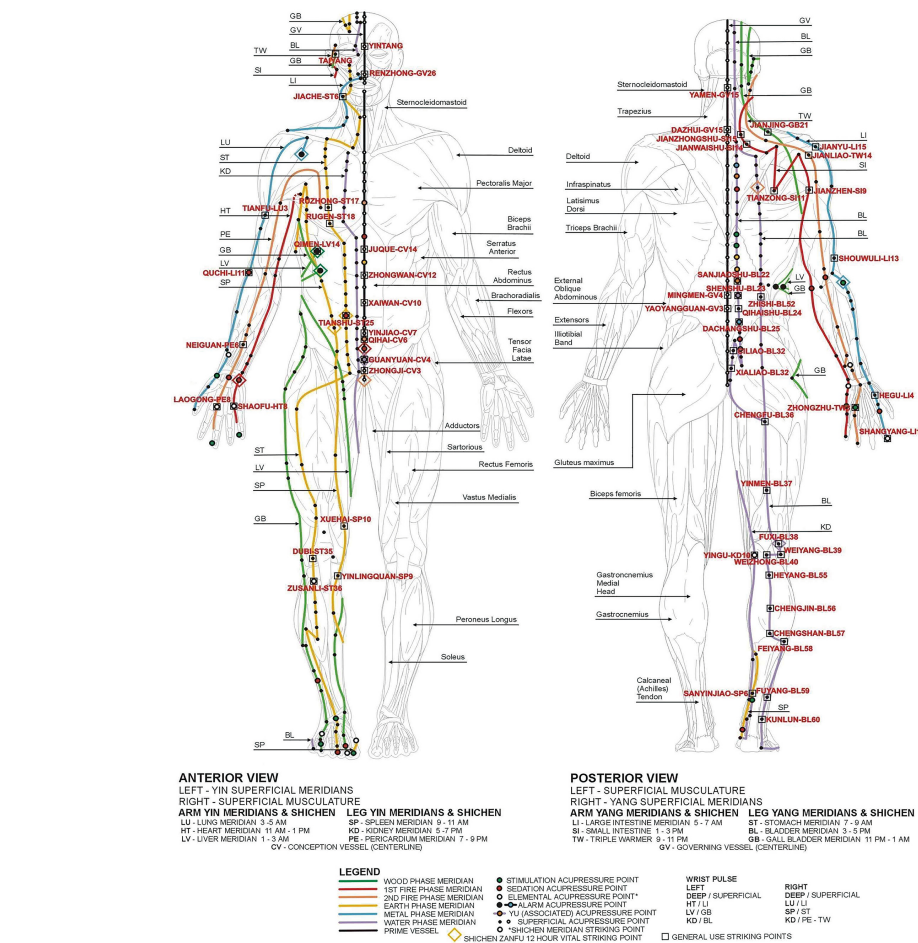


Figure 1: Acupoint locations as interpreted in Traditional Chinese Medicine teaching, illustrating anatomical reference points relevant to both research applications and clinical acupuncture practice.^[49]

2.2.3 Xing Nao Kai Qiao

XNKK (regaining consciousness and opening orifice) is a needling method invented by Prof. Shi Xuemin that has been shown to be effective for motor recovery in ischemic stroke patients.^[56] The principal acupoints stimulated during this acupuncture procedure are DU-26 (ren2 zhong1), PC 6 (nei4 guan1), SP 6 (san1 yin1 jiao1), HT 1 (ji2 quan2), LU 5 (chi3 ze2), and BL 40 (wei3 zhong1), shown in **Figure 1**.^[57] DU-26 corresponds to the buccal branches of the facial nerve and the second branch of the trigeminal nerve, whereas PC 6 corresponds to the median nerve and SP 6 to the tibial nerve.^[58] Additionally, LU 5 is associated with the lung meridian,^[59] HT 1 with the radial side of the axillary artery, and BL 40 is located at the midpoint of the transverse crease

of the popliteal fossa and associated with the tibial nerve.^[60] A study using XNKQ showed that ischemic stroke patients receiving acupuncture had fewer dependencies.^[61] This is supported by a meta-analysis study that showed the XNKQ needling significantly reduced disability and enhanced patients' activity of daily living, noting the selective and measurement biases detected.^[62] Another study showed that adjunctive XNKQ acupuncture may be associated with improved cognitive performance and daily living activities in acute ischemic stroke patients.^[63]

2.2.4 Adjunctive Acupuncture

Adjunctive acupuncture refers to the use of acupuncture as an additional therapy in conjunction with another medical intervention. It is not the primary treatment, rather, it is used alongside conventional treatments to enhance the effects. Acupuncture adjunct to Western medicine, was shown to reduce mortality risk in stroke patients compared to routine care alone.^[64] Furthermore, adjunctive acupuncture showed reductions in medical expenditure and re-hospitalization rates.^[65] Therefore, adjunctive acupuncture seems promising for stroke recovery and reducing the economic burden of stroke.

Physiotherapy, encompassing structured physical rehabilitation, is one of the most frequently employed interventions alongside acupuncture in stroke recovery. Its role is critical in counteracting inactivity, which can increase the risk of recurrent stroke.^[66] Active physiotherapy, as opposed to passive physiotherapy (which is limited to assisted muscle contractions and requires minimal patient effort), includes activities such as aquatic therapy or gait training and has been shown to elevate physical activity levels both during and after sessions.^[67]

Evidence comparing physiotherapy and acupuncture suggests mixed results. In one trial involving 120 ischemic stroke patients, physiotherapy outperformed acupuncture treatment on the FMA and Modified Barthel Index (MBI), and combining the two did not yield superior outcomes over

physiotherapy alone.^[68] Similarly, a larger study of 274 stroke patients treated over four weeks found no synergistic effect of combined therapy compared with physiotherapy by itself.^[69]

In contrast, another investigation assessing FMA, Mini-Mental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA) scores reported that adding acupuncture to conventional rehabilitation produced additional gains in swallowing, lower-limb motor function, and cognitive performance.^[70] The timing of intervention also appears crucial, with evidence indicating that earlier mobilization is associated with better recovery outcomes.^[71]

2.2.5 Timepoint of Intervention

The importance of a critical time window for stroke recovery has been determined.^{[72][73][74]} However, there is evidence against the widely accepted belief that stroke recovery only occurs within the first six months after the lesion.^[75;76] In a study investigating the functional and structural recovery in 219 hemiparetic stroke patients, the critical period for neuroplasticity extended beyond 12 months.^[77] Furthermore, it has been shown that acupuncture influences the reorganization of functional and structural connections in adult stroke patients in the subacute and chronic phase.^[78;79]

Adding to the complexity of determining the most appropriate time for intervention is investigating whether rehabilitation outcomes are due to spontaneous recovery or to the treatment intervention, especially early after the stroke onset.^[80–83] A study that teased apart spontaneous recovery and treatment to determine rehabilitation outcomes showed that treatment contributed twice as much to defect reduction compared to spontaneous recovery.^[80] Major factors influencing spontaneous recovery are the physiological phenomena such as blood-brain barrier (BBB) disruption and reperfusion injury.^[84]

2.2.6 Blood Brain Barrier

The BBB is a dynamic physical and metabolic barrier that regulates communication between the central nervous system and the peripheral body, creating a tightly controlled microenvironment that is vital for maintaining homeostasis.^[85–87] Ischemic stroke has been shown to contribute to BBB disruption, which is associated with the altered expression of tight junction proteins. This results in cerebral edema, increased infiltration of leukocytes, and hemorrhagic transformation, exacerbating ischemic injury.^[88]

Increased BBB permeability is suggested to be the final converging mechanism of reperfusion injury in ischemic stroke.^[82] Reperfusion injury occurs in 3 stages (**Figure 2**):

1. Loss of cerebral autoregulation
2. Hypoperfusion
3. A biphasic rise in paracellular permeability

The first phase of paracellular permeability is characterized by an inflammatory response (3 - 8 hours post-reperfusion), and during the second phase, angiogenesis and vasogenic edema take place (18 - 96 hours post-reperfusion).^[89] A meta-analysis study showed that acupuncture within 48 hours post-stroke had superior beneficial effects compared to acupuncture after 2 days.^[90] At first glance, acupuncture intervention within the second phase of the third stage of reperfusion injury might seem like the preferred option. However, it has been shown that there is no significant difference between acupuncture intervention at 48h, 49-72h, and 4-10 days.^[91] Therefore, conclusions about the intervention time of acupuncture are inconsistent.

2.2.7 Duration of Intervention

In a study of 251 ischemic stroke patients assigned to one of three groups based on acupuncture needle-retention times (20, 40, and 60 minutes), those in the 60-minute group experienced

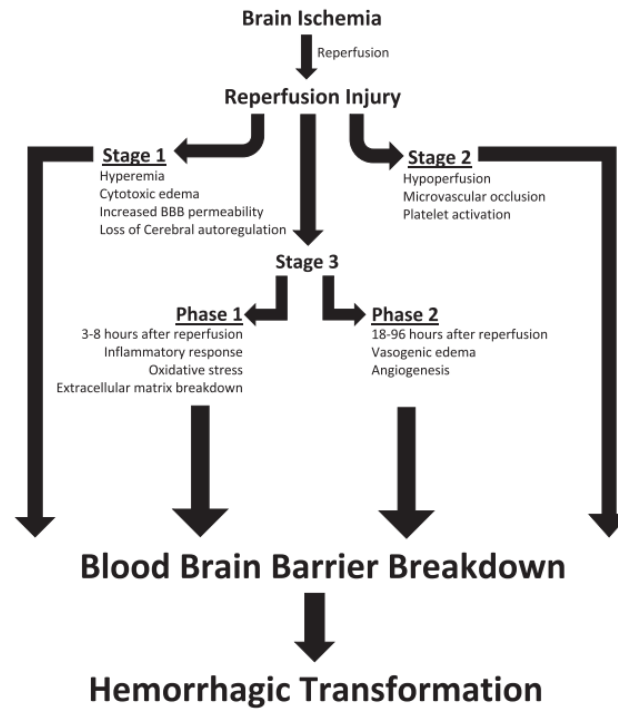


Figure 2: **Flow chart depicting the mechanism of reperfusion injury, outlining three stages, with the third stage comprising two distinct phases.** [76]

the most favorable outcomes.^[92] Another study showed that 24-hour needle retention had overall better motor outcomes compared to control (no acupuncture) and 30-minute needle retention.^[93] However, after 3 months, there was no significant difference between the three groups when comparing patients' balance. Therefore, it might be suggested that despite different retention times at the initial stages, with time, there is a point of convergence where the balance in patients is similar. The outcomes of acupuncture can be further affected by the acupuncture modality.

2.2.8 Acupuncture Modality

Manual acupuncture (MA) includes manipulation techniques such as rotation, lifting, and thrusting and can range from subtle to vigorous, rapid, and forceful.^[94] On the other hand, electroacupuncture (EA) applies electrical currents to acupuncture needles using a stimulator, which allows for continuous and controlled stimulation.^[95] EA has been shown to have a superior effect compared to MA when evaluating the cerebral blood flow and motor function.^[96] However, it is unclear

whether the EA stimulation time was the same as for MA when doing body acupuncture. Therefore, the stimulation duration could be a confounding factor. Additionally, EA has the advantage of controlling various parameters such as stimulation frequency and intensity.^[95] Nevertheless, both MA and EA result in physiological responses characterized by molecular signaling pathways.^[94]

2.2.9 Acupuncture and Physiological Changes

After an ischemic stroke, the levels of Brain-Derived Neurotrophic Factor (BDNF) decline significantly compared to healthy controls.^[97] BDNF, the primary neurotrophin in the adult brain, plays a critical role in synapse development, synaptic plasticity, and the preservation of cognitive abilities.^[98] It therefore plays a role in the pathogenesis and rehabilitation of stroke. Without further investigation, BDNF might seem like a promising therapeutic target for stroke rehabilitation, however, challenges such as low transport rate across the BBB, off-target increase in apoptosis, and weight loss as a side-effect encourage exploration of other interventions.^[98]

Acupuncture is a safe treatment compared to prescription drugs, however, not without some adverse effects such as haematoma, swelling and bruising.^[99] Nevertheless, acupuncture regulates cerebral blood flow, has anti-apoptotic effects, regulates neurochemicals, improves long-term potentiation (LTP), and promotes neurogenesis.^[53] Notably, ischemic rats treated with EA showed increased expression of BDNF.^[100] Similarly, a study showed that EA increased BDNF and additionally decreased the expression of Nogo-A, an inhibitor of neurite outgrowth in the ischemic penumbra of rats.^[101] Furthermore, a clinical study showed that high Nerve Growth Factor (NGF) serum levels were associated with better functional prognosis after 3 months of ischemic stroke incidence.^[102] NGF promotes neuronal growth and survival and plays a role in maintaining functional neuronal integrity.^[103] Moreover, EA significantly increased the passage of NGF through the BBB in rats with middle cerebral artery occlusion.^[104] Taken together, both BDNF and NGF play a role in neuroplasticity after acupuncture treatment in ischemic stroke.

Chapter 3

Neuroplasticity and Structural Changes

Neuroplasticity is the functional and structural changes of the CNS due to endogenous and exogenous stimuli in the context of development, learning, aging, and trauma.^[105] These structural changes include axon regeneration and oligodendrogenesis, which are crucial for the myelination of axons.^[106] Ischemic stroke results in local and remote white matter tract degeneration.^[78] Specifically, Wallerian degeneration and diaschisis occur, which are the self-destructive degeneration of axonal injury and the neurophysiological changes due to neuronal deactivation, respectively.^[107;108]

The effects of Wallerian degeneration and diaschisis might be hindered by acupuncture, which promotes the upregulation of synaptophysin and postsynaptic density 95 (PSD-95) in rodent models.^[109] Furthermore, PSD-95 is involved in Hebbian plasticity, which refers to the synaptic changes that code information associated with learning and memory.^[110–112] Interestingly, a study published in 2022 showed that Hebbian plasticity is not only limited to synaptic plasticity but includes myelin plasticity.^[113] Additionally, myelin plasticity affects the conduction velocity and synchronization of signals across the brain.^[114] Therefore, changes to myelin can affect functional activity across brain regions. Indeed, a study showed that motor function correlates with white matter structure.^[115]

3.1 White Matter

White matter is the axons wrapped in an insulating plasma membrane, myelin, which connects different brain regions.^[116] It can be described by its volume and connectivity, referring to the amount of white matter through which myelinated axons pass and the starting and ending points of

these tracts, respectively.^[117] Furthermore, in patients with right hemispheric subcortical infarcts, needling at GB34 (Yanglingquan) resulted in the enhancement of functional connectivity between the premotor cortex/supplementary motor area (SMA) and the supramarginal gyrus (SMG), which are the origin and termination of the superior longitudinal fasciculus-frontoparietal part, respectively.^[117]

3.2 Ischemic Core versus Penumbra

Underlying the structural changes are the physiological changes that differentiate the ischemic core from the penumbra. The ischemic core is comprised of the irreversible necrotic tissue, and the penumbra is the reversible hypoperfused tissue extending from the potentially salvageable core.^[118] The penumbra can be salvaged by increased perfusion, which promotes neuroplasticity via acupuncture. Interestingly, it was shown that the size of the ischemic core, but not the penumbra, was strongly associated with motor impairment.^[119] Importantly, the authors mentioned that the location of the penumbra, rather than its size, might affect motor impairment. When the infarct is adjacent to major white matter tracts, multi-motor deficits can occur. A few of these major tracts include the corpus callosum, anterior commissure, internal capsule, superior and middle longitudinal fasciculus.^[119] The corticospinal tract (CST) is the major descending motor pathway controlling the voluntary movement of the limb. It originates in the precentral gyrus and descends through the internal capsule towards the pyramids of the medulla, where decussation occurs.^[120] Decussation is the crossing of axonal fibers at the midline so that fibers originating on the ipsilateral side will control motor function on the contralateral side and vice versa.^[121] Whether patients present with ipsilateral or contralateral hemiplegia or hemiparesis will depend on whether the crossing or non-crossing fibers of the corticospinal tract are damaged. Nevertheless, to effectively predict motor outcomes in stroke patients, rigorous methods are needed to study the underlying damaged structure and repair after intervention. A well-known method is DTI and tractography.^[122]

3.3 DTI and Tractography

Diffusion Tensor Imaging (DTI) is the modeling of Diffusion Weighted Imaging (DWI) using a diffusion tensor model to indirectly measure the degree of anisotropy based on the random diffusion of water molecules in tissues, known as Brownian motion.^[123;124] The degree of anisotropy might reflect a biological basis such as myelination and tissue orientation.^[125] For example, water molecules diffuse more freely in the ventricles compared to more restricted diffusion in the white matter tracts.^[126] Thus, DTI can be used to indirectly infer the WM microstructure. In detail, the diffusion tensor model can be decomposed into eigenvectors and eigenvalues, which characterize the main axis and magnitude of diffusion in each voxel, respectively. Based on these values, the following DTI metrics can be derived: Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD), where FA is a measure of the anisotropic diffusion and ranges from 0 (isotropic) to 1 (anisotropic) diffusion (**Figure 3**).

In a study where ischemic stroke patients received the conventional treatment (antiplatelet aggregation drugs) or the conventional treatment with acupuncture (AG), those receiving AG had a greater improvement in motor function.^[54] However, there was no significant difference in FA, and unexpectedly, there was no correlation between FA and motor function after one month of intervention. In contrast, another study showed a positive correlation between FA and FMA in the frontoparietal part of the superior longitudinal fasciculus (SLF-FP) for ischemic stroke patients.^[127] Interestingly, a study showed that there was a positive correlation between motor recovery and FA over a period of 3 months in ischemic stroke patients who did not receive acupuncture.^[55] Another study showed that there was a significant correlation between reduced FA and the affected white matter tracts as measured by using 3D fiber tractography.^[128]

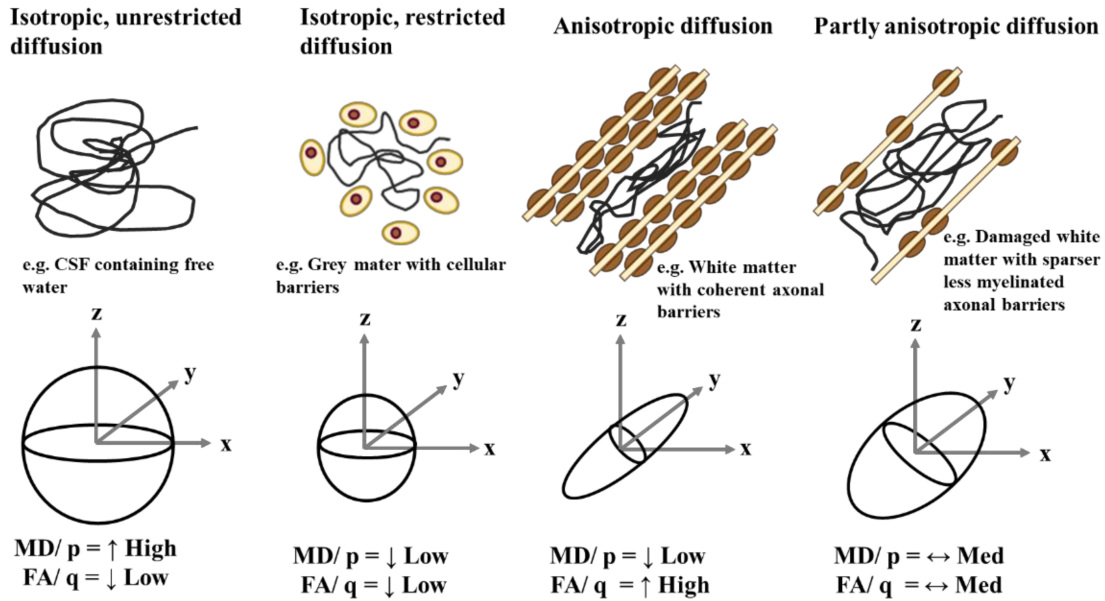


Figure 3: **Diagram illustrating diffusion tensors from various brain microstructures, highlighting their impact on isotropic (p and MD) and anisotropic (q and FA) tensor derivatives.** The isotropic component, p , represents the average diffusion within a voxel, similar to Mean Diffusivity (MD), while the anisotropic component, q , reflects directional diffusion, akin to Fractional Anisotropy (FA). Note that the fourth image demonstrates how FA changes when white matter is damaged, such as in ischemic stroke.^[119]

3.3.1 Tractography

Tractography is a method used to infer and reconstruct WM fiber pathways by mapping the trajectories of voxel-wise WM fiber orientations. While voxel-wise DTI analysis investigates the WM microstructural integrity, tractography reveals the anatomical organization of the structural connectivity in the brain.^[129] A recent study used diffusion tensor tractography (DTT) and showed that electroacupuncture significantly improved FA in the corticospinal tract in stroke patients compared to the control group.^[130] However, it is not enough to understand the structural changes; the functional changes should also be investigated to have a full picture of neuroplasticity in ischemic stroke patients.

Chapter 4

Neuroplasticity and Functional Changes

Neuroplasticity, involving the complex interplay between cellular and molecular changes, results in neuronal activity that gives rise to functional remodelling.^[131] The remodelling is characterised by the reorganization of functional networks that compensate for the lost function in ischemic stroke patients. It involves the enhanced activity in preexisting networks to compensate for motor deficits.^[132] To investigate the functional changes, Functional magnetic resonance imaging (fMRI) is used.

4.1 Functional Magnetic Resonance Imaging (fMRI)

Where DTI makes use of FA to indirectly determine the microstructure, fMRI makes use of the Blood-Oxygen-Level-Dependent (BOLD) contrast signal to indirectly determine the neuronal activity.^[133] The BOLD contrast relies on the magnetic properties of haemoglobin that differ depending on whether it is bound to oxygen, and hence it is a measure of metabolic demand.^[134;135] In short, when neurons become active, they demand more oxygen and glucose. This triggers an increase in Cerebral Blood Flow (CBF) to the engaged brain region, delivering an excess supply of oxygen. Because the rise in CBF outweighs the actual oxygen consumption, the proportion of oxygenated hemoglobin increases and the concentration of deoxygenated hemoglobin decreases.^[136] The oxygenation state of hemoglobin influences the tissue-relaxation parameters, T1 and T2, which describe how the net magnetization of tissue recovers after an RF pulse and creates contrast in MRI images.^[137] Since deoxyhemoglobin is paramagnetic, a natural contrasting agent and distorts the MRI signal more than oxyhemoglobin, this shift in oxygenation alters the local magnetic

field.^[138] The MRI detects this change as the BOLD contrast.^[139] The BOLD signal, recorded as a time series for each voxel, follows the Hemodynamic Response Function (HRF), characterized by a brief delay of about 2-6 seconds, a peak, and a gradual return to baseline, as illustrated in

Figure 4.

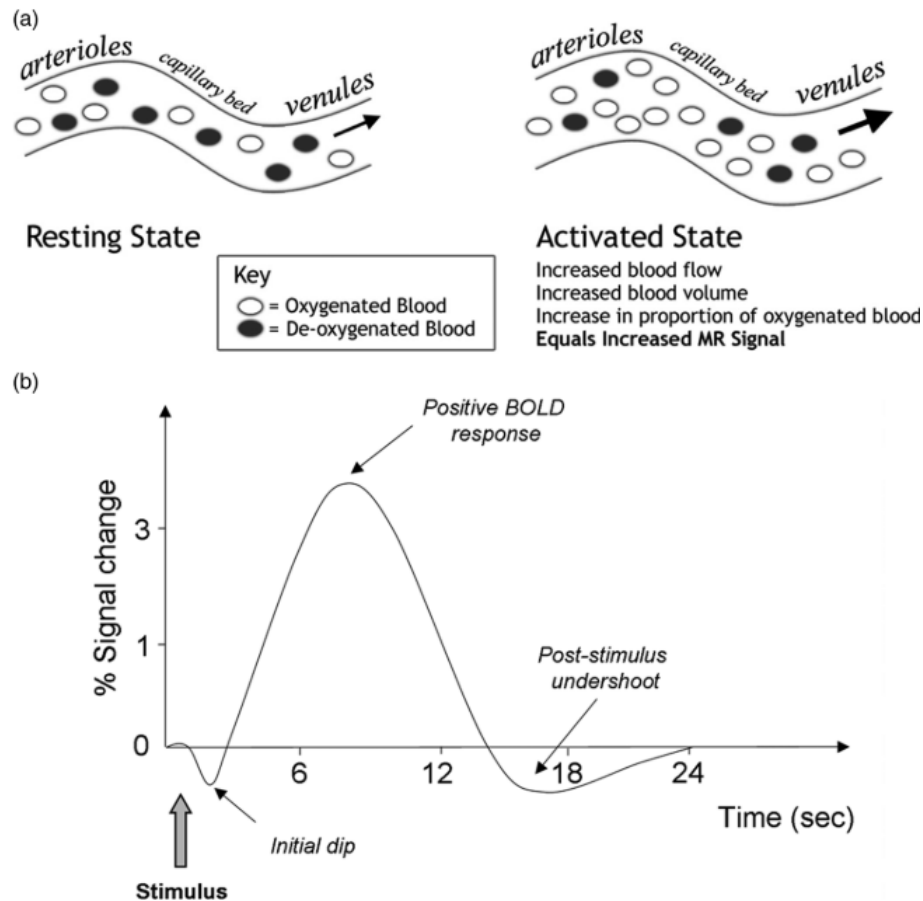


Figure 4: **Diagram illustrating (a) the hemodynamic processes driving the blood oxygenation level-dependent (BOLD) signal, and (b) the BOLD hemodynamic response function.** In part (a), the BOLD signal arises from changes in blood flow and oxygenation. Neural activity triggered by a stimulus increases oxygen demand, causing an initial dip in oxygenated blood as deoxyhemoglobin levels rise briefly. This is followed by an influx of oxygenated blood, reducing deoxyhemoglobin and enhancing the BOLD signal. Part (b) shows the BOLD response function, which captures the temporal dynamics of this signal, reflecting the initial dip and subsequent rise in signal intensity due to the interplay of oxygenated and deoxygenated blood.^[129]

In Ischemic stroke patients, the HRF presents a delay in the peak latency, which is related to deterioration of cerebral hemodynamics. This hemodynamic lag impacts different rs-fMRI metrics.^[140;141] Rs-fMRI is a non-invasive method to measure the spontaneous low-frequency fluctuations (0.01 - 0.1 Hz) by detecting changes in the BOLD signal, while the subject is not performing any task (i.e., "at rest").^[142] On the other hand, task-based fMRI, measures brain activity while

subjects perform a specific task (finger tapping, viewing images or memory tasks). The BOLD signal is analysed in relation to the timing of the task to identify regions that are associated with the task. Rs-fMRI is preferred for studying ischemic stroke because it demands minimal effort from patients, who often experience cognitive, language, and motor impairments.^[143] Requiring patients to perform tasks can cause stress and may lead to unreliable data. Moreover, task-based fMRI depends on the subject's ability to perform the task well, which can introduce noise and bias. In contrast, rs-fMRI bypasses these issues by not requiring active participation. Additionally, rs-fMRI provides a more comprehensive view of functional connectivity across the entire brain, whereas task-based fMRI focuses only on networks engaged by the specific task.^[144] Various analytical methods have been developed to study resting-state fMRI (rs-fMRI), each utilizing the BOLD time series in distinct ways. One common approach is seed-based correlation analysis, in which the average BOLD signal within a selected region of interest (ROI) is correlated with signals from all other voxels in the brain.^[145] Another widely used method is independent component analysis (ICA), which requires fewer a priori assumptions and identifies spatially independent networks by maximizing statistical independence among components.^[146] A third approach involves graph-theoretical analysis, which models the brain as a complex network in which ROIs represent nodes and the correlations between their BOLD time series define the edges connecting them.^[147]

A systematic review showed that there is a relationship between the FA and BOLD signal when investigating the sensory and motor systems.^[148] Hence, functional changes might reflect structural changes. A meta-analysis study showed that acupuncture resulted in hyperactivation of the basal ganglia, which was associated with motor improvement.^[149] Another study used machine learning to identify ROIs that could predict motor recovery, and showed that acupuncture could eliminate abnormal functional connectivity and modulate activity across both hemispheres in ischemic stroke patients.^[150] Thus, acupuncture is considered to modulate functional connectivity

between brain regions, brain networks, and hemispheres. To determine how changes to the structural organization can give rise to the emergent functional expression and how different elements in the brain interact, graph theory is utilized.^[151]

Chapter 5

Graph Theory

The power of an experiment is only as strong as the clarity of the basic assumptions that underlie it.^[152] Assumptions are important as they directly or indirectly guide evidence, conclusions, and the selection of methodology. This is particularly important in understanding the complexity of the brain. The two main assumptions about the brain's underlying neural tissue are^[153]:

1. The neural tissue is continuous
2. The neural tissue is made up of "cables" connecting different entities (neurons)

When the first assumption is made, the underlying dynamical system can be treated as a field as shown by this equation:

$$F = (\vec{s}, t) \tag{1}$$

Here, $\vec{s}$ denotes a location in anatomical space and $t \in \mathbb{R}$ represents physical time, spanning scales from observational to developmental and evolutionary. F may take the form of a scalar, vector, or tensor field, and can additionally exhibit further structure, such as symmetries. It can also be characterized in the system's frequency domain or phase space. For the second assumption, the dynamics of the brain can be represented as a graph or network. The following equation is used for a graph N :

$$N = (V, E) \quad (2)$$

Where V is the vertices or nodes and E is the edges or connections. Advantages of treating the brain as a network include versatility, allowing for the understanding of network features as statistical properties, and the ability to perturb the network.^[153]

5.1 Classification of Networks

Brain graphs can be classified as directed or undirected based on whether or not there is a distinction between inward and outward connections.^[154] They can further be classified as weighted or binary, where the former takes on different values for the edges, such as size or density, that can be used to differentiate between strong and weak connections. Many studies use unweighted networks by thresholding a weighted network to minimize the noise level and the computational cost.^[155] However, thresholding a network is a challenge, as there is no consensus on which method is the best. The 3 main methods used are: 1) Fixed threshold, 2) fixed average degree, and 3) fixed edge density.^[156] However, the problem that arises with these thresholding methods includes differences in the total connectivity, irrelevant connections as the connectivity of networks can vary, and modification of the topology.^[156]

5.2 Topology

Topology refers to the arrangement of nodes and edges in a network and can be classified into three main types: 1) Regular, 2) small-world, and 3) random^[157] (**Figure 5**). These topological network architectures are based on their clustering coefficient and characteristic path length.^[158] The clustering coefficient is the measure of the number of edges that exist between a node and its nearest neighbors, whereas the characteristic path length is the minimal number of edges that need to be traversed to form a direct connection between two nodes.

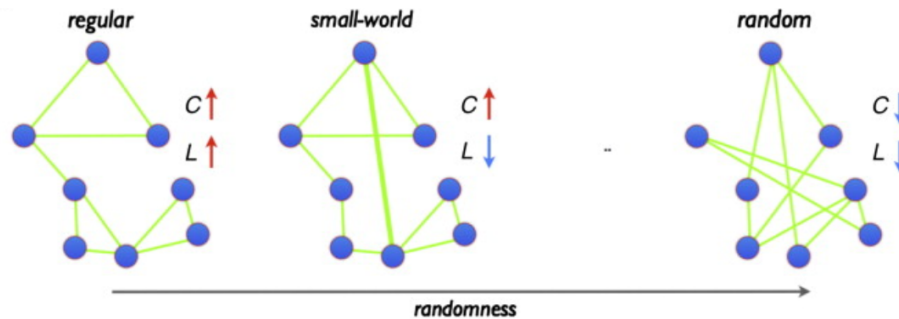


Figure 5: **Network topologies.** From left to right: Regular Network (high clustering coefficient C , long characteristic path length L), Small-World Network (high C , short L), and Random Network (low C , short L), illustrating distinct patterns of connectivity and efficiency in complex systems. ^[133]

5.2.1 Small-world Network

It is commonly accepted that the brain is a small-world network. ^[159;160] This is due to its complex structure across spatial and temporal scales, and its ability to maximize efficiency while minimizing wiring and processing costs. ^[161] Small-world networks are neither random nor highly ordered but instead have a high clustering coefficient and short characteristic path length. ^[162] Such an architecture facilitates efficient information segregation and integration at low wiring and energy costs. ^[158;163] Segregation, measured by using the metrics clustering coefficient and local efficiency, refers to the specialized processing in interconnected subsystems, also known as clusters, whereas integration, measured by using global efficiency and the characteristic path length, is the effective global cooperation between different subsystems. ^[164;165] Small-world topological organizations undergo changes in the context of brain disorders. Several studies have shown abnormal functional segregation and integration in ischemic stroke patients. ^[166;167]

5.2.2 Network Topology in Stroke Patients

A study using functional connectivity (FC) to investigate the network organization in acute ischemic stroke patients indicated that severely injured individuals showed high segregation but low integration. ^[167] Thus, a network organization that favors a state where information could easily travel from one component to another in the same domain (intra-domain connectivity) was preferred

among these patients. Additionally, the excessive recruitment of motor areas could indicate network reorganization. Similarly, structural network analysis in 30 ischemic stroke patients showed a decrease in integration and an increase in segregation, where the topological changes were associated with motor impairment.^[168]

Supporting this, a longitudinal study of 20 ischemic stroke patients (assessed at one-month intervals) showed that small-world topology was initially similar between stroke patients and healthy controls, although nodal properties were significantly different.^[169] After one month, the recovery in the ischemic stroke patients resulted in nodal properties that were not significantly different from the healthy controls. A potential explanation was spontaneous recovery, however, it is not clear, as the neural plasticity mechanisms were not investigated. Importantly, all patients received antiplatelet therapy, thus, it is challenging to determine whether the effect on the network properties was purely due to the antiplatelet, and critical interpretation of the results is necessary.

5.3 Graph-Theory-Based Measures

Global efficiency (E_g) is inversely related to the topological distance between nodes and is a measure of how effectively information is integrated across the entire network (**Figure 6**).^[170] G is the network and L_{ij} is the shortest path length between each pair of nodes i and j . N is the number of nodes in the network.

$$E_g(G) = \frac{1}{N(N-1)} \sum_{\substack{i,j \in G \\ i \neq j}} \frac{1}{L_{ij}}$$

Local efficiency (E_{loc}) indicates how effectively information is integrated between the immediate neighbors.^[171] G_i is the subgraph consisting of the neighbors of node i .

$$E_{\text{loc}}(G) = \frac{1}{N} \sum_{i=1}^N E_{\text{glob}}(G_i)$$

Clustering coefficient (Cp) is the interconnectedness of nodes with the same neighbor^[172]. k_i is the number of edges by node i and E_i is the number of edges existing in G_i .

$$C_p(i) = \frac{1}{N} \sum_{i \in G} \frac{E_i}{\frac{k_i(k_i-1)}{2}}$$

Characteristic path length (Lp) is the average shortest path lengths across all possible pairs of nodes in the network and reflects global integration.^[158;173]

$$L_p(G) = \left(\frac{1}{N(N-1)} \sum_{\substack{i,j \in G \\ i \neq j}} \frac{1}{L_{ij}} \right)^{-1}$$

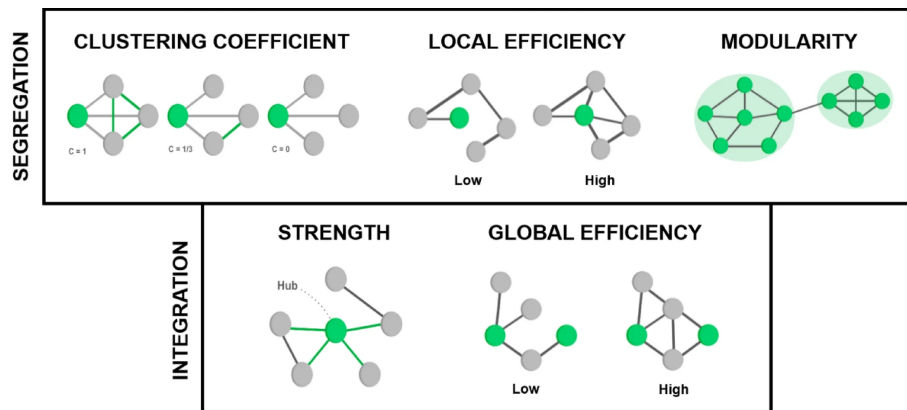


Figure 6: **Graph theory metrics used to characterize brain network organization in neuroimaging data.** The metrics are divided into two categories: segregation and integration, reflecting distinct aspects of network connectivity and efficiency. Segregation is measured using clustering coefficient, local efficiency and modularity. Integration is measured by strength, and global efficiency. ^[149]

5.3.1 Effect of Acupuncture on Network Topology

When the functional network parameters were investigated in ischemic stroke patients, they showed reduced small-world topology compared to healthy controls.^[174] When these stroke patients received acupuncture, the clustering coefficient and mean local efficiency increased, while small-worldness and global efficiency remained unchanged. While there are several studies investigating the changes in structural topology in stroke patients, there are no studies specifically investigating the effect of acupuncture, let alone adjunctive acupuncture, on structural topology in ischemic stroke.

5.4 Structural and Functional Connectivity

5.4.1 Structural Connectivity

Structural connectivity (SC) is the anatomical connections in the brain formed by white matter tracts consisting of axons. Mathematically, it is represented as a SC adjacency matrix A of size $N^2 - N$, where the entry w_{ij} represents the connection strength between elements i and j .^[175]

For $N = 4$ regions, the matrix A is given by:

$$A = \begin{bmatrix} 0 & w_{12} & w_{13} & w_{14} \\ w_{21} & 0 & w_{23} & w_{24} \\ w_{31} & w_{32} & 0 & w_{34} \\ w_{41} & w_{42} & w_{43} & 0 \end{bmatrix} \quad (3)$$

- $N^2 = 16$ total entries.
- Minus 4 self-connections: $16 - 4 = 12$ possible edges.

The $(-N)$ is included, as self-connections are not meaningful in structural connectivity. If the connections are undirected, then only half of the matrix is unique. The number of nodes will

depend on the parcellation atlas used to divide the brain into ROIs. For example, the AAL116 atlas will yield a 116 by 116 matrix.^[176] The cell entries represent the network edges (connections) between pairs of nodes. An unweighted matrix will contain 0 or 1 in the cells, whereas a weighted matrix will contain values representing the strength or quality of the connections. Common edge strength metrics include: 1) Fractional anisotropy, 2) streamline count (number of fiber tracts), 3) mean diffusivity, 4) axial diffusivity (AD) and 5) fiber length or density.^[177;178] When using the software Tolerably Obsessive Registration and Tensor Optimization Indolent Software Ensemble (TORTOISE) for processing DWI data, FA is a common choice for edge strength because it reflects fiber tract integrity.^[179] Complementing this is functional connectivity (FC), with notable differences in edge values, ways of measurement, and interpretations.

5.4.2 Functional Connectivity

FC measures the statistical dependence or correlation between the activity of different brain regions, based on synchronised BOLD signals.^[180] Unlike SC, where edge weights represent metrics derived from reconstructed physical tracts, FC typically defines edge weights by computing the Pearson correlation between regional BOLD time series. It is also mathematically represented by matrices and captures how brain regions work together. To determine how an intervention affects SC and how it may relate to FC, structural-functional connectivity (SC-FC) coupling can be calculated.^[181] However, the nodes should correspond between the matrices and therefore, the same atlas should be used during the processing steps. Any thresholding methods should be consistent and carefully chosen.

5.4.3 Structural-Functional Connectivity Coupling

Some studies have shown a direct structural-functional connectivity (SC-FC) correlation across the brain,^[182;183] but notable discrepancies remain. For example, fixed structural connections can produce diverse functional patterns.^[184] Another study showed that interhemispheric FC can be

preserved even in the absence of major commissural fibers.^[185] These findings challenge the assumption that no anatomical pathway means no functional communication.^[186]

In ischemic stroke patients, abnormal SC-FC coupling and significantly lower structural global efficiency have been observed compared to healthy controls.^[181] Additionally, significantly lower local efficiency was associated with greater motor impairment. While these findings highlight the critical role of SC-FC coupling and how it relates to motor impairment after ischemic stroke, research is still lacking on how adjunctive acupuncture influences this relationship and its potential impact on motor recovery. Consequently, it limits our ability to evaluate its potential role in enhancing motor recovery via modulation of structural and functional brain connectivity.

Chapter 6

Problem statement

The mechanisms of adjunctive acupuncture in patients with unilateral limb dysfunction due to ischemic stroke remain unclear. A review of the literature indicates that the effect of adjunctive acupuncture on structural connectivity (SC) and functional connectivity (FC), as well as the SC-FC coupling, has not previously been examined. Furthermore, knowledge of network changes measured using graph theory for structural connectivity data and their correlation with clinical variables is limited. This gap provides a novel avenue for research.

6.1 Hypotheses

We hypothesize that adjunctive acupuncture will modulate structural and functional networks on a whole-brain scale in ischemic stroke patients so that it more closely resembles a small-world network topology compared with pre-treatment conditions. We further hypothesize that the increased functional brain connections will be involved in motor function. Furthermore, functional connectivity changes will reflect structural connectivity changes. Finally, we expect these neural changes to be associated with improved motor recovery, reflected by higher FMA values in ischemic stroke patients with unilateral limb dysfunction.

6.2 Aim and Objectives

6.2.1 Aim

To determine the effect of adjunctive acupuncture on the SC, FC, SC-FC coupling, and brain network topology, and to assess how these measures relate to motor recovery in ischemic stroke patients with unilateral limb dysfunction.

6.2.2 Objectives

Objective 1: Calculate FC networks using mean correlated time series, and SC networks using mean FA values across brain regions defined by the AAL116 atlas, before and after treatment in ischemic stroke patients with unilateral limb dysfunction, randomized into three groups: (1) True Acupuncture (TA), (2) TA combined with Physiotherapy (PT), and (3) PT combined with Sham Acupuncture (SA; placebo or non-acupoint stimulation).

Objective 2: Identify and compare the functional brain connections that show significant changes after versus before treatment, for the following groups: (1) TA, (2) TA+PT, and (3) SA+PT, and extract the mean FA values of the corresponding connections from the SC matrices.

Objective 3: Compare the SC-FC coupling across significant brain connections in the three treatment groups.

Objective 4: Compare brain network topology using graph theoretical measures in the 1) TA, 2) TA+PT, and 3) SA+PT groups before and after treatment.

Objective 5: Evaluate the relationship between graph-theoretical measures and motor recovery, as measured by the FMA, across the three treatment groups for both SC and FC networks.

Chapter 7

Methodology

7.1 Study-cohort

Nineteen right-handed male ischemic stroke patients, aged (57 ± 7.51) with basal ganglia infarcts displaying unilateral limb dysfunction were recruited from the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine.

7.1.1 Inclusion Criteria

First-time male ischemic stroke survivors with unilateral limb dysfunction following a moderate ischemic stroke in the basal ganglia or motor cortex were recruited. Enrollment occurred within two weeks of the stroke onset, employing WHO standards for stroke diagnosis. Eligible candidates exhibited sufficient unilateral limb dysfunction, allowing them to walk using a walking frame and perform dressing tasks independently or with assistance. Recruitment exclusively targeted individuals eligible for a 15-session rehabilitation program (5 sessions each week for 3 weeks).

7.1.2 Exclusion Criteria

Patients presenting with intracerebral hemorrhage were not considered for inclusion. Additionally, individuals meeting any of the following criteria were excluded from participating in the study: (1) experiencing a stroke in the corpus callosum, (2) reliant on assistance for daily activities before the current stroke event, (3) unable to complete the prescribed treatment regimen, (4) exhibiting infections at acupuncture sites, (5) suffering from severe aphasia or unconsciousness that hinders comprehension or cooperation in the study's assessments, (6) affected by other serious complications or concurrent health conditions such as heart or renal failure, (7) having HIV-positive status

or diabetic limbs (diabetes-affected legs or foot with complications such as neuropathy, poor circulation, ulcers or infection), (8) unwillingness to undergo acupuncture or hypersensitivity to sensory stimuli, (9) multiple stroke occurrences, (10) previously diagnosed with clinical stroke, including minor strokes detected in the MRI obtained for the study, (11) undergoing thrombolysis and/or thrombectomy procedures, and (12) having any metal implants.

7.2 Treatment Intervention: Adjunctive Acupuncture

The Xing Nao Kai Qiao needling method, effective for motor recovery in ischemic stroke patients, was used. The following acupoints were manually stimulated and included (**Figure 7**): PC6, GV26, SP6, and BL40 acupoints. Electroacupuncture was used for the GB34 and GB40 acupoints, which involved passing a small electric current between two needles inserted as electrodes, with at least one at the acupoint. For sham acupuncture, the stimulation occurred 10 mm lateral to acupoints, except for GV26, where stimulation was 5 mm horizontally. Deqi was achieved for true acupuncture but not for sham acupuncture.

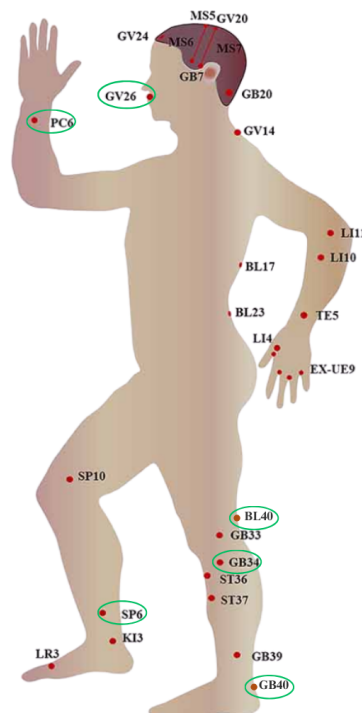


Figure 7: **Locations of the six acupoints (green oval) used in the Xing Nao Kai Qiao needling method.** The acupoints PC6, GV26, SP6, and BL40 were manually stimulated, while electroacupuncture was applied to the GB34 and GB40 acupoints. Figure adapted from. Ma et al.^[110]

7.3 Study Design

Participants were randomized into three treatment groups: 1) True Acupuncture (TA; n=5), 2) TA and Physiotherapy (PT; n=6), and 3) PT and Sham Acupuncture (SA, a placebo or non-acupoint acupuncture; n=8). Patients underwent 5 acupuncture and physiotherapy sessions per week for 3 weeks, resulting in 15 sessions in total conducted by an acupuncturist and physiotherapist, respectively. The treatment groups are represented in **Table 1** and involved:

- **True Acupuncture (TA):** Patients in this group received acupuncture stimulation 5 times per week for 3 weeks at the six acupoints and experienced Deqi, but did not receive physiotherapy.
- **TA and Physiotherapy (PT):** Patients in this group received acupuncture stimulation at the six acupoints, experienced Deqi, and also received physiotherapy for 5 sessions per week for 3 weeks.

- **PT and Sham Acupuncture (SA):** Patients in this group received acupuncture stimulation at non-acupoints (placebo or non-acupoint acupuncture), where Deqi was not expected, and also received physiotherapy for 5 sessions per week for 3 weeks.

Table 1: **Treatment design for ischemic stroke patients.** Treatment regimen for patients with moderate basal ganglia infarcts and unilateral limb dysfunction following recruitment. The regimen was administered over 4 weeks, starting at Week 0 and continuing through Week 3. *TA= True Acupuncture, SA=Sham Acupuncture, PT=Physiotherapy, PE=Physical Examination, MRI=Magnetic Resonance Imaging*

Treatment groups	Week 0	Week 1	Week 2	Week 3
TA (n=5)	MRI+PE	TA(x5)	TA(x5)+PE	TA(x5)+MRI+PE
TA+PT (n=6)	MRI+PE	TA+PT(x5)	TA+PT(x5)+PE	TA+PT(x5)+MRI+PE
SA+PT (n=8)	MRI+PE	SA+PT(x5)	SA+PT(x5)+PE	SA+PT(x5)+MRI+PE

7.4 Physical Examination

7.4.1 Fugl-Meyer Assessment (FMA)

The Fugl-Meyer Assessment (FMA) is a quantitative method for measuring the recovery in hemiplegic stroke patients.^[187] It is superior to other methods such as the National Institutes of Health Stroke Scale (NIHSS) because it captures the dynamic process of motor recovery and can detect changes in response to different treatments. The FMA uses a 226-point scale divided into 5 domains: 1) motor function, 2) sensation, 3) balance, 4) joint range of motion, and 5) joint pain. The scoring is based on direct observation of performance, with the items for each domain classified using an ordinal scale: 0: cannot perform, 1: performs partially and 2: performs fully. Scores for each domain are calculated as follows:^[187]

- Motor score: 0-100 points (66 upper extremity, 34 lower extremity).
- Sensation: 0-24 points (8 light touch, 16 position sense).
- Balance: 0-14 points (6 sitting, 8 standing).
- Joint range of motion: 0-44 points.
- Joint pain: 0-44 points.

It is important to note that the FMA is used to measure motor recovery and not functional recovery.^[188] Motor recovery is the restoration of movement, balance, and coordination, whereas functional recovery refers to the restoration of daily activities such as walking and eating. This means that even if stroke patients regain motor control on one side of their body, they might not be able to perform all functional tasks. The recruited patients underwent FMA before and after treatment. These assessments were conducted by a physiotherapist.

7.5 Image-and Data Acquisition

The patients were scanned and imaged on a 3T Skyra MRI (Siemens, Erlangen, Germany) before and after treatment intervention. The DTI parameters were: (1 reference image with $b=0$ s/mm² and 64 direction with $b=1000$ s/mm², 70 slices, TR=8200 ms, TE=75 ms, FOV=220 mm, voxel size $2.0 \times 2.0 \times 2.0$ mm³) with posterior-anterior (PA) phase encoding. T1-weighted structural imaging was performed using an MPRAGE sequence (TR=2000 ms, TE=1.97 ms, flip angle=8°, FOV=256 mm, voxel size $1.0 \times 1.0 \times 1.0$ mm³). For rs-fMRI data, a gradient echo EPI sequence (TR = 2000 ms, TE = 30 ms, flip angle 90°, FOV= 220 mm, voxel size $3.4 \times 3.4 \times 3.0$ mm³, was used.

7.6 Functional Connectivity

7.6.1 Neuroimage Pre-processing: rs-fMRI data

Pre- and post-processing of the rs-fMRI data was performed (**Figure 8**) using the CONN Toolbox (version 18.b, standalone edition for Windows), created by the Massachusetts Institute of Technology (MIT).^[189] Firstly, the experiment was set up in the “Basic” window of the toolbox as follows (**Appendix A, Figure 33**): Number of subjects = 19, number of sessions = 2, repetition time (TR) = 2, acquisition type = continuous. The T1 images in NIfTI format (before data = session 1 and after data = session 2) were imported. The same was done for the rs-fMRI data. The AAL116 atlas^[176] was imported into the ROI section and assigned to all subjects (“Session-specific ROI” instead of “Subject-specific ROI”) from which mean BOLD time series were extracted.^[189] The AAL116 atlas brain regions are shown in **Appendix A (Table 25 and Figure 34)**. The files were visually inspected by selecting the montage from the “Structural Tools” and “Functional Tools” buttons, respectively (**Appendix A, Figure 35-36**). CONN’s default MNI pipeline was used for preprocessing.

Functional and anatomical data underwent preprocessing through an extensive pipeline,^[190] slice-timing correction, outlier identification, direct segmentation, normalization to MNI space, and smoothing. Susceptibility distortion correction (SDC) was not performed because no field maps were acquired. In detail, functional data realignment utilized the Statistical Parametric Mapping (SPM12) realign and unwarp method,^[191] coregistering all scans to the first scan of the initial session via a least squares approach with a 6-parameter rigid body transformation,^[192] followed by resampling with b-spline interpolation to address motion and susceptibility artifacts. To correct temporal misalignment in the functional data, acquired in Siemens interleaved order, the SPM12 slice-timing correction (STC) procedure^[193;194] was applied, using sinc interpolation to align each slice's BOLD time series to a common mid-acquisition time point.

Potential outlier scans were detected using the Artifact Detection Tools (ART),^[195] flagging acquisitions with framewise displacement (FD) exceeding 0.5 mm or global BOLD signal changes surpassing 3 standard deviations.^[196;197] These flagged outlier volumes were stored as dummy variables and automatically included in the denoising pipeline and regressed out. Thus, the variance uniquely associated with these outlier volumes was modeled and removed, without altering the number of volumes. In CONN, motion covariates were calculated as the average maximum framewise displacement (FD) across sessions (pre- and post-treatment) for each subject, using raw data prior to scrubbing and denoising. FD was derived from the six motion parameters by summing absolute differences between consecutive volumes. These covariates were later controlled for at the second-level analysis to account for voxel shifts and artificial signal fluctuations. The technique above differs from the Euclidean method for the DWI data as it uses absolute values and not geometric distances. The Euclidean method captures translational motion, which is the primary source of misalignment in DWI data, whereas the FD method captures translational and rotational motion.^[198] For each subject, a reference BOLD image was generated by averaging all

scans, excluding identified outliers. Functional and anatomical data (T1 images) were normalized into standard MNI space, segmented into grey matter, white matter, and CSF tissue classes, and resampled to 1 mm and 2 mm isotropic voxels for the anatomical and functional data, respectively (**Appendix A, Figure 37-42**). This was done directly after a normalization procedure^[197;199] using the SPM12 unified segmentation and normalization algorithm.^[200;201] Last, functional data were smoothed using spatial convolution with a Gaussian kernel of 6 mm full-width half maximum (FWHM).^[202] The experimental conditions were defined as shown in **Table 2 and Appendix A (Figure 43-44)**.

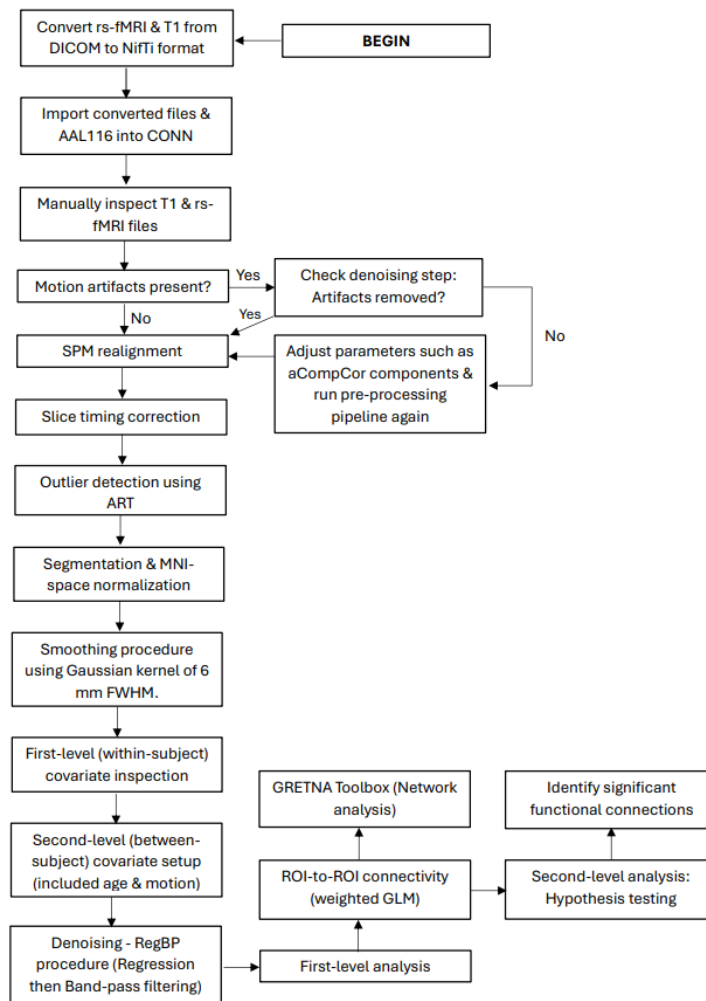


Figure 8: **Processing pipeline for functional connectivity analysis using the CONN toolbox for all three groups, before and after treatment.** Groups included: TA (n=5), TA+PT (n=6), and SA+PT (n=8). Resting-state fMRI data were preprocessed with QC to mitigate motion artifacts (e.g., blurring, ghosting). FC matrices captured time-invariant correlations between AAL116 regions, and are exported to GRETNA for graph-theory analysis, and integrated with structural connectivity (SC) data from the same AAL116 atlas parcellation to evaluate SC-FC coupling.

Table 2: **Experimental design for first-level functional connectivity analysis in the CONN toolbox.** Pre-post setup with Session 1 (before) and Session 2 (after) data across three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8).

Design	Condition Name	Session 1		Session 2	
		Onset	Duration	Onset	Duration
Pre-post design	Pre	0	inf	–	–
	Post	–	–	0	inf

After running the pre-processing pipeline, first-level (within-subject) covariates, namely scrubbing, realignment, and quality QC timeseries were created (**Appendix A, Figure 45**). Scrubbing included outlier scans identified by ART-based scrubbing, while realignment contained the 6 rigid-body parameters characterizing the estimated subject motion for each subject/session. Functional data were denoised using a standard pipeline,^[190] involving regression of confounding effects, including white matter time series (5 Component-based Noise Correction (CompCor) components), CSF time series (5 CompCor noise components), motion parameters with their first-order derivatives (12 factors),^[203;204] outlier scans (up to 30 factors),^[196] session effects with their first-order derivatives (2 factors), and linear trends (2 factors) within each functional run. The BOLD time series was then subjected to bandpass filtering between 0.01 Hz and 0.1 Hz.^[205] Thus, a Regression followed by Band-Pass (RegBP) procedure, where band-pass filtering is done after regression, was used for denoising. Linear detrending was used, but despiking was not applied, as despiking has been shown to cause misalignment in brain volumes.^[206] CompCor^[207;208] noise components within white matter and Cerebral Spinal Fluid (CSF) were estimated by computing the average BOLD signal as well as the largest principal components orthogonal to the BOLD average, motion parameters, and outlier scans within each subject's eroded segmentation masks. Quality control (**Appendix A, Figure 46**) showed no adjustments were needed as the denoising did a sufficient job at removing outliers as seen in **Appendix A (Figure 47)**. For first-level functional connectivity analysis, the RRC (ROI-to-ROI Connectivity) was chosen to compute the ROI-to-ROI correlation matrices between the set regions of interest. RRC matrices were estimated, charac-

terizing the patterns of functional connectivity with 116 AAL ROIs. Functional connectivity strength was represented by Fisher-transformed bivariate correlation coefficients from a weighted general linear model (GLM),^[190] defined separately for each pair of target areas, modeling the association between their BOLD signal time series. To determine the significant brain connections for each hypothesis (**Appendix A, Table 26**), the threshold parameters included: selected all ROIs for analysis, threshold ROI-ROI connections by intensity, one-sided (positive) and one-sided (negative) that were thresholded at a significance level of $p < 0.05$ (FDR-corrected) to control for multiple comparisons at the seed-level.

Importantly, the one-sided negative test reflected a relative reduction in correlation strength as determined by the specified contrast (e.g., condition A $>$ condition B), rather than an absolute designation of the connections' initial state (ie. BOLD anti-correlated signals) as positive or negative.^[189;209;210] For example, connections that shift from positive to less positive, from negative to less negative, or from positive to negative are considered decreased connections. The connections between ROIs in the brain were visualized using "Display3d".^[195] A list of the 116 ROI names was imported to display on the brain images for those connections that survived.

7.7 Structural Connectivity

7.7.1 Neuroimage Pre-processing: DWI data

Diffusion-Weighted Imaging (DWI) and T1-weighted images were acquired before and after the 15 treatment sessions. The images were converted from DICOM to NIfTI (Appendix B. 12.2.1) using the dcm2niix tool.^[211] Subjects moving during a scan could introduce motion artifacts that could cause misaligned volumes, disrupting the consistency needed for calculating diffusion directions. Therefore, motion was considered a potential confounder as it introduces unwanted variability that can either obscure or mimic brain signals of interest. Motion parameters were calculated using the mcflirt command from the FSL suite.^[212] The output was a text file with the 6 degrees of freedom

in a rigid-body transformation: 1) rotation around x-axis (pitch, in radians), 2) rotation around y-axis (roll, in radians), 3) rotation around z-axis (yaw, in radians), 4) translation along x-axis (mm), 5) translation along y-axis (mm), 6) translation along z-axis (mm).^[192] The maximum values for each of the six motion parameters were calculated across all the volumes separately for the pre-treatment and post-treatment data. The Euclidean norm (square root of the sum of squares) was calculated by treating each motion parameter as contributing to a single value, the maximum total displacement. This was done for all subjects across all groups and saved for use in statistical analysis.

The converted images were quality-checked. No volumes were removed, as none had motion artifacts. The images were then imported into TORTOISE using ImportNIFTI, which produced the following four output files: 1) info file, 2) bmtxt file, 3) list file, and 4) nii file.^[179] Automatic Anterior Commissure-Posterior Commissure (AUTO ACPC) Detect was used to align the T1-weighted (T1w) contrast images to the default T1 template.^[213] The DIFF_PREP module was used for correcting DWI artifacts such as motion and eddy current distortion.^[213] Then the auto-acpc-aligned T1w image was used as a template to reorient the T2-weighted (T2w) image to the same standard position.

The distortion and motion-corrected (DMC) files were inspected for any interleave slice motion. Translation along the y-axis contributed mostly to the Euclidean norm (enorm) indicated in green (**Appendix B, Figure 48**). Echo-Planar Imaging (EPI) distortion correction using DR_BUDDI was not performed as data was not acquired in both AP and PA directions.^[214] Instead of using DIFF_CALC for tensor fitting, the fat_proc_dwi_to_dt command from the FATCAT (Functional And Tractographic Connectivity Analysis Toolbox) module within AFNI was used.^[215] Before proceeding, the GradFlipTest was performed to ensure all the gradients were “flipped”, if needed, to be consistent with processing in AFNI.^[216] The fat_proc_dwi_to_dt command used the preprocessed DWI

data and computed the diffusion tensor for each voxel in the brain. The T1 MRI images were processed using the Freesurfer command, recon-all, performed by an MSc graduate, Mrs Soné Fouché, to perform cortical reconstruction and subcortical segmentation, generating a variety of brain volume measures.^[217] The estimated total intracranial volume (eTIV) was used as a potential covariate, as differences in brain size across subjects can influence tractography results and network analysis. For example, more WM could result in spurious longer tracts.^[218] An overview of the DTI processing pipeline is shown in **Figure 9**. with coding script provided in **Appendix B**.

7.7.2 Tractography

The network nodes or ROIs were defined using the automated anatomical labeling (AAL116) atlas, which is in Montreal Neurological Institute (MNI) space, with brain regions defined in **Appendix A (Table 25)**. The output nodes were then inversely warped back to the native space of each participant.^[176] 3dTrackID was used to trace and quantify white matter fiber tracts between the predefined 116 ROIs in the brain, based on diffusion tensor data obtained using fat_proc_dwi_to_dt.^[215] 3dTrackID uses three modes: 1) deterministic, 2) mini-probabilistic, and 3) (full) probabilistic. The (full) probabilistic mode was used, which utilizes the uncertainty in the estimated diffusion tensor ellipsoids and Monte-Carlo simulations to find likely WM voxels associated with the ROI. The termination condition for the fiber tracking was kept as the default: 1) the max angle for turning when going to a new voxel during propagation was 60°, and 2) the threshold for the Fractional Anisotropy (FA) map was 0.2.^[219] The resulting .grid file was used in the subsequent step as it contains all the structural connectivity matrices for the different DTI parameters (FA, MD, L1, and RD), where quantities were mean values in each tract. The matrices for the FA parameter for all participants were extracted and inspected to ensure that they were 116 by 116.

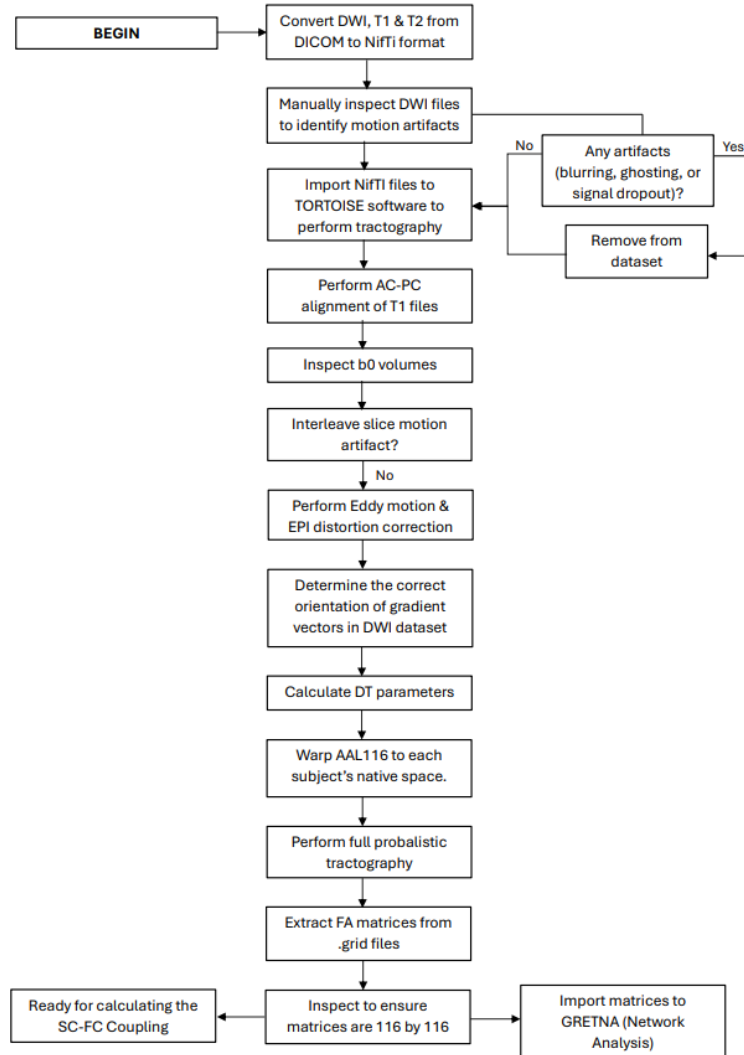


Figure 9: **Overview of Diffusion Tensor Imaging (DTI) processing pipeline across groups, before and after treatment.** The workflow for processing DWI data for all subjects in three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8). In short the pipeline shows DWI, T1, and T2 images were converted from DICOM to Nifti format, quality control (QC) was performed to identify and address any artifacts in the imaging data, the AAL116 atlas was warped to each subject's native space for region-specific analysis, tractography was conducted to calculate fractional anisotropy (FA) values, mapping white matter tracts. Connectivity matrices were generated based on the tractography results, providing structural connectivity data. The connectivity matrices were used for downstream analyses, including graph theoretical calculations and assessment of coupling strengths, performed consistently across all subjects in the three different groups.

7.8 Graph-Theory-Based Analysis for Structural and Functional Networks

The Graph Theoretical Network Analysis (GRETNA) toolbox (v2.0.0) was used to calculate the same graph theoretical measures for both the structural and functional connectivity matrices.^[220]

The following parameters were set: 1) the sign of the network: positive, 2) thresholding method:

network sparsity, 3) threshold sequence: 1 x 10 double, 4) network type: weighted, 5) random network generation: 1000.

Network sparsity refers to the ratio of the number of actual edges divided by the maximum possible number of edges in a network. A positive network was chosen to negate any detrimental effects on Test-Retest (TRT) reliability.^[221] The threshold sequence specifies the range of continuous threshold values applied in the analysis. The weighted network type is preferred because it is more robust in identifying subtle network organizations compared to binary networks. Finally, random network refers to networks generated for comparison purposes to test whether real brain networks are significantly non-random. These random networks are created using a Markov wiring algorithm, which preserves the same number of nodes and edges and maintains the degree distribution of the real networks.^[220]

The following global network metrics were calculated: small-worldness and efficiency. For the nodal network metrics, the clustering coefficient, shortest path length, and local efficiency were calculated.

7.9 Statistical analysis

7.9.1 Demographics

Between-group differences in age, framewise displacement (motion for rs-fMRI data), Euclidean norm (motion for DWI data), and brain volume between the three groups were assessed using one-way ANOVA (**Appendix C.12.3.1-2**). Within-group differences were assessed using the paired t-test. Brain volume was quantified as the estimated total intracranial volume (eTIV), which was reported in cubic millimeters (cm³). Fisher's Exact Tests were conducted to examine whether the distribution of laterality (Left vs. Right) differed across the three treatment groups, TA (n=5), TA+PT (n=6), and SA+PT (n=8). The laterality variable was then recoded such that values of 1 and 2 corresponded to "Right" and "Left," respectively (**Appendix C, Table 27**). When the infarct side was unknown, "0" was used.

Pairwise group comparisons were performed for the following combinations: (i) TA vs. TA+PT, (ii) TA vs. SA+PT, and (iii) TA+PT vs. SA+PT. For each comparison, a 2×2 contingency table was generated, representing the counts of Left and Right for each group. Fisher's Exact Test was applied to these tables to calculate the odds ratio and corresponding p-value, providing a measure of association between treatment group and laterality (**Appendix C.12.3.4**). In addition, 95% confidence intervals for the odds ratios were computed using the standard log-transformation method. In cases where any cell of the contingency table contained a zero, confidence intervals were not estimated to avoid division-by-zero errors. A significance threshold of $p \leq 0.05$ was applied.

7.9.2 Functional Connectivity Analysis

Functional connectivity (FC) was quantified using bivariate correlation coefficients of the resting-state time series. Differences in connectivity between conditions (e.g., pre- vs. post-intervention) within the same subjects were assessed using a general linear model (GLM) implemented in the

CONN Toolbox. For within-subject contrasts, the GLM employed a paired t-test framework applied to the Fisher-transformed correlation coefficients. For post-treatment between-group comparisons, a two-sample t-test was performed. The resulting output included T-statistics for each ROI-to-ROI connection, along with uncorrected (p-unc) and false discovery rate-corrected (p-FDR) p-values. Age and motion were adjusted for, and statistical analysis was performed in the CONN Toolbox. FC matrices were extracted from the CONN toolbox, with each matrix entry representing a Fisher Z-transformed correlation coefficient between a pair of ROIs. The diagonal elements of these matrices were NaN as a result of self-correlations. Before importing the matrices into the GRETNA toolbox, the Fisher Z values were converted to Pearson correlation coefficients (r -values) using the inverse Fisher transformation:

$$r = \frac{e^{2z} - 1}{e^{2z} + 1},$$

where z is the Fisher Z-transformed correlation coefficient. For statistical analysis, these r -values were then reconverted to Fisher Z values.

$$z = \frac{1}{2} \ln \frac{1 + r}{1 - r},$$

where r represents the Pearson correlation coefficient. This procedure ensured that the diagonal elements were no longer NaN and also helped approximate a more normal distribution of the connectivity values, which is desirable for parametric statistical analyses.^[222] This was performed in MATLAB R2018a (v. 9.4.0.813654, MathWorks, Natick, MA, USA) (**Appendix C.12.3.5-6**).

7.9.3 Structural Connectivity Analysis

The structural connectivity (SC) matrices obtained from the 3dTrackID analysis were used to extract mean FA values. The selection of these regions was not exploratory but rather hypothesis-

driven, guided by the significant functional connections identified in the CONN toolbox. In other words, the SC analysis focused only on the regions associated with the FC results, and no additional exploratory statistical tests were conducted on the SC matrices to independently identify significant brain regions. For each treatment group, FA values of all subjects were first aligned by connection and then averaged. Specifically, connections were grouped within each treatment arm (TA, TA+PT, SA+PT), and the arithmetic mean of the FA values was computed separately for the pre-treatment (“Before”) and post-treatment (“After”) conditions. These group-averaged values were then used to populate the symmetric adjacency matrices, providing a representation of the mean structural connectivity strength between brain regions for each group before and after treatment (**Appendix C.12.3.7**)

7.9.4 Structural-Functional Connectivity Coupling

The statistical analysis was conducted to evaluate the relationship between SC, expressed as FA values, and FC, expressed as Fisher Z-transformed correlation coefficients. Importantly, the absolute Fisher Z-values were used as raw rs-fMRI data might show anti-correlations (not investigated in this study). For each subject, SC and FC values were extracted for the same 13 brain region connections that had been identified as significant in the FC analysis and involved in the 5 FMA domains: 1) motor function, 2) sensation, 3) balance, 4) joint range of motion, and 5) joint pain. Pearson correlation analyses were then performed separately for each treatment group, TA (n=5), TA+PT (n=6), SA+PT (n=8), and for each time point (pre-treatment and post-treatment). Importantly, no averaging across subjects or connections was performed; instead, the raw subject-level values were used directly. This means that each scatter plot contained one point per connection per subject, resulting in a total of 13 data points for each subject (**Appendix C.12.3.8 and Figure 51-52**). Consequently, each Pearson correlation analysis was based on 65 data points for the

TA group (13 connections $\times$ 5 subjects), 78 data points for the TA+PT group (13 connections $\times$ 6 subjects), and 104 data points for the SA+PT group (13 connections $\times$ 8 subjects).

For each group and time point, linear regression lines were fitted to the data, and Pearson's correlation coefficient (r) together with its associated p -value were calculated to assess the strength and statistical significance of the SC–FC relationship. A p -value of ≤ 0.05 was considered statistically significant.

7.9.5 Graph Theoretical Measurements

Graph measures were compared before and after treatment for the three independent groups, TA ($n=5$), TA+PT ($n=6$), and SA+PT ($n=8$) in both the SC and FC data. For the SC data, the following covariates were considered: Side, age, motion, and brain volume. For the FC data, the following covariates were considered: Side, age, and motion. Brain volume was not chosen as a potential covariate for the FC data, as rs-fMRI data were normalized to MNI space, aligning brain volumes across subjects and ensuring the region-based BOLD signal is comparable. Although several covariates showed significant associations with the dependent variables after the Pearson correlation analysis, motion was prioritized for the SC and FC data (**Appendix C. 12.3.9-12**). This decision reflects the fact that, despite being addressed during preprocessing, motion effects are not fully eliminated and residual artifacts can persist, potentially biasing the final results.^[223] Although overall head motion was minimal across the five motion parameters for DWI data, translation along the y -axis (in mm) showed the greatest variability and contributed most to the Euclidean norm (**Appendix B, Figure 48**). Only the motion covariate was chosen, as the sample size was very small, and including multiple covariates could reduce the statistical power and increase the chance of multicollinearity.^[224] Repeated-measures ANCOVA was used to adjust for motion within groups, where motion was defined as the mean of pre- and post-treatment values, since motion did not significantly change after treatment compared to baseline in the DWI data (**Table 3**). One-way

ANCOVA was used for between-group analysis, with pre-treatment motion values from the DWI data included as a covariate for baseline comparisons and post-treatment motion values included for follow-up comparisons (after treatment). For functional graph measures, the same motion covariates obtained from the CONN toolbox were used for within and between-group analysis. Given the influence of age on brain networks,^[225] age was included as a covariate in the GLM to control for its effects when identifying significant functional connections in the CONN toolbox.

The following assumptions of ANCOVA were tested first: normality, linearity, homogeneity of regression slopes, homogeneity of variance, and multicollinearity.^[226] The assumption of homogeneity of regression slopes was violated for some of the dependent variables (**Appendix C.12.3.13-14**); however, ANCOVA was still used with the severity of the violation discussed in the limitations section.^[227] The t-test for pairwise comparisons of adjusted means (post-ANCOVA) was used for significant between-group differences (p-value ≤ 0.05) to determine exactly where the differences occurred.^[228] Importantly, the winsorized data were used for statistical analysis due to the small sample size. Winsorization is a procedure that reduces the influence of extreme values (outliers) by replacing the most extreme data points with the nearest values that fall within a specified range.^[229] Specifically, values below the lower bound are set to the lower bound, and values above the upper bound are set to the upper bound (**Appendix C.12.3.15**). In this study, the lower bound was defined as $Q_1 - 1.5 \times \text{IQR}$ and the upper bound as $Q_3 + 1.5 \times \text{IQR}$, where Q_1 and Q_3 are the first and third quartiles, and IQR is the interquartile range ($Q_3 - Q_1$). This approach preserves the rank order of most data points while limiting the impact of extreme values on statistical analyses. It was also used to preserve the sample size, as the groups were small: TA (n=5); TA+PT (n=6); SA+PT (n=8), and removing outliers would have further reduced statistical power.

7.9.6 Correcting for Multiple Comparisons

Pairwise Sidak tests were used as a post-hoc test to correct for multiple comparisons. Sidak was chosen as it is a more robust test for small sample sizes and less conservative than the Bonferroni test.^[230] Pairwise Sidak tests were applied for all statistical analyses, while p-FDR correction was used to account for multiple comparisons in the GLM when identifying significant functional brain connections.

For all analyses, the raw data were plotted instead of the winsorized data. Mean values with standard deviations (SD), standard error of the mean (SEM), and effect sizes, and p-values were recorded for all statistical analyses. For ANCOVA, three different p-values were recorded: the p-unadj (before adjusting for motion), p-adj (after adjusting for motion), and p-Sidak (after adjusting for motion and correcting for multiple comparisons). The effect size for ANCOVA was recorded as partial eta-squared (η^2_p) and for ANOVA as eta-squared (η^2). For pairwise comparisons, the effect sizes, absolute Cohen's d and Hedge's g (correcting for small sample size) was provided. Statistically significant p-values were shown in bold in the tables.

7.9.7 Pearson Correlations of FMA with Graph Measures

Pearson correlation was used to investigate the relationship between FMA and the structural and functional graph measures, respectively. Due to the small sample size, the pre-treatment and post-treatment data were pooled together to increase the effective sample size and obtain a more reliable estimate of the correlations between graph measures and FMA scores. The paired t-test was used for within-group analysis to compare the pre-treatment and post-treatment FMA scores. The FMA post-treatment results were compared between the three different groups using ANOVA. The effect size, Cohen's d was recorded as an absolute value where the T-statistic gave the direction of the effect. All statistical analysis and generation of figures were done in Google Colab

(Python 3.10, Google LLC, Mountain View, CA, USA), with the code script in **Appendix C.12.3.16-17**.

All mean values in the tables were reported to three significant figures, with the corresponding standard deviation (SD) and standard error of the mean (SEM) rounded to the same number of decimal places as the mean. Descriptive statistics such as p-values were generally reported to two decimal places, unless the second decimal place did not represent a significant digit.

Chapter 8

Results

Age showed significant differences between the three treatment groups ($F = 5.81$, $\eta^2 = 0.42$, $p = 0.01$): TA, TA+PT and SA+PT (**Table 3**), with the highest mean value observed in the TA+PT group (mean $\pm$ SD (SEM): 63.83 ± 5.49 (2.24)) followed by the SA+PT group (54.75 ± 6.67 (2.36)) and the lowest value observed in the TA group (52.40 ± 5.59 (2.50)). A total of 42% (η^2) of the age variance was explained by the treatment group, showing a large effect, with the F statistic greater than 3, indicating that most of the variance was explained by the treatment group with minimal unexplained variance. FMA between groups, pre-treatment before) was not significantly different ($F = 0.34$, $\eta^2 = 0.04$, $p = 0.72$). For FMA, the analysis between groups (post-treatment comparisons) did not show significant differences ($F = 1.02$, $\eta^2 = 0.11$, $p = 0.38$). In contrast, all within-group comparisons showed significant differences ($p \leq 0.05$) with FMA after treatment being higher compared to before treatment, as indicated by a negative t-statistic (**Table 3**). The effect was small for the TA+PT ($t = -6.23$) and SA+PT ($t = -6.23$) groups, but medium for the TA group ($t = -14.06$). For structural connectivity data, the Euclidean norm (motion) and brain volume were compared between groups. Both showed no statistical significance between the groups ($p = 0.83$ and 0.40 , respectively). Within-group analysis also showed no statistically significant difference between the Euclidean norm post-treatment compared to pre-treatment.

Framewise displacement (motion) was adjusted for functional connectivity data, which was not statistically significant ($t = 0.53$, $\eta^2 = 0.06$, $p = 0.60$). The infarct hemisphere (side) did not show significant differences between the groups (**Table 4**). The number of patients with left infarcts in the

SA+PT and TA group was equal ($n = 2$). The low odds ratio (OR) in the SA+PT group compared to the TA group (OR = 0.40, $p = 0.58$, CI: [0.03,5.15]) was due to the high number of right infarcts compared to left infarcts. The odds of a left infarct in the SA+PT group compared to the TA+PT group was very small (OR = 0.08, $p = 0.10$, CI: [0.01, 1.19]). Finally, the odds of a left infarct in the TA group were 20% of those in the TA+PT group and not statistically significant, meaning that the observed difference could easily be due to chance, given the small sample size (OR = 0.20, $p = 0.5$, CI: [0.01, 3.66]).

Table 3: **Structural and functional demographic data across ischemic stroke study groups.** This table presents between-group and within-group differences for structural and functional data across three groups: TA (n=5), TA+PT (n=6), SA+PT (n=8). It includes variables such as brain volume, age, Fugl-Meyer Assessment (FMA), and motion, with mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group. Statistical metrics include p-values, Eta-squared (η^2), F-statistics for between-group comparisons, and absolute Cohen's d and T-statistics for within-group comparisons (before vs. after treatment). Note: Framewise displacement is a motion variable for rs-fMRI, and the Euclidean norm, a motion variable for DWI data. A p-value of ≤ 0.05 , was considered statistically significant.

Variables	TA (n=5)	TA+PT (n=6)	SA+PT (n=8)	p-value	η^2	F-statistic
	Mean $\pm$ SD (SEM)	Mean $\pm$ SD (SEM)	Mean $\pm$ SD (SEM)			
Between-group differences						
Age	52.40 $\pm$ 5.59 (2.50)	63.83 $\pm$ 5.49 (2.24)	54.75 $\pm$ 6.67 (2.36)	0.01	0.42	5.81
FMA (before)	20.80 $\pm$ 0.84 (0.37)	19.17 $\pm$ 4.71 (1.92)	19.00 $\pm$ 4.63 (1.64)	0.72	0.04	0.34
FMA (after)	29.00 $\pm$ 1.22 (0.55)	30.00 $\pm$ 2.90 (1.18)	26.62 $\pm$ 6.37 (2.25)	0.38	0.11	1.02
Euclidean norm (mm)	4.38 $\pm$ 1.26 (0.56)	3.92 $\pm$ 1.71 (0.70)	3.96 $\pm$ 1.22 (0.43)	0.83	0.02	0.18
Framewise displacement (mm)	2.78 $\pm$ 3.19 (1.43)	1.61 $\pm$ 1.05 (0.43)	1.78 $\pm$ 1.65 (0.58)	0.60	0.06	0.53
Brain volume (cm ³)	1487.67 $\pm$ 106.62 (47.68)	1425.95 $\pm$ 83.39 (34.05)	1517.16 $\pm$ 148.88 (52.64)	0.40	0.11	0.98

Group	Before		After		p-value	Cohen's d	T-statistic
	Mean ± SD (SEM)		Mean ± SD (SEM)				
Within-group differences							
FMA							
TA (n=5)	20.80 ± 0.84	(0.37)	29.00 ± 1.22	(0.55)	0.000	6.29	-14.06
TA+PT (n=6)	19.17 ± 4.71	(1.92)	30.00 ± 2.90	(1.18)	0.001	2.54	-6.23
SA+PT (n=8)	19.00 ± 4.63	(1.64)	26.62 ± 6.37	(2.25)	0.000	2.20	-6.23
Euclidean norm (mm)							
TA (n=5)	4.55 ± 1.86	(0.83)	4.21 ± 1.27	(0.57)	0.71	−0.176	−0.39
TA+PT (n=6)	3.74 ± 1.67	(0.68)	4.09 ± 1.85	(0.76)	0.38	0.39	0.95
SA+PT (n=8)	3.60 ± 1.05	(0.37)	4.32 ± 1.51	(0.53)	0.055	0.81	2.30

Table 4: **Group comparisons of hemispheric infarcts.** This table presents comparisons between groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8) based on the number of subjects with left and right hemispheric infarcts. The Fisher's Exact test was used for each comparison (TA vs TA+PT, TA vs SA+PT, TA+PT vs SA+PT). The table includes the count of subjects with left and right infarcts, along with statistical metrics: p-value, Odds Ratio, and 95% Confidence Interval. A p-value of ≤ 0.05 was considered statistically significant.

Group	Left	Right	p-value	Odds Ratio	95% CI
TA vs TA+PT					
TA	2	2	0.50	0.20	[0.01, 3.66]
TA+PT	5	1			
TA vs SA+PT					
SA+PT	2	5	0.58	0.40	[0.03, 5.15]
TA	2	2			
TA+PT vs SA+PT					
SA+PT	2	5	0.10	0.08	[0.01, 1.19]
TA+PT	5	1			

Note: Patients with unknown (0) infarcts were not included, thus the TA group only has 4 subjects and the SA+PT group only 7. Patient 3 and Patient 19's brain infarct was unknown as seen in Appendix C (Table 24).

8.1 Functional Connectivity

8.1.1 Within-group differences

To investigate the hypothesis (After > Before) that specifies that post-treatment yields significant functional brain connections compared to pre-treatment in the different groups: TA(n=5), TA+PT (n=6), and SA+PT (n=8), the CONN Toolbox was used. For the TA group, the following significantly increased functional connections (p-value < 0.05) were observed, post-treatment (**Figure.10.a**): Vermis_7 to Caudate_R, Vermis_7 to Thalamus_R and Hippocampus_R to Temporal_Inf_R (**Table 5**). However, significantly decreased connections were also observed (**Figure.10.b**). The decreased functional brain connections included the Supp_Motor_Area_L to the following brain regions: Heschl_L, R; Thalamus_R; Temporal_Sup_L, R; Rolandic_Oper_R, L, and the Cerebelum_7b_R to Cerebelum_8_L. Thus, these regions were significantly increased before treatment compared to post-treatment. Importantly, the hypothesis (After > Before) vs. (Before > After) informs which connections are classified as increased or decreased, making this classifica-

tion relative rather than indicating whether the raw data showed positive or negative mean time series, which were not investigated in the present study. For the TA+PT group, only the Insula_R to Vermis_1_2 ($t=4.37$, $p\text{-unc}=0.0002$, $p\text{-FDR}=0.0276$) showed a significantly increased functional connection (**Figure.11.a and Table 6**), while multiple significantly decreased connections were also observed ($p\text{-value} < 0.05$): Temporal_Pole_Mid_L to Frontal_Sup_L, Frontal_Sup_Medial_R, Cerebelum_7b_L; Amygdala_L to Frontal_Sup_Orb_L, R and Temporal_Mid_L to Vermis_7 (**Figure.11.b**). For the SA+PT group, the following significantly increased brain connections were observed: Amygdala_L to Frontal_Sup_Orb_R, Frontal_Mid_Orb_R, Frontal_Sup_R; Amygdala_R to Frontal_Sup_R, L, Cerebelum_Crus2_L, Frontal_Sup_Medial_R, Cerebelum_6_R; Supp_Motor_Area_L to Frontal_Sup_Orb_L, Fusiform_L, R; Frontal_Sup_R to Temporal_Pole_Mid_L and Olfactory_L (**Figure.12.a and Table 7**). The only decreased connection was the ParaHippocampal_R to Cerebelum_10_L ($t=-6.25$, $p\text{-unc}<0.0001$, $p\text{-FDR}=0.0007$) (**Figure.12.b**).

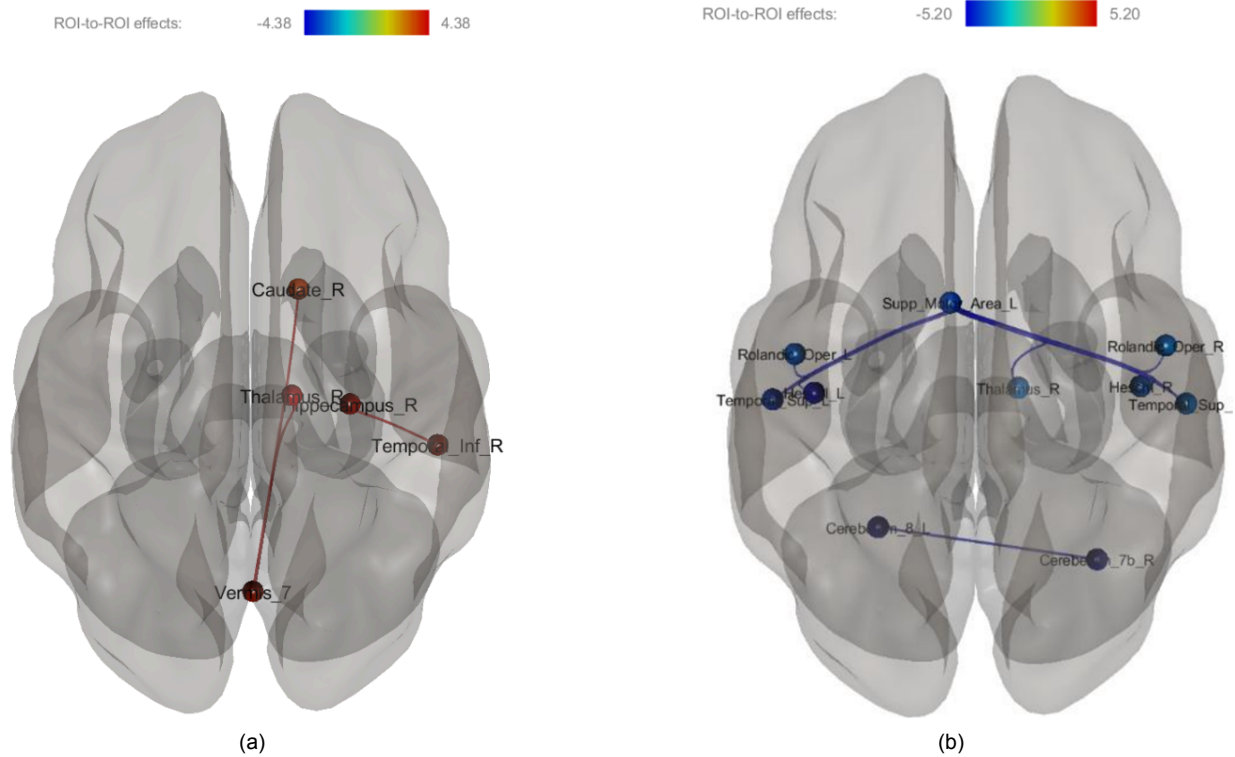


Figure 10: **Significant changes in brain functional connectivity post-treatment in the TA group, depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed at each node in the image. A color bar indicates the T-statistic values for the color-coding of brain regions.

Table 5: **Significantly increased and decreased functional connections between brain regions of interest (ROIs) were identified using a paired t-test on Fisher-transformed bivariate correlation coefficients derived from a general linear model (GLM), post-treatment compared to pre-treatment in the TA Group.** The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p -uncorrected (p -unadj) and p -FDR (False Discovery Rate-corrected) values. A p -value < 0.05 was considered statistically significant.

Connection	T-statistics	p-unc	p-FDR
<i>Increased Connections</i>			
Vermis_7-Caudate_R	4.38	0.0002	0.0218
Vermis_7-Thalamus_R	4.15	0.0004	0.0218
Hippocampus_R-Temporal_Inf_R	4.35	0.0002	0.0286
<i>Decreased Connections</i>			
Supp_Motor_Area_L-Heschl_L	-5.20	0.0000	0.0051
Cerebellum_7b_R-Cerebellum_8_L	-4.87	0.0001	0.0098
Supp_Motor_Area_L-Heschl_R	-4.30	0.0003	0.0157
Supp_Motor_Area_L-Thalamus_R	-3.92	0.0006	0.0209
Supp_Motor_Area_L-Temporal_Sup_L	-3.84	0.0007	0.0209
Supp_Motor_Area_L-Rolandic_Oper_R	-3.47	0.0016	0.0361
Supp_Motor_Area_L-Rolandic_Oper_L	-3.36	0.0020	0.0378
Supp_Motor_Area_L-Temporal_Sup_R	-3.20	0.0028	0.0459

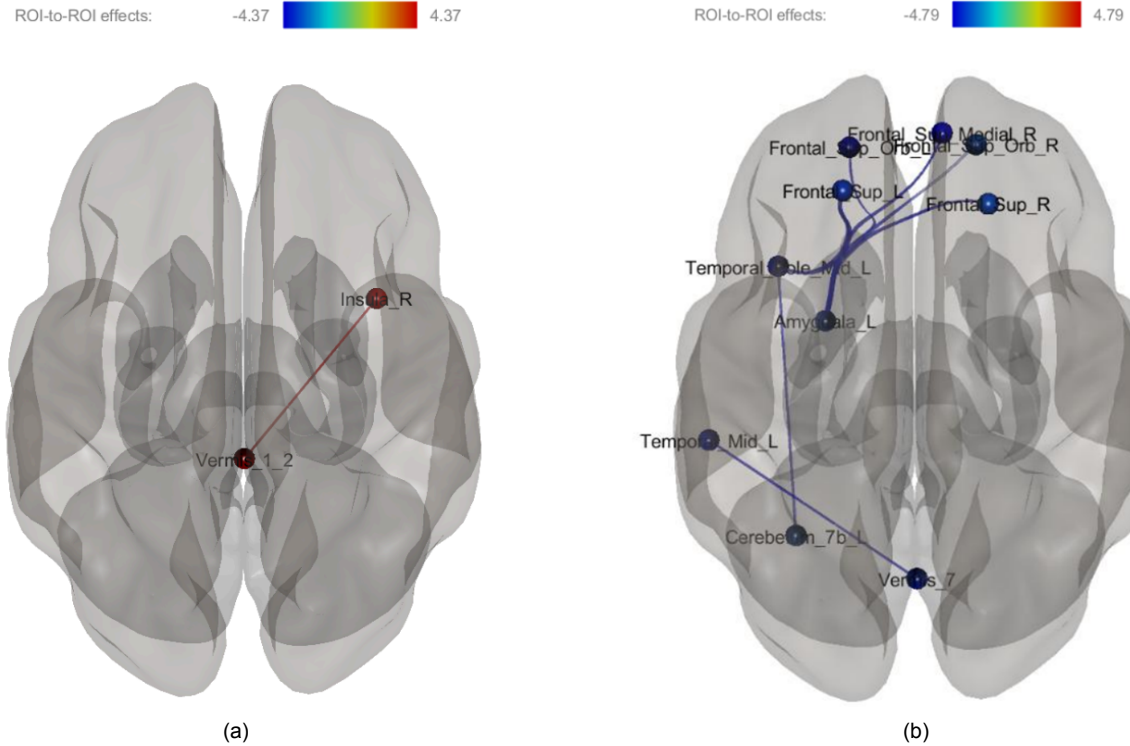


Figure 11: **Significant changes in brain functional connectivity post-treatment in the TA+PT group, depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.

Table 6: **Significantly increased and decreased functional connections between brain regions of interest (ROIs) were identified using a paired t-test on Fisher-transformed bivariate correlation coefficients derived from a general linear model (GLM), post-treatment compared to pre-treatment in the TA+PT Group.** The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p -uncorrected (p -unadj) and p -FDR (False Discovery Rate-corrected) values. A p -value < 0.05 was considered statistically significant.

Connection	T-statistics	p-unc	p-FDR
<i>Increased Connection</i>			
Insula_R-Vermis_1_2	4.37	0.0002	0.0276
<i>Decreased Connections</i>			
Temporal_Mid_L-Vermis_7	-4.79	0.0001	0.0114
Amygdala_L-Frontal_Sup_Orb_L	-4.27	0.0003	0.0325
Amygdala_L-Frontal_Sup_Orb_R	-3.96	0.0006	0.0325
Temporal_Pole_Mid_L-Frontal_Sup_L	-3.84	0.0007	0.0343
Temporal_Pole_Mid_L-Frontal_Sup_Medial_R	-3.75	0.0009	0.0343
Temporal_Pole_Mid_L-Cerebelum_7b_L	-3.74	0.0009	0.0343

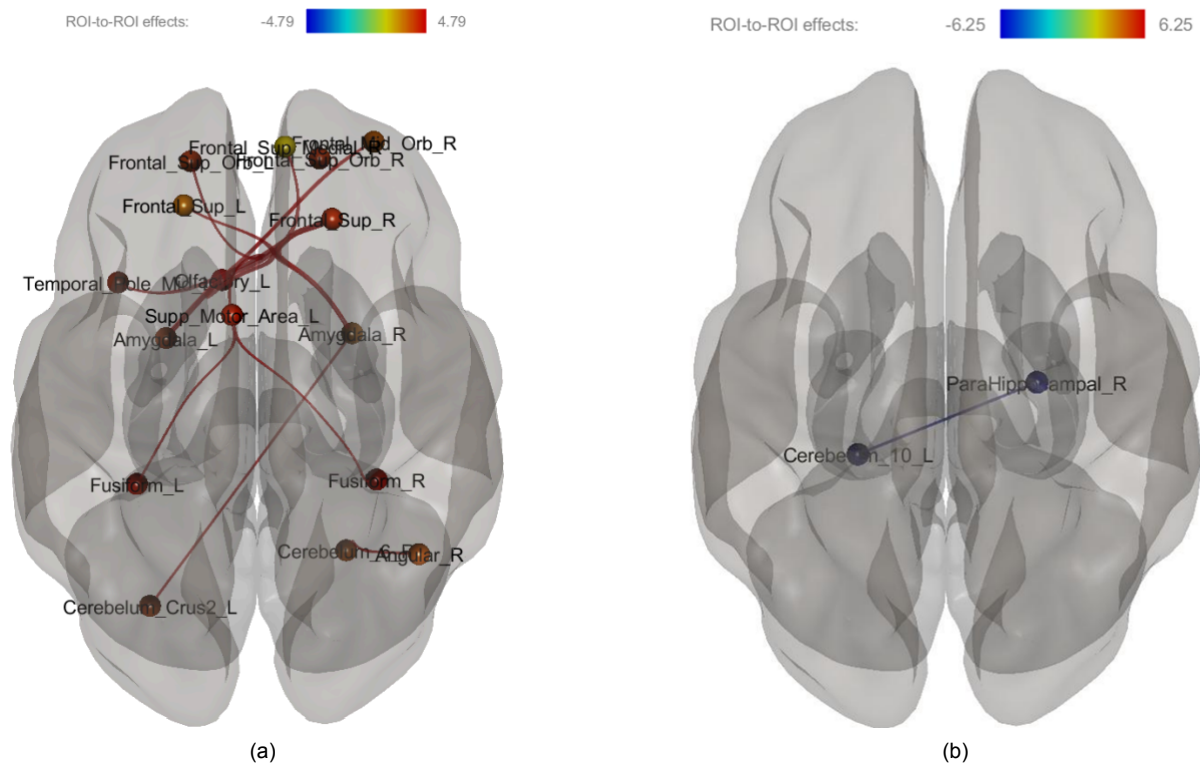


Figure 12: **Significant changes in brain functional connectivity post-treatment in the SA+PT group, depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.

Table 7: **Significantly increased and decreased functional connections between brain regions of interest (ROIs) were identified using a paired t-test on Fisher-transformed bivariate correlation coefficients derived from a general linear model (GLM), post-treatment compared to pre-treatment in the SA+PT Group.** The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p-uncorrected (p-unadj) and p-FDR (False Discovery Rate-corrected) values. A p-value < 0.05 was considered statistically significant.

Connection	T-statistics	p-unc	p-FDR
<i>Increased Connections</i>			
Amygdala_L-Frontal_Sup_Orb_R	4.79	0.0001	0.0089
Amygdala_L-Frontal_Mid_Orb_R	4.42	0.0002	0.0089
Amygdala_L-Frontal_Sup_R	4.38	0.0002	0.0089
Frontal_Sup_R-Amygdala_R	4.04	0.0005	0.0273
Amygdala_R-Frontal_Sup_L	3.79	0.0008	0.0293
Amygdala_R-Cerebelum_Crus2_L	3.73	0.0009	0.0293
Amygdala_R-Frontal_Sup_Medial_R	3.68	0.0010	0.0293
Supp_Motor_Area_L-Frontal_Sup_Orb_L	3.98	0.0005	0.0336
Supp_Motor_Area_L-Fusiform_L	3.94	0.0006	0.0336
Supp_Motor_Area_L-Fusiform_R	3.70	0.0010	0.0370
Angular_R-Cerebelum_6_R	4.17	0.0004	0.0418
Frontal_Sup_R-Temporal_Pole_Mid_L	3.62	0.0012	0.0445
Frontal_Sup_R-Olfactory_L	3.43	0.0017	0.0492
<i>Decreased Connection</i>			
ParaHippocampal_R-Cerebelum_10_L	-6.25	0.0000	0.0007

8.1.2 Between-group differences

To determine which brain regions were significantly different between the treatment groups, post-treatment, the following hypotheses were tested: 1) SA+PT > TA, 2) TA+PT > SA+PT and 3) TA+PT > TA. For the first hypothesis, functional connections in the SA+PT group significantly decreased compared to the TA group, post-treatment, with no significantly increased connections observed (**Figure 13**). The decreased brain regions included: Rectus_R and Cerebelum_6_L, Cerebelum_10_L and Parietal_Inf_L, Cerebelum_10_L and Amygdala_R, Cerebelum_6_L and Rectus_L, Hippocampus_R and Temporal_Inf_R, Cerebelum_Crus1_R and Cerebelum_4_5_L, Cerebelum_10_L and Parietal_Sup_L, Cerebelum_10_L and Cerebelum_8_L, as well as between

the Parietal_Inf_L and Cerebelum_10_L (**Table 8**). The second hypothesis showed predominantly increased functional connections: Cerebelum_Crus2_L and Frontal_Mid_L, Cerebelum_Crus2_L and Frontal_Inf_Tri_L, Frontal_Mid_L and Cerebelum_Crus2_L, Cerebelum_Crus2_L and Frontal_Inf_Oper_L, Frontal_Inf_Oper_L and Cerebelum_9_R, Vermis_9 and Vermis_3, Vermis_9 and Vermis_6, Vermis_3 and Putamen_L, Cerebelum_9_R and Paracentral_Lobule_L, as well as between the Caudate_R and Temporal_Inf_R (**Table 9**). The only significantly decreased connection involved the Olfactory_L and Angular_L. The third hypothesis revealed that the TA+PT group exhibited significantly increased functional connectivity compared to the TA group, involving the Vermis_6 and Pallidum_L, Cerebelum_Crus2_L and Frontal_Inf_Oper_L, as well as the Cerebelum_Crus2_L and Frontal_Inf_Tri_L (**Figure 15 and Table 10**). The significantly decreased connections were between the Temporal_Sup_L and Amygdala_L, Cerebelum_6_L and Cerebelum_Crus1_L, Cerebelum_6_L and Cerebelum_Crus2_L, Cerebelum_Crus1_L and Cerebelum_6_L, Amygdala_L and Temporal_Mid_L, Cerebelum_Crus2_L and Cerebelum_6_L, as well as between the Cerebelum_6_L and Cerebelum_Crus1_R.

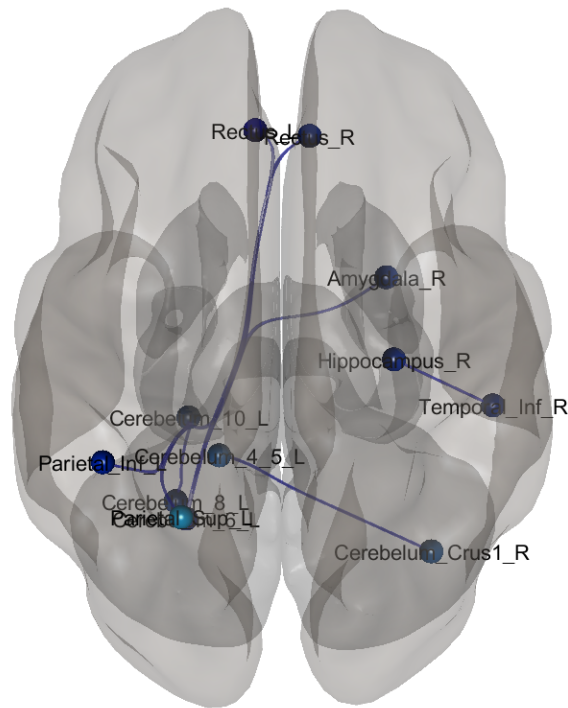


Figure 13: **Significantly decreased brain functional connectivity in the SA+PT group compared to the TA group, post-treatment.** The 3D superior view illustrates the decreased (blue lines) functional connections between regions of interest (AAL116 atlas). Spheres represent brain regions color-coded according to their T-statistic values. Lines indicate significant pairwise connections (p -value < 0.05). A color bar denotes the T-statistic range for the color-coded nodes. The names of the involved brain regions are listed alongside the figure.

Table 8: **Significantly decreased functional connections between the SA+PT and TA groups, post-treatment, identified using a two-sample t-test on Fisher-transformed correlation coefficients derived from a GLM.** The connected brain regions (defined by the AAL116 atlas) are listed, along with their corresponding T-statistic, p -uncorrected (p -unadj) and p -FDR (False Discovery Rate-corrected) values. A p -value < 0.05 was considered statistically significant.

Connection	T-statistic	p -unc	p -FDR
Rectus_R–Cerebellum_6_L	-4.53	0.0002	0.0229
Cerebellum_10_L–Parietal_Inf_L	-4.15	0.0004	0.0251
Cerebellum_10_L–Amygdala_R	-4.14	0.0004	0.0251
Cerebellum_6_L–Rectus_L	-4.11	0.0005	0.0267
Hippocampus_R–Temporal_Inf_R	-4.32	0.0003	0.0352
Cerebellum_Crus1_R–Cerebellum_4_5_L	-4.21	0.0004	0.0431
Cerebellum_10_L–Parietal_Sup_L	-3.59	0.0013	0.0444
Cerebellum_10_L–Cerebellum_8_L	-3.52	0.0015	0.0444
Parietal_Inf_L–Cerebellum_10_L	-4.15	0.0004	0.0490

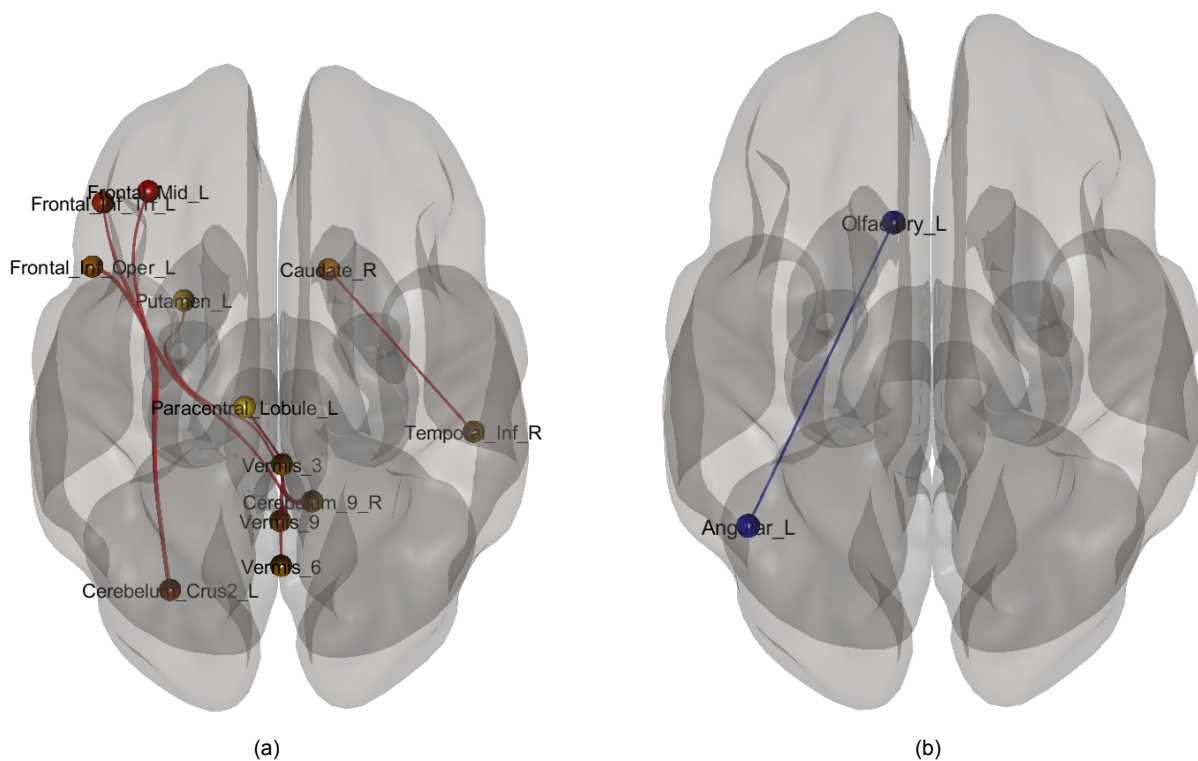


Figure 14: **Significant changes in brain functional connectivity in the TA+PT group compared to the SA+PT group, post-treatment depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.

Table 9: **Significantly increased and decreased functional connections in the TA+PT group compared to the SA+PT group, post-treatment, identified using two-sample t-test on Fisher-transformed correlation coefficients derived from a general linear model (GLM).** The table lists the brain region pairs (AAL116 atlas), their corresponding T-statistics, uncorrected (p-unc), and FDR-corrected (p-FDR) p-values. A p-value < 0.05 was considered statistically significant.

Connection	T-statistic	p-unc	p-FDR
<i>Increased Connections</i>			
Cerebelum_Crus2_L–Frontal_Mid_L	4.78	0.0001	0.0091
Cerebelum_Crus2_L–Frontal_Inf_Tri_L	4.64	0.0002	0.0091
Frontal_Mid_L–Cerebelum_Crus2_L	4.78	0.0001	0.0141
Cerebelum_Crus2_L–Frontal_Inf_Oper_L	4.03	0.0005	0.0209
Frontal_Inf_Oper_L–Cerebelum_9_R	4.02	0.0006	0.0319
Vermis_9–Vermis_3	4.00	0.0006	0.0389
Vermis_9–Vermis_6	3.92	0.0007	0.0389
Vermis_3–Putamen_L	3.85	0.0008	0.0449
Cerebelum_9_R–Paracentral_Lobule_L	3.85	0.0008	0.0456
Caudate_R–Temporal_Inf_R	4.18	0.0004	0.0460
<i>Decreased Connections</i>			
Olfactory_L–Angular_L	-4.33	0.0003	0.0340

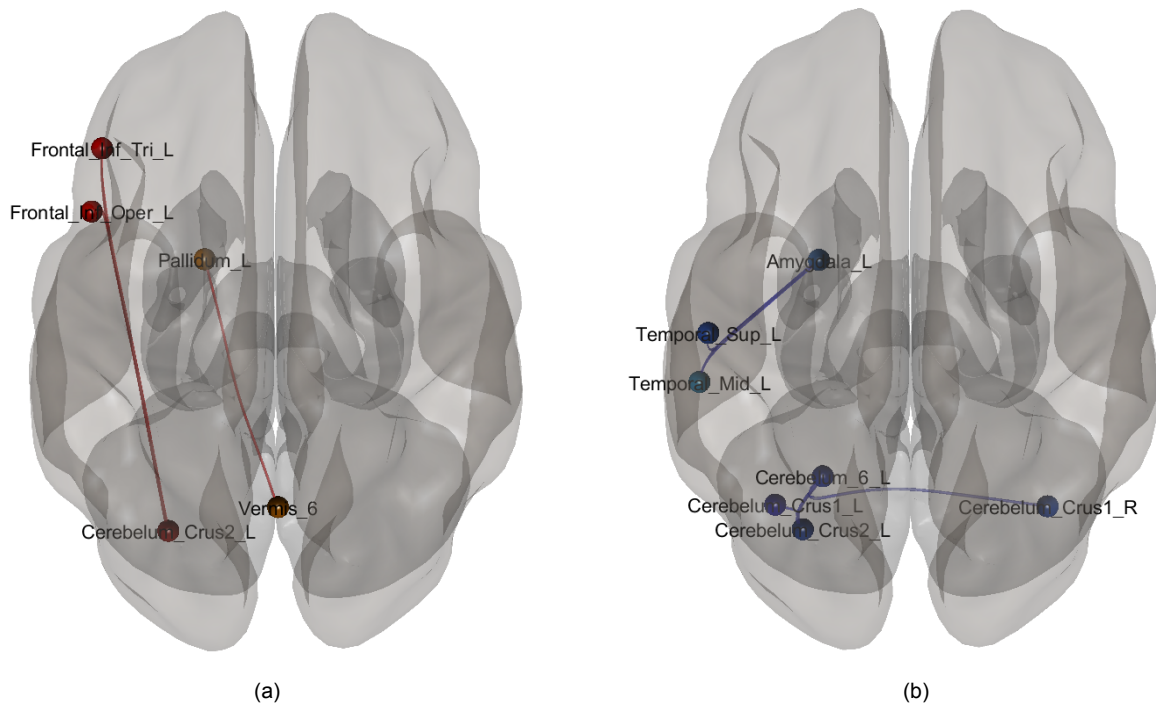


Figure 15: **Significant changes in brain functional connectivity in the TA+PT group compared to the TA group, post-treatment depicted in two 3D superior view representations.** (a) Red Connections: Illustrates significant increases in functional connectivity, with spheres representing brain regions (defined by the AAL116 atlas) color-coded according to the T-statistic color bar. (b) Blue Connections: Shows significant decreases in functional connectivity, with brain regions similarly color-coded. In both images, lines connecting spheres indicate significant connections (p -value < 0.05) but are not color-coded by T-statistic values. The names of the involved brain regions are listed alongside each image. A color bar indicates the T-statistic values for the color-coding of brain regions.

Table 10: **Significantly increased and decreased functional connections in the TA+PT group compared to the TA group, post-treatment, identified using two-sample t-tests on Fisher-transformed correlation coefficients derived from a general linear model (GLM).** The table lists the brain region pairs (AAL116 atlas), their corresponding T-statistics, uncorrected (p-unc), and FDR-corrected (p-FDR) p-values. A p-value < 0.05 was considered statistically significant.

Connection	T-statistic	p-unc	p-FDR
<i>Increased Connections</i>			
Vermis_6–Pallidum_L	4.42	0.0002	0.0284
Cerebelum_Crus2_L–Frontal_Inf_Oper_L	4.01	0.0006	0.0343
Cerebelum_Crus2_L–Frontal_Inf_Tri_L	3.99	0.0006	0.0343
<i>Decreased Connections</i>			
Temporal_Sup_L–Amygdala_L	–4.74	0.0001	0.0151
Cerebelum_6_L–Cerebelum_Crus1_L	–4.51	0.0002	0.0175
Cerebelum_6_L–Cerebelum_Crus2_L	–4.32	0.0003	0.0175
Cerebelum_Crus1_L–Cerebelum_6_L	–4.51	0.0002	0.0240
Amygdala_L–Temporal_Mid_L	–3.98	0.0006	0.0344
Cerebelum_Crus2_L–Cerebelum_6_L	–4.32	0.0003	0.0351
Cerebelum_6_L–Cerebelum_Crus1_R	–3.70	0.0011	0.0414

8.2 Structural Connectivity

The brain region connections identified from the within-group hypotheses during functional connectivity analysis, directly involved in the 5 domains of the FMA, were further investigated. The brain regions were: Supp_Motor_Area_L–Thalamus_R , Supp_Motor_Area_L–Rolandic_Oper_R , Supp_Motor_Area_L–Rolandic_Oper_L, Vermis_7–Caudate_R , Cerebelum_7b_R–Cerebelum_8_L, Vermis_7–Thalamus_R , Insula_R–Vermis_1_2 , Temporal_Mid_L–Vermis_7, Amygdala_L–Frontal_Sup_Orb_L, Amygdala_L–Frontal_Sup_Orb_R, Amygdala_R–Frontal_Sup_Orb_L, Amygdala_R–Frontal_Sup_Orb_R, Amygdala_L–Frontal_Mid_Orb_R. The mean FA was extracted from the SC matrices corresponding to these brain connections and represented in adjacency matrices (**Figures 16-18**).

Qualitatively, the structural brain connections showed noticeable changes post-treatment compared to pre-treatment. In the TA group, all brain connections increased except for the Supp_

Motor_Area_L-Rolandic_Oper_L, Supp_Motor_Area_L-Rolandic_Oper_R and the Amygdala_L-Frontal_Mid_Orb_R which decreased post-treatment, while all the other connections involving the amygdala showed little to no changes (**Figure 16**). For the TA+PT group all brain connections did not show noticeable changes post-treatment compared to pre-treatment, except for the Insula_R-Vermis_1_2 and the Temporal_Mid_L-Vermis_7, which decreased post-treatment, while the Cerebelum_7b_R-Cerebelum_8_L and Vermis_7-Caudate_R increased post-treatment (**Figure 17**). For the SA+PT group, all brain connections decreased except for the Supp_Motor_Area_L-Rolandic_Oper_L, the Amygdala_L-Frontal_Sup_Orb_R, and Amygdala_R-Frontal_Sup_Orb_L that showed no noticeable changes post-treatment compared to pre-treatment (**Figure 18**). Additionally, the Temporal_Mid_L-Vermis_7 increased post-treatment compared to pre-treatment. The lowest FA value observed in the adjacency matrices was 0.36, which falls within the expected range for white matter (typically ≥ 0.20). FA values below 0.20 are generally associated with grey matter and were not present in the data.

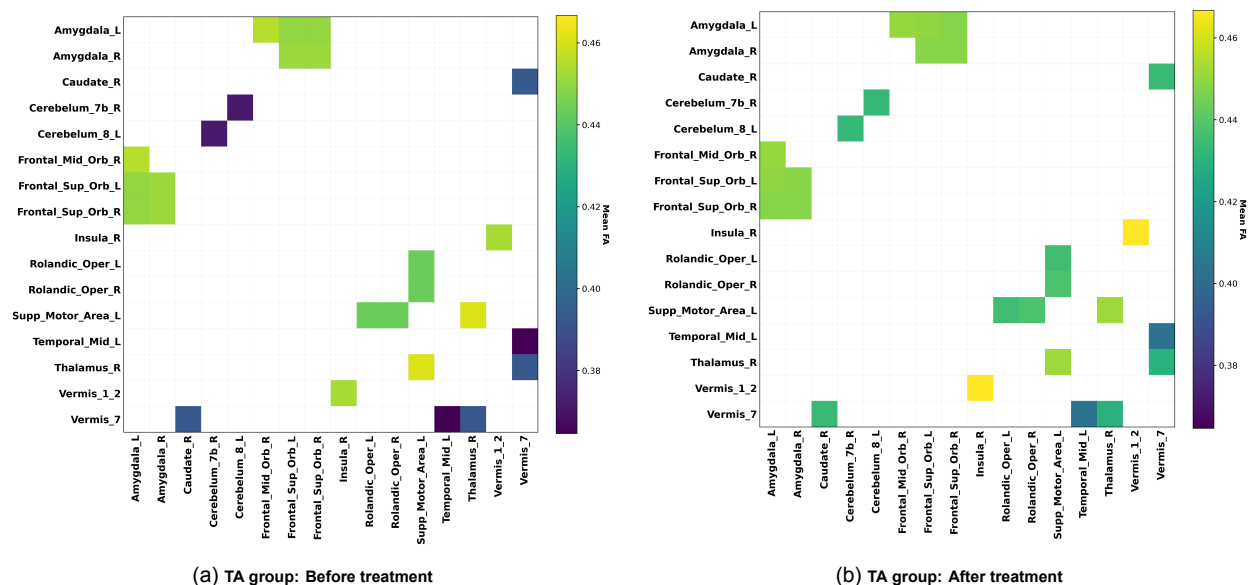


Figure 16: **Structural connectivity adjacency matrices for the TA group (n=5). (A) Before treatment, (B) After treatment. The indices in the matrices are color-coded based on FA values between brain regions.**

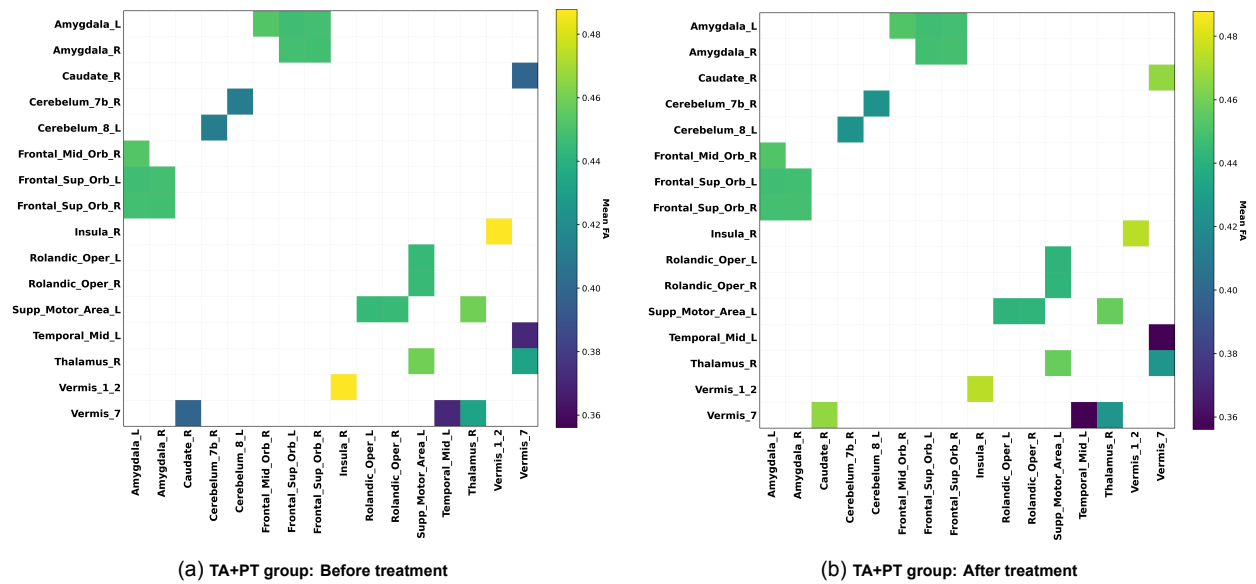


Figure 17: **Structural connectivity adjacency matrices for the TA+PT group (n=6). (A) Before treatment, (B) After treatment. The indices in the matrices are color-coded based on FA values between brain regions.**

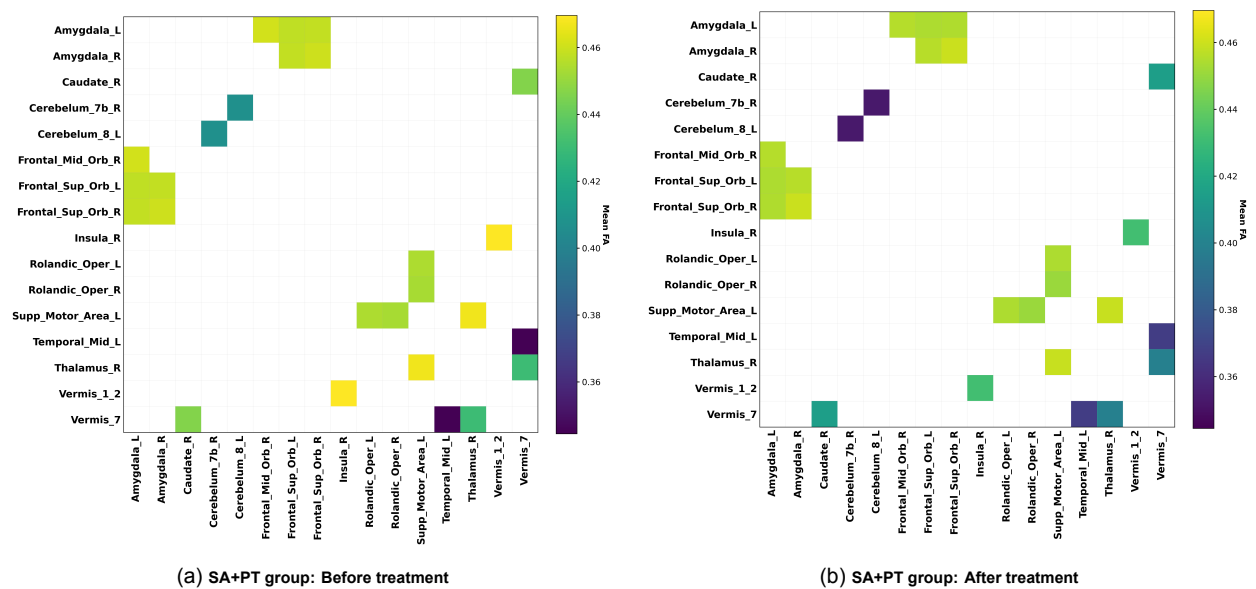


Figure 18: **Structural connectivity adjacency matrices for the SA+PT group (n=8). (A) Before treatment, (B) After treatment. The indices in the matrices are color-coded based on FA values between brain regions.**

8.3 Structural-Functional Connectivity Coupling

The structural-functional connectivity coupling (SC-FC Coupling) showed negative correlations for all three groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8) pre- and post-treatment (**Figure 19.a-c**). The SC-FC coupling had a weak negative correlation for the TA and TA+PT group, pre-

treatment (before), with the TA+PT group the only significant correlation among the three groups (TA: $r=-0.21$, $p=0.1$, CI [0.02, 0.64]; TA+PT: $r=-0.35$, $p=0.002$, CI [-0.04, 0.34]). The SA+PT group, pre-treatment, had a very weak negative correlation that was not significant ($r=-0.18$, $p=0.06$, CI [0.011, 0.44]) (**Figure 19.c**). Post-treatment, all three groups showed very weak negative correlations, with none statistically significant (TA: $r=-0.15$, $p=0.24$, CI [0.002, 0.43]; TA+PT: $r=-0.07$, $p=0.55$, CI [0.11, 0.38]; SA+PT: $r=-0.11$, $p=0.28$, CI [0.12, 0.51]). There was a tendency for the SC data to cluster around the FA values between 0.40 and 0.45, and this was observed in all three groups. The highest absolute Fisher Z-values appeared to be associated with lower FA values (below 0.40), whereas low FC values were associated with high and low FA values, seen across all three groups.

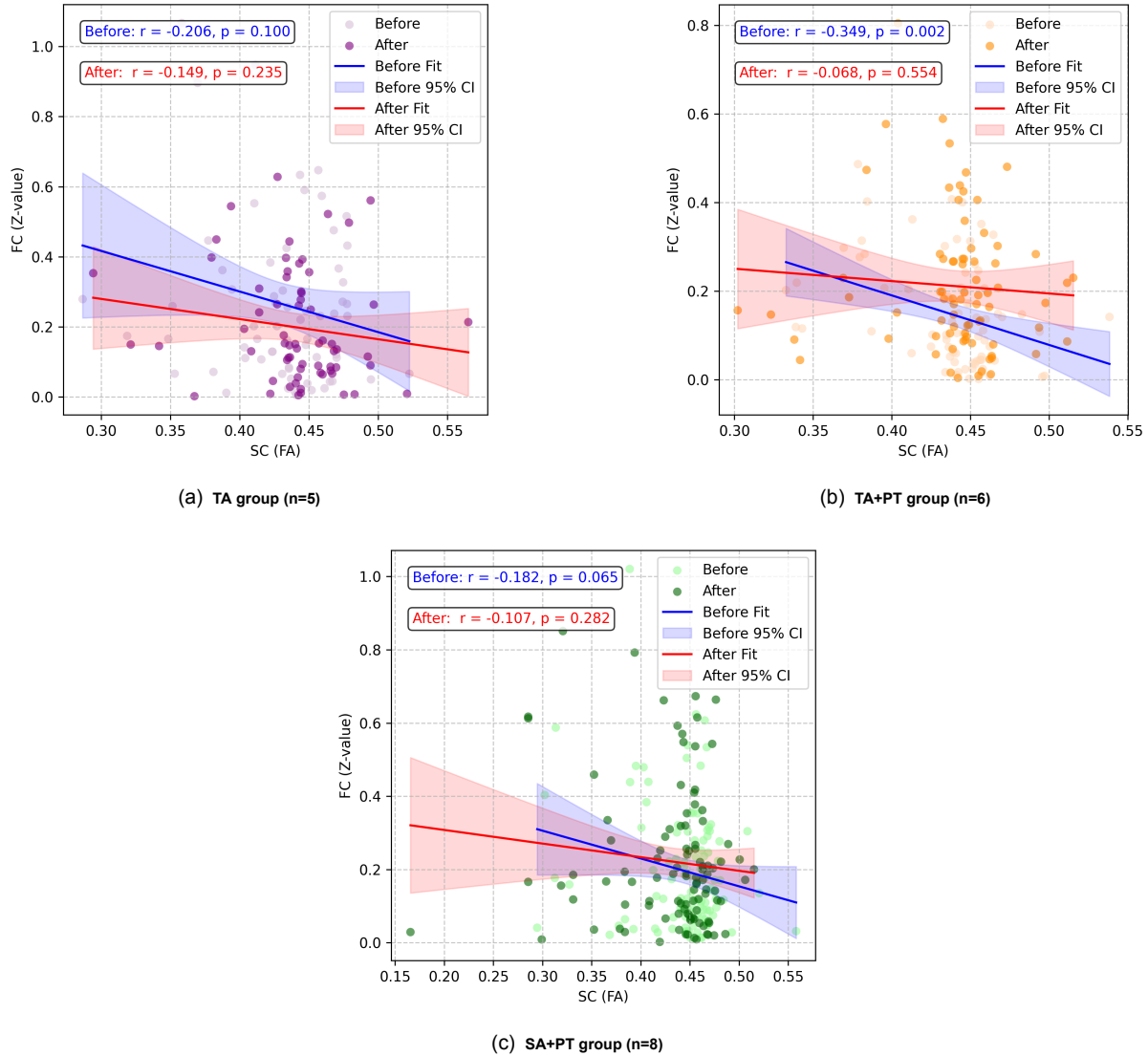


Figure 19: **Structural-Functional Connectivity Coupling (SC-FC Coupling) across three groups: (a) TA, (b) TA+PT, and (c) SA+PT.** Scatter plots illustrate Pearson correlations between fractional anisotropy (FA) values and absolute Fisher Z-transformed values of correlated mean time series from 13 brain regions, assessed before (blue) and after (red) treatment interventions. Lines of best fit are shown, with corresponding r-values (Pearson correlation coefficients) and p-values indicated for each group and time point. A p-value of ≤ 0.05 was considered statistically significant.

8.4 Functional Connectivity Graph Theoretical Measurements

For within-group comparisons (**Figure 20 and Table 11**), L_p ($F = 6.72$, $\eta^2 p = 0.53$, $p\text{-unadj} = 0.045$) was the only graph measure that showed a statistically significant decrease after treatment (mean $\pm$ SD (SEM): 1.93 ± 0.16 (0.06)) compared to pre-treatment (mean $\pm$ SD (SEM): 2.01 ± 0.12 (0.04)) for the SA+PT group before adjusting for the covariate, motion, and after ($p\text{-adj} = 0.041$).

In total, 53% of the remaining variance was explained by the treatment group, after adjusting for motion. The significance was lost after correcting for multiple comparisons (p -Sidak = 0.19). No other graph measures showed significant within-group differences.

The between-group differences, pre-treatment showed no statistically significant differences between the graph measures after adjusting for motion (**Table 12**), except for Lp ($F=4.46$, $\eta^2p = 0.37$, p -unadj=0.02, p -adj=0.03, p -Sidak = 0.14). The SA+PT group significantly increased (**Table 14**) compared to the TA (p -unc=0.02, $g=1.49$) and TA+PT groups (p -unc=0.05, $g=1.09$), where g is the effect size, Hedge's g (small sample size correction). The significance was still observed for Lp after correcting for multiple comparisons (p -Sidak = 0.04), comparing the TA and SA+PT groups. Eg showed a significant difference between groups, pre-treatment, before adjusting for motion (p -unadj=0.04), but not after (p -adj=0.051). Between-group analysis for the post-treatment data did not show any statistically significant differences (**Table 13**).

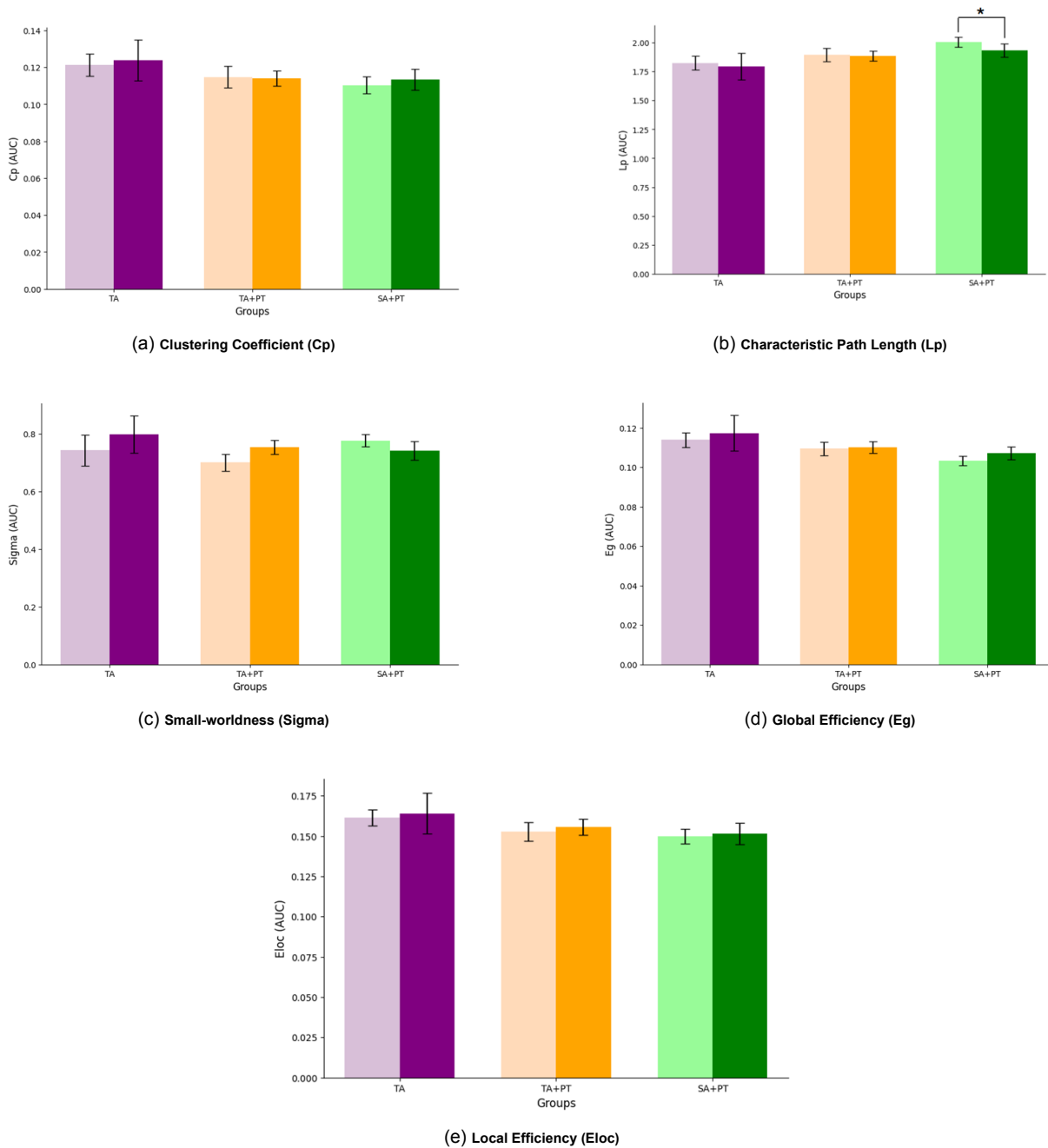


Figure 20: **Graph theoretical measures of functional connectivity networks before and after treatment across three groups, using weighted connections between the 116 brain regions where the weights represent the correlated mean time series values and represented by bar plots.** Data are shown for three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8), with the keys indicating the specific group. Measures were calculated using the GRETNA Toolbox, and mean values were reported as the area under the curve (AUC). (A) Clustering Coefficient (Cp), (B) Characteristic Path Length (Lp), (C) Sigma, (D) Global Efficiency (Eg), and (E) Local Efficiency (Eloc). Mean $\pm$ SD and SEM are given as error bars. A p-value of ≤ 0.05 was considered statistically significant and is indicated with an asterisk (*), after adjusting for motion. Note: Significant baseline differences are not shown.

Table 11: **Within-group analysis of the functional connectivity graph measures.** Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.

Group	Variable	Before Mean $\pm$ SD (SEM)	After Mean $\pm$ SD (SEM)	p-unadj	p-adj	p-Sidak	η_p^2	F
TA (n=5)								
	Cp	0.123 $\pm$ 0.009 (0.004)	0.124 $\pm$ 0.025 (0.011)	0.93	0.94	1.00	0.002	0.007
	Lp	1.83 $\pm$ 0.14 (0.06)	1.79 $\pm$ 0.26 (0.12)	0.77	0.79	1.00	0.03	0.08
	Sigma	0.743 $\pm$ 0.121 (0.054)	0.811 $\pm$ 0.117 (0.052)	0.46	0.52	0.98	0.15	0.52
	Eg	0.114 $\pm$ 0.008 (0.004)	0.117 $\pm$ 0.020 (0.009)	0.67	0.71	1.00	0.05	0.16
	Eloc	0.164 $\pm$ 0.006 (0.003)	0.164 $\pm$ 0.028 (0.013)	0.98	0.99	1.00	0.0001	0.0003
TA+PT (n=6)								
	Cp	0.115 $\pm$ 0.015 (0.006)	0.114 $\pm$ 0.010 (0.004)	0.95	0.95	1.00	0.001	0.004
	Lp	1.88 $\pm$ 0.05 (0.02)	1.90 $\pm$ 0.04 (0.02)	0.61	0.65	0.99	0.06	0.25
	Sigma	0.700 $\pm$ 0.073 (0.030)	0.754 $\pm$ 0.058 (0.024)	0.21	0.23	0.73	0.34	2.02
	Eg	0.110 $\pm$ 0.006 (0.002)	0.109 $\pm$ 0.002 (0.001)	0.65	0.68	1.00	0.048	0.20
	Eloc	0.154 $\pm$ 0.012 (0.005)	0.154 $\pm$ 0.009 (0.004)	0.96	0.96	1.00	0.0007	0.003
SA+PT (n=8)								
	Cp	0.110 $\pm$ 0.013 (0.005)	0.114 $\pm$ 0.016 (0.006)	0.58	0.61	0.99	0.05	0.29
	Lp	2.01 $\pm$ 0.12 (0.04)	1.93 $\pm$ 0.16 (0.06)	0.045	0.041	0.19	0.53	6.72
	Sigma	0.778 $\pm$ 0.060 (0.021)	0.742 $\pm$ 0.091 (0.032)	0.46	0.47	0.96	0.09	0.59
	Eg	0.103 $\pm$ 0.007 (0.002)	0.107 $\pm$ 0.009 (0.003)	0.07	0.06	0.28	0.46	5.17
	Eloc	0.150 $\pm$ 0.013 (0.005)	0.151 $\pm$ 0.019 (0.007)	0.80	0.80	1.00	0.01	0.07

Table 12: **Pre-treatment between-group analysis for functional connectivity graph measures.** Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion, p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η^2p), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.

Measure	TA: mean $\pm$ SD (SEM)	TA+PT: mean $\pm$ SD (SEM)	SA+PT: mean $\pm$ SD (SEM)	p-unadj	p-adj	p-Sidak	η^2p	F
Cp	0.123 $\pm$ 0.009 (0.004)	0.115 $\pm$ 0.014 (0.006)	0.110 $\pm$ 0.013 (0.004)	0.26	0.31	0.84	0.14	1.27
Lp	1.83 $\pm$ 0.14 (0.06)	1.88 $\pm$ 0.05 (0.02)	2.01 $\pm$ 0.12 (0.04)	0.02	0.03	0.14	0.37	4.46
Sigma	0.743 $\pm$ 0.121 (0.054)	0.700 $\pm$ 0.072 (0.030)	0.778 $\pm$ 0.060 (0.021)	0.25	0.28	0.80	0.16	1.41
Eg	0.114 $\pm$ 0.008 (0.004)	0.110 $\pm$ 0.006 (0.002)	0.103 $\pm$ 0.007 (0.002)	0.04	0.051	0.23	0.33	3.64
Eloc	0.164 $\pm$ 0.006 (0.003)	0.154 $\pm$ 0.012 (0.005)	0.150 $\pm$ 0.013 (0.005)	0.11	0.16	0.58	0.22	2.10

Table 13: **Post-treatment, between-group analysis for functional connectivity graph measures.** Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion, p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η^2p), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.

Measure	TA: mean $\pm$ SD (SEM)	TA+PT: mean $\pm$ SD (SEM)	SA+PT: mean $\pm$ SD (SEM)	p-unadj	p-adj	p-Sidak	η^2p	F
Cp	0.124 $\pm$ 0.025 (0.011)	0.114 $\pm$ 0.010 (0.004)	0.114 $\pm$ 0.016 (0.006)	0.55	0.58	0.99	0.070	0.57
Lp	1.79 $\pm$ 0.26 (0.12)	1.90 $\pm$ 0.04 (0.02)	1.93 $\pm$ 0.16 (0.06)	0.37	0.50	0.97	0.09	0.72
Sigma	0.811 $\pm$ 0.117 (0.052)	0.754 $\pm$ 0.058 (0.024)	0.742 $\pm$ 0.091 (0.032)	0.41	0.53	0.98	0.08	0.67
Eg	0.117 $\pm$ 0.020 (0.009)	0.109 $\pm$ 0.002 (0.001)	0.107 $\pm$ 0.009 (0.003)	0.32	0.42	0.94	0.11	0.91
Eloc	0.164 $\pm$ 0.028 (0.013)	0.154 $\pm$ 0.009 (0.004)	0.151 $\pm$ 0.019 (0.007)	0.52	0.58	0.99	0.07	0.56

Table 14: **Pre-treatment, pairwise comparisons for Lp functional graph measure for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8).** The Adj. mean order corresponds to the order in the comparison column. T-statistic with degrees of freedom, p-unc (p-value before correcting for multiple comparisons), p-Sidak (p-value after correcting for multiple comparisons), and the effect sizes: absolute Cohen's d and Hedge's g are given. A p-value of ≤ 0.05 was considered statistically significant.

Measure	Comparison	Adj mean 1	Adj mean 2	t(df)	p-unc	p-Sidak	Cohen's d	Hedge's g
Lp	TA vs TA+PT	1.83	1.88	-0.699 (15)	0.50	0.87	0.44	-0.40
Lp	SA+PT vs TA	2.00	1.83	2.74 (15)	0.02	0.04	1.60	1.49
Lp	SA+PT vs TA+PT	2.00	1.88	2.15 (15)	0.05	0.14	1.16	1.09

8.5 Structural Connectivity Graph Theoretical Measurements

Comparing post-treatment data to pre-treatment data for the five different graph measures (Cp, Lp, Sigma, Eg and Eloc) for structural connectivity within the three independent groups (**Figure.21**): TA (n=5), TA+PT (n=6) and SA+PT (n=8), there was no statistically significance observed after adjusting for the covariate motion (**Table 15**), except in the TA group for the graph measure Sigma ($F=12.10$, $\eta^2p = 0.80$, $p\text{-unadj}=0.89$, $p\text{-adj}=0.04$, $p\text{-Sidak}=0.12$) which decreased post-treatment (mean $\pm$ SD (SEM): 0.243 ± 0.133 (0.060) compared to pre-treatment (mean $\pm$ SD (SEM): 0.258 ± 0.111 (0.049)). Additionally, Eloc in the TA group showed a statistically significant increase ($F=13.30$, $\eta^2p = 0.82$, $p\text{-unadj}=0.82$, $p\text{-adj}=0.04$, $p\text{-Sidak}=0.10$) post-treatment compared to pre-treatment. No significant differences were observed after correcting for multiple comparisons ($p\text{-Sidak} > 0.05$).

The SA+PT group did not show any statistically significant differences after adjusting for motion (**Figure 21 and Table 15**), but Sigma and Eloc did significantly increase post-treatment compared to pre-treatment, before adjusting for motion ($p_{\text{unadj}}=0.04$ and 0.02 , respectively, indicated by "+"). The following graph measures were significantly different between groups, pre-treatment, before and after adjusting for motion (**Table 16**): Cp ($F=8.96$, $\eta^2p = 0.54$, $p_{\text{unadj}}=0.02$, $p_{\text{adj}}=0.003$, $p_{\text{Sidak}}=0.01$), Sigma ($F=10.02$, $\eta^2p = 0.57$, $p_{\text{unadj}}=0.02$, $p_{\text{adj}}=0.002$, $p_{\text{Sidak}} = 0.01$), and Eloc ($F=6.32$, $\eta^2p = 0.46$, $p_{\text{unadj}}=0.03$, $p_{\text{adj}}=0.01$, $p_{\text{Sidak}}=0.05$). These measures were still statistically significant after correcting for multiple comparisons. Lp was not significantly different between groups, pre-treatment ($p_{\text{unadj}}=0.12$, $p_{\text{adj}}=0.08$). Eg, only showed significance after adjusting for motion ($F=3.71$, $\eta^2p = 0.33$, $p_{\text{unadj}}=0.11$, $p_{\text{adj}}=0.049$, $p_{\text{Sidak}}=0.22$).

Pairwise comparison (**Table 18**) showed that Cp was significantly decreased in the SA+PT group compared to the TA ($p_{\text{unc}}=0.001$) and TA+PT groups ($p_{\text{unc}}=0.03$), with Cp still significant in the SA+PT group compared to the TA group after correcting for multiple comparisons ($p_{\text{Sidak}}=0.003$). Sigma was significantly increased in the TA group compared to the TA+PT group ($p_{\text{unc}}=0.01$, $p_{\text{Sidak}}=0.04$) and the SA+PT group ($p_{\text{unc}}=0.0004$, $p_{\text{Sidak}}=0.001$). There was a very strong difference between Sigma in the TA and SA+PT group, with the difference unlikely due to chance ($d=2.65$). The SA+PT and the TA+PT group was not significantly different ($p_{\text{unc}}=0.10$, $p_{\text{Sidak}}=0.28$).

Eg only showed a significant difference between the TA and TA+PT group (**Table 18**), with the TA+PT group showing a statistically significant increase ($p_{\text{unc}}=0.02$, $p_{\text{Sidak}}=0.05$). Eloc was significantly decreased in the SA+PT group compared to the TA ($p_{\text{unc}}=0.004$, $p_{\text{Sidak}}=0.01$) and TA+PT groups ($p_{\text{unc}}=0.05$, $p_{\text{Sidak}}=0.14$), with only the SA+PT group vs. the TA group still showing significance after correcting for multiple comparisons.

Between-group analysis for post-treatment data (**Table 17**) showed that Lp ($F = 3.73$, $\eta^2p = 0.33$, $p_{\text{unadj}} = 0.046$) and Eg ($F = 3.63$, $\eta^2p = 0.33$, $p_{\text{unadj}} = 0.048$) were statistically significant before

adjusting for motion, where Lp was also statistically significant after adjustment for the motion ($p\text{-adj} = 0.05$), but not Eg ($p\text{-adj} = 0.052$). No statistically significant difference was found after correcting for multiple comparisons ($p\text{-Sidak} > 0.05$). In total, 33.2% (rounded to 33%) of the variance for Lp was uniquely explained by the group, compared to 32.6% (rounded to 33%) for Eg, after adjusting for motion. The pairwise comparison showed that Lp in the SA+PT group (mean $\pm$ SD (SEM): 1.87 ± 0.31 (0.11)) was significantly increased compared to TA (mean $\pm$ SD (SEM): 1.58 ± 0.14 (0.06), $p\text{-unc}=0.04$, $d=1.28$, $g=-1.22$) and TA+PT (mean $\pm$ SD (SEM): 1.58 ± 0.13 (0.06), $p\text{-unc}=0.03$, $d=1.26$, $g=-1.20$), where TA and TA+PT were not statistically different ($p\text{-unc}=0.97$) (**Table 19**).

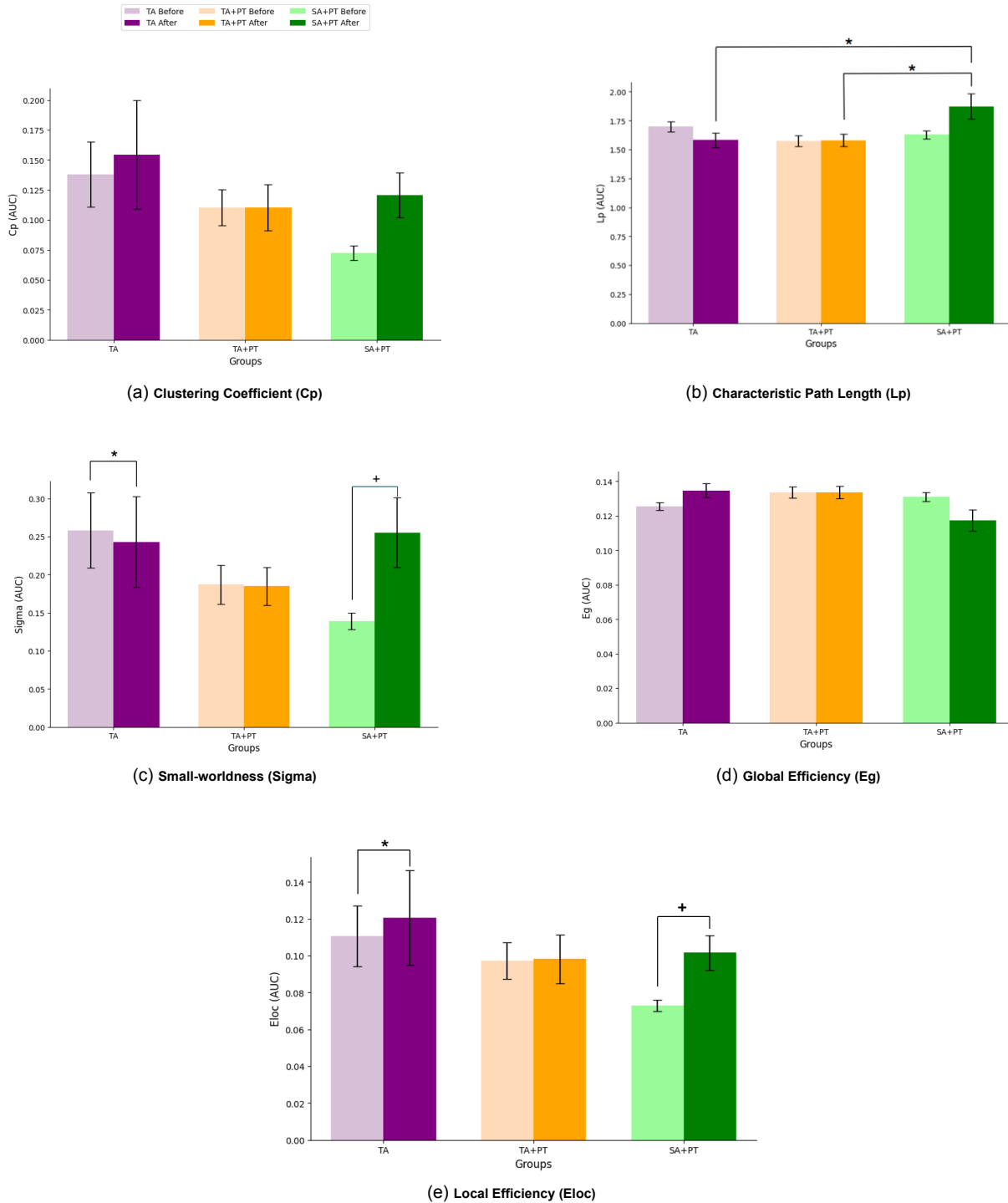


Figure 21: **Graph theoretical measures of structural connectivity networks before and after treatment across three groups, using weighted connections between the 116 brain regions where the weights represent fractional anisotropy (FA) values and represented by bar plots.** Data are shown for three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8), with the keys indicating the specific group. Measures were calculated using the GREYNA Toolbox, and mean values were reported as the area under the curve (AUC). (A) Clustering Coefficient (Cp), (B) Characteristic Path Length (Lp), (C) Sigma, (D) Global Efficiency (Eg), and (E) Local Efficiency (Eloc). Mean $\pm$ SD and SEM are given as error bars. A p-value of ≤ 0.05 was considered statistically significant and is indicated with an asterisk (*). "(+)" indicates significance before adjusting for motion (but not after). Note: Significant baseline differences are not shown.

Table 15: **Within-group analysis of the structural connectivity graph measures for the TA (n=5), TA+PT (n=6) and SA+PT (n=8) groups.** Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.

Group	Variable	Before Mean $\pm$ SD (SEM)	After Mean $\pm$ SD (SEM)	p-unadj	p-adj	p-Sidak	η_p^2	F
TA (n=5)								
	Cp	0.138 $\pm$ 0.061 (0.027)	0.154 $\pm$ 0.101 (0.045)	0.81	0.05	0.15	0.76	9.76
	Lp	1.70 $\pm$ 0.10 (0.04)	1.58 $\pm$ 0.143 (0.064)	0.248	0.08	0.22	0.70	6.91
	Sigma	0.258 $\pm$ 0.111 (0.049)	0.243 $\pm$ 0.133 (0.060)	0.89	0.04	0.12	0.80	12.10
	Eg	0.126 $\pm$ 0.005 (0.002)	0.135 $\pm$ 0.009 (0.004)	0.11	0.06	0.16	0.75	9.15
	Eloc	0.111 $\pm$ 0.0365 (0.016)	0.121 $\pm$ 0.0574 (0.026)	0.82	0.04	0.10	0.82	13.30
TA+PT (n=6)								
	Cp	0.113 $\pm$ 0.027 (0.011)	0.110 $\pm$ 0.0464 (0.019)	0.84	0.41	0.80	0.17	0.83
	Lp	1.57 $\pm$ 0.12 (0.05)	1.58 $\pm$ 0.134 (0.055)	0.76	0.62	0.94	0.07	0.30
	Sigma	0.186 $\pm$ 0.054 (0.022)	0.185 $\pm$ 0.0613 (0.025)	0.94	0.48	0.86	0.13	0.61
	Eg	0.134 $\pm$ 0.008 (0.003)	0.134 $\pm$ 0.009 (0.004)	0.99	0.61	0.94	0.07	0.31
	Eloc	0.0973 $\pm$ 0.0245 (0.0100)	0.0982 $\pm$ 0.0322 (0.0131)	0.90	0.51	0.88	0.11	0.51
SA+PT (n=8)								
	Cp	0.074 $\pm$ 0.015 (0.006)	0.121 $\pm$ 0.053 (0.019)	0.05	0.31	0.67	0.17	1.23
	Lp	1.64 $\pm$ 0.07 (0.02)	1.87 $\pm$ 0.309 (0.109)	0.08	0.09	0.24	0.41	4.17
	Sigma	0.139 $\pm$ 0.030 (0.011)	0.255 $\pm$ 0.129 (0.046)	0.04	0.26	0.60	0.20	1.51
	Eg	0.130 $\pm$ 0.005 (0.002)	0.117 $\pm$ 0.017 (0.006)	0.10	0.08	0.21	0.43	4.53
	Eloc	0.0728 $\pm$ 0.0087 (0.0031)	0.102 $\pm$ 0.027 (0.009)	0.02	0.57	0.92	0.06	0.37

Table 16: **Pre-treatment, between-group analysis for structural connectivity graph measures.** Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.

Measure	TA: mean $\pm$ SD (SEM)	TA+PT: mean $\pm$ SD (SEM)	SA+PT: mean $\pm$ SD (SEM)	p-unadj	p-adj	p-Sidak	η_p^2	F
Cp	0.138 $\pm$ 0.060 (0.027)	0.113 $\pm$ 0.027 (0.011)	0.074 $\pm$ 0.015 (0.005)	0.02	0.003	0.01	0.54	8.96
Lp	1.70 $\pm$ 0.10 (0.04)	1.57 $\pm$ 0.12 (0.05)	1.64 $\pm$ 0.07 (0.03)	0.12	0.08	0.34	0.29	3.01
Sigma	0.258 $\pm$ 0.110 (0.049)	0.186 $\pm$ 0.054 (0.022)	0.139 $\pm$ 0.030 (0.011)	0.02	0.002	0.01	0.57	10.02
Eg	0.126 $\pm$ 0.005 (0.002)	0.134 $\pm$ 0.008 (0.003)	0.130 $\pm$ 0.005 (0.002)	0.11	0.049	0.22	0.33	3.71
Eloc	0.111 $\pm$ 0.037 (0.016)	0.097 $\pm$ 0.025 (0.010)	0.073 $\pm$ 0.009 (0.003)	0.03	0.01	0.05	0.46	6.32

Table 17: **Post-treatment, between-group analysis for structural connectivity graph measures.** Values are given as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8). p-unadj (p-value before adjusting for motion), p-adj (p-value after adjusting for motion), and p-Sidak (p-value after correcting for multiple comparisons), the effect size, partial eta-squared (η_p^2), and the F-statistic are given. A p-value of ≤ 0.05 was considered statistically significant.

Measure	TA: mean $\pm$ SD (SEM)	TA+PT: mean $\pm$ SD (SEM)	SA+PT: mean $\pm$ SD (SEM)	p-unadj	p-adj	p-Sidak	η_p^2	F
Cp	0.154 $\pm$ 0.101 (0.045)	0.110 $\pm$ 0.046 (0.019)	0.121 $\pm$ 0.053 (0.019)	0.53	0.44	0.94	0.10	0.87
Lp	1.58 $\pm$ 0.14 (0.06)	1.58 $\pm$ 0.13 (0.06)	1.87 $\pm$ 0.31 (0.11)	0.05	0.05	0.22	0.33	3.73
Sigma	0.243 $\pm$ 0.133 (0.060)	0.185 $\pm$ 0.061 (0.025)	0.255 $\pm$ 0.129 (0.046)	0.51	0.49	0.96	0.09	0.75
Eg	0.135 $\pm$ 0.009 (0.004)	0.134 $\pm$ 0.009 (0.004)	0.117 $\pm$ 0.017 (0.006)	0.048	0.052	0.23	0.33	3.63
Eloc	0.121 $\pm$ 0.057 (0.026)	0.098 $\pm$ 0.032 (0.013)	0.102 $\pm$ 0.027 (0.009)	0.60	0.52	0.98	0.08	0.68

Table 18: **Pre-treatment pairwise comparisons for Cp, Lp, Eg, and Eloc structural graph measures across groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8).** The Adj. mean order corresponds to the order in the comparison column. T-statistic with degrees of freedom, p-unc (p-value before correcting for multiple comparisons), p-Sidak (p-value after correcting for multiple comparisons), and the effect sizes: absolute Cohen's d and Hedge's g are given. A p-value of ≤ 0.05 was considered statistically significant.

Measure	Comparison	Adj mean 1	Adj mean 2	t(df)	p-unc	p-Sidak	Cohen's d	Hedge's g
Cp	TA vs TA+PT	0.146	0.111	1.82 (15)	0.09	0.24	1.13	1.07
Cp	TA vs SA+PT	0.146	0.070	4.15 (15)	0.001	0.003	2.45	2.33
Cp	TA+PT vs SA+PT	0.111	0.0699	2.45 (15)	0.03	0.08	1.32	1.26
Sigma	TA vs TA+PT	0.276	0.182	2.76 (15)	0.01	0.04	1.71	1.62
Sigma	TA vs SA+PT	0.276	0.13	4.48 (15)	0.0004	0.001	2.65	2.52
Sigma	TA+PT vs SA+PT	0.182	0.131	1.74 (15)	0.10	0.28	0.94	0.89
Eg	TA vs TA+PT	0.124	0.134	-2.71 (15)	0.02	0.05	1.68	-1.59
Eg	TA vs SA+PT	0.124	0.131	-1.87 (15)	0.08	0.22	1.11	-1.05
Eg	TA+PT vs SA+PT	0.134	0.131	1.05 (15)	0.31	0.67	0.57	0.54
Eloc	TA vs TA+PT	0.115	0.096	1.41 (15)	0.18	0.44	0.88	0.83
Eloc	TA vs SA+PT	0.115	0.071	3.46 (15)	0.004	0.01	2.04	1.94
Eloc	TA+PT vs SA+PT	0.096	0.071	2.16 (15)	0.05	0.14	1.17	1.11

Table 19: **Post-treatment pairwise comparisons for the Lp structural graph measure for each group: TA (n=5), TA+PT (n=6), and SA+PT (n=8).** The Adj. mean order corresponds to the order in the comparison column. T-statistic with degrees of freedom, p-unc (p-value before correcting for multiple comparisons), p-Sidak (p-value after correcting for multiple comparisons), and the effect sizes: absolute Cohen's d and Hedge's g are given. A p-value of ≤ 0.05 was considered statistically significant.

Comparison	Adj mean 1	Adj mean 2	t(df)	p-unc	p-Sidak	Cohen's d	Hedges' g
TA vs TA+PT	1.58	1.59	-0.04 (15)	0.97	1.00	0.02	-0.02
TA vs SA+PT	1.58	1.87	-2.25 (15)	0.04	0.12	1.28	-1.22
TA+PT vs SA+PT	1.59	1.87	-2.33 (15)	0.03	0.10	1.26	-1.20

8.6 Correlations of Structural and Functional Graph Measures with FMA

For the functional connectivity data, significant correlations between FMA and Cp, Lp, Eg, and Eloc were observed only in the SA+PT group (**Figure.22-23, 25-26**). A very weak positive correlation and a significant moderate positive correlation were observed in the TA ($r = 0.13$, $p = 0.72$, CI: [-0.54, 0.70]) and SA+PT groups for Cp ($r = 0.60$, $p = 0.01$, CI: [0.15, 0.84]), respectively (**Table 20 and Figure.19.a+c**). Additionally, a very weak negative correlation between FMA and Cp in the TA+PT group was observed ($r = -0.08$, $p = 0.8$, CI: [-0.63, 0.52]) (**Figure.22.b**). A very weak negative correlation between FMA and Lp was observed in the TA ($r = -0.18$, $p = 0.62$, CI: [-0.73, 0.51]) and in the TA+PT ($r = -0.03$, $p = 0.92$, CI: [-0.60, 0.55]) groups, with a significant strong negative correlation in the SA+PT group ($r = -0.71$, $p = 0.002$, CI: [-0.89, -0.34]) (**Figure.23**). For sigma, a very weak positive and moderate positive correlation were seen in the TA ($r = 0.21$, $p = 0.55$, CI: [-0.48, 0.74]) and TA+PT groups ($r = 0.51$, $p = 0.09$, CI: [-0.09, 0.84]), respectively (**Figure.24.a+b**). The SA+PT group showed a weak negative correlation ($r = -0.19$, $p = 0.48$, CI: [-0.63, 0.34]) between FMA and Sigma (**Figure.24.c**). A very weak positive correlation between FMA and Eg was seen in the TA ($r = 0.20$, $p = 0.57$, CI: [-0.49, 0.74]) and TA+PT ($r = 0.03$, $p = 0.94$, CI: [-0.56, 0.59]) groups (**Figure.25.a+b**). Additionally, a significantly strong positive correlation ($r = 0.71$, $p = 0.002$, CI: [0.32, 0.89]) was observed for the SA+PT group (**Figure.25.c**). Similarly, very weak positive correlations between FMA and Eloc were seen for the TA ($r = 0.14$, $p = 0.69$, CI: [-0.53, 0.71]) and TA+PT ($r = 0.09$, $p = 0.79$, CI: [-0.51, 0.63]) groups, respectively (**Figure.26.a+b**). A significant moderate positive correlation was seen in the SA+PT group ($r = 0.58$, $p = 0.02$, CI: [0.12, 0.85]) (**Figure.26.c**). For all the statistically significant correlations (Cp, Lp, Eg and Eloc) in the SA+PT group, "0" was not part of the 95% confidence interval and therefore, there is 95% certainty that the relationship is real.

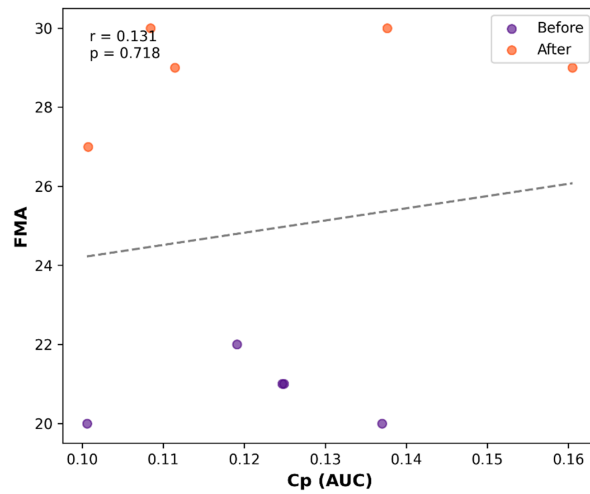
For the structural connectivity data, FMA was not significantly correlated with any of the 5 graph measures for the groups: TA (n=10), TA+PT (n=12) or SA+PT (n=16) with p-values > 0.05 (**Table 21 and Figure.24-28**). For Cp, the TA ($r = 0.17$, $p = 0.65$, CI: [-0.52, 0.72]) and SA+PT groups ($r = 0.19$, $p = 0.48$, CI: [-0.34, 0.63]) both showed very weak positive correlations (**Figure.27.a+c**). The TA+PT group showed a weak negative correlation ($r = -0.42$, $p = 0.18$, CI: [-0.80, 0.21]) (**Figure.27.b**). For the graph measure Lp, the TA ($r = -0.54$, $p = 0.11$, CI: [-0.87, 0.14]) and TA+PT group ($r = -0.01$, $p = 0.97$, CI: [-0.58, 0.57]) showed moderate and very weak negative correlations, respectively (**Figure.28.a+b**). The SA+PT group showed a moderate positive correlation ($r = 0.40$, $p = 0.13$, CI: [-0.12, 0.74]) (**Figure.28.c**). The Sigma graph measures showed a negative correlation for both the TA ($r = -0.01$, $p = 0.97$, CI: [-0.64, 0.62]) and TA+PT group ($r = -0.47$, $p = 0.12$, CI: [-0.82, 0.14]), with TA being very weakly negative and the TA+PT group being weakly negative (**Figure.29.a+b**). A very weak positive correlation was observed for the SA+PT group ($r = 0.28$, $p = 0.28$, CI: [-0.24, 0.68]) (**Figure.29.c**). In contrast, Eg was positively correlated with FMA for the TA ($r = 0.63$, $p = 0.05$, CI: [0.00, 0.90]) and TA+PT group ($r = 0.04$, $p = 0.90$, CI: [-0.55, 0.60]) with a weak negative correlation in the SA+PT group ($r = -0.36$, $p = 0.17$, CI: [-0.73, 0.16]) (**Figure.30.a-c**). Eloc showed a very weak positive and weak positive correlation in the TA ($r = 0.18$, $p = 0.63$, CI: [-0.51, 0.72]) and SA+PT group ($r = 0.33$, $p = 0.21$, CI: [-0.2, 0.71]), respectively (**Figure.31.a+c**). The TA+PT group showed a weak negative correlation ($r = -0.30$, $p = 0.34$, CI: [-0.75, 0.33]) (**Figure.31.b**).

Table 20: **Pearson correlations between FMA and functional graph measures.** Shown are correlation coefficients (r), p -values, and 95% confidence intervals (CI) for each group: TA ($n=10$), TA+PT ($n=12$), and SA+PT ($n=16$), with the pre-treatment and post-treatment data pooled together. A p -value of ≤ 0.05 was considered statistically significant.

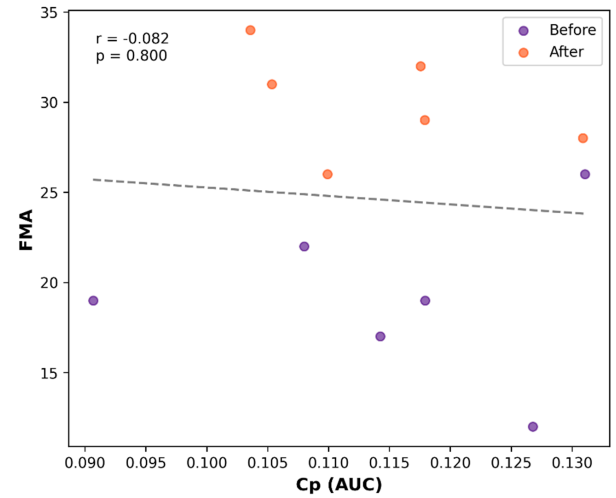
Measure	Group	r-value	p-value	95% CI
Cp	TA	0.13	0.72	[-0.54, 0.70]
	TA+PT	-0.08	0.80	[-0.63, 0.52]
	SA+PT	0.60	0.01	[0.15, 0.84]
Lp	TA	-0.18	0.62	[-0.73, 0.51]
	TA+PT	-0.03	0.92	[-0.60, 0.55]
	SA+PT	-0.71	0.002	[-0.89, -0.34]
Sigma	TA	0.21	0.55	[-0.48, 0.74]
	TA+PT	0.51	0.09	[-0.09, 0.84]
	SA+PT	-0.19	0.48	[-0.63, 0.34]
Eg	TA	0.20	0.57	[-0.49, 0.74]
	TA+PT	0.03	0.94	[-0.56, 0.59]
	SA+PT	0.71	0.002	[0.32, 0.89]
Eloc	TA	0.14	0.69	[-0.53, 0.71]
	TA+PT	0.09	0.79	[-0.51, 0.63]
	SA+PT	0.58	0.02	[0.12, 0.85]

Table 21: **Pearson correlations between FMA and structural graph measures.** Shown are correlation coefficients (r), p -values, and 95% confidence intervals (CI) for each group: TA ($n=10$), TA+PT ($n=12$), and SA+PT ($n=16$), with the pre-treatment and post-treatment data pooled together. A p -value of ≤ 0.05 was considered statistically significant.

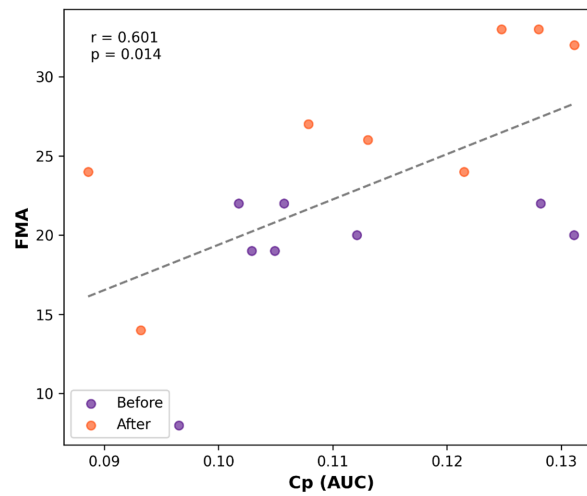
Measure	Group	r-value	p-value	95% CI
Cp	TA	0.17	0.65	[-0.52, 0.72]
	TA+PT	-0.42	0.18	[-0.80, 0.21]
	SA+PT	0.19	0.48	[-0.34, 0.63]
Lp	TA	-0.54	0.11	[-0.87, 0.14]
	TA+PT	-0.01	0.97	[-0.58, 0.57]
	SA+PT	0.40	0.13	[-0.12, 0.74]
Sigma	TA	-0.01	0.97	[-0.64, 0.62]
	TA+PT	-0.47	0.12	[-0.82, 0.14]
	SA+PT	0.28	0.28	[-0.24, 0.68]
Eg	TA	0.63	0.05	[0.00, 0.90]
	TA+PT	0.04	0.90	[-0.55, 0.60]
	SA+PT	-0.36	0.17	[-0.73, 0.16]
Eloc	TA	0.18	0.63	[-0.51, 0.72]
	TA+PT	-0.30	0.34	[-0.75, 0.33]
	SA+PT	0.33	0.21	[-0.20, 0.71]



(a) TA group (n=10)

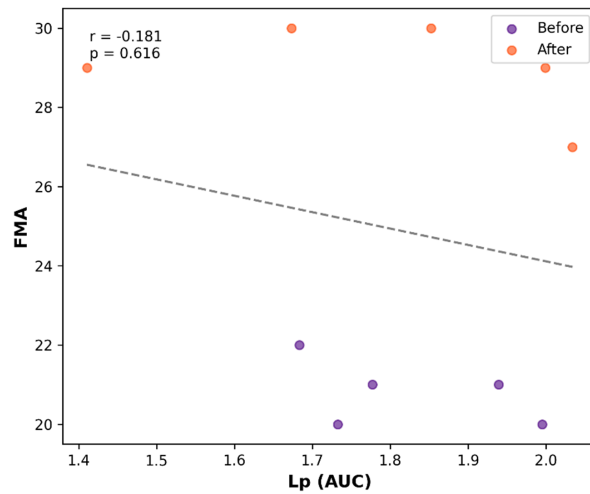


(b) TA+PT group (n=12)

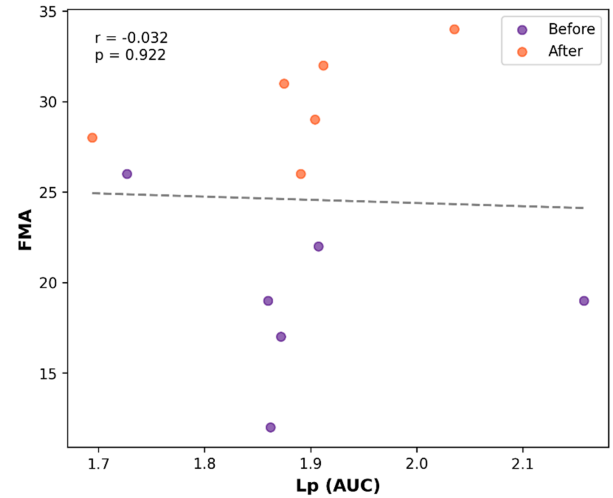


(c) SA+PT group (n=16)

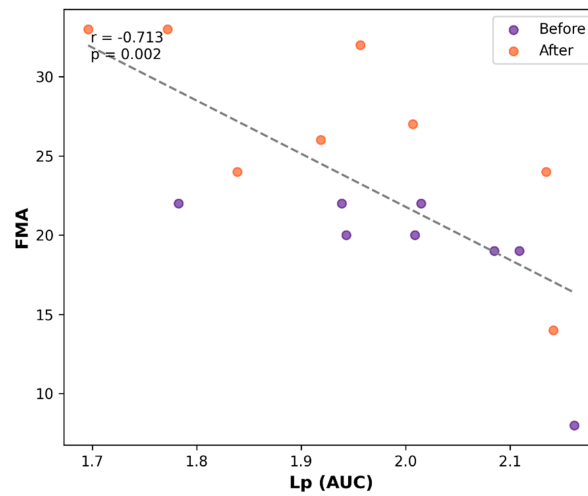
Figure 22: **Functional connectivity graph measure, Cp, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the Clustering Coefficient (Cp), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant



(a) TA group (n=10)

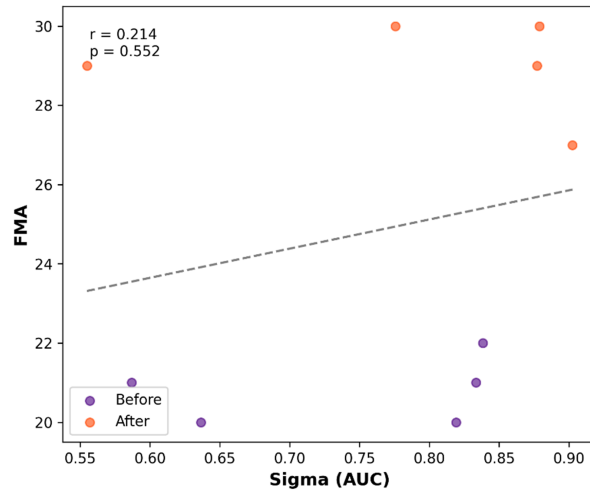


(b) TA+PT group (n=12)

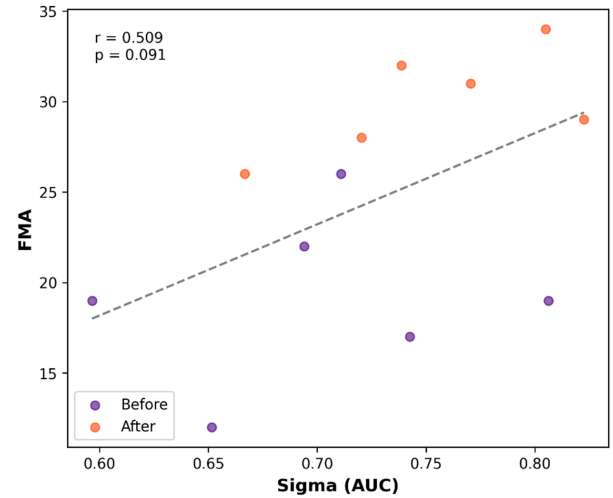


(c) SA+PT group (n=16)

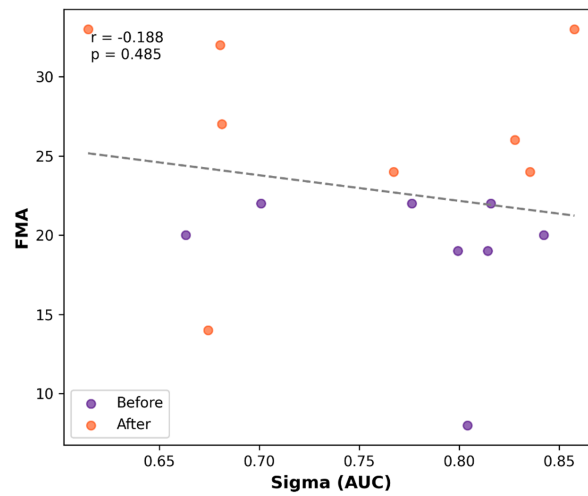
Figure 23: **Functional connectivity graph measure, Lp, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the Characteristic path length (Lp), a graph theoretical measure of network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p -value are provided. A p -value ≤ 0.05 was considered statistically significant



(a) TA group (n=10)

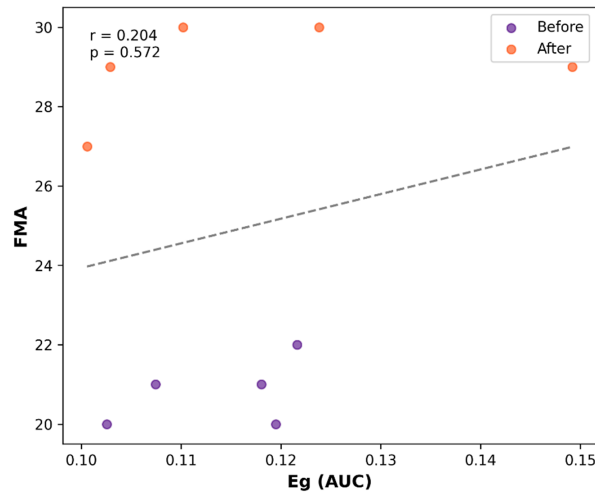


(b) TA+PT group (n=12)

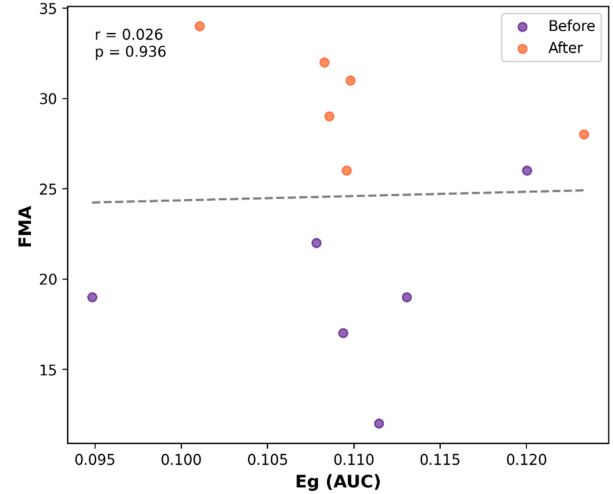


(c) SA+PT group (n=16)

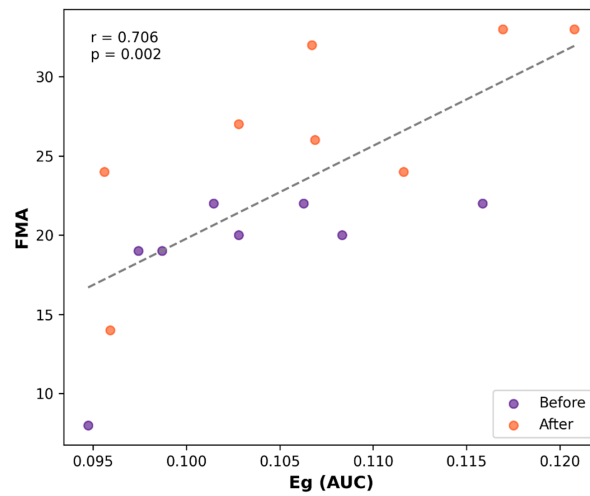
Figure 24: **Functional connectivity graph measure, Sigma, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Sigma, a graph theoretical measure of small-worldness, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant



(a) TA group (n=10)



(b) TA+PT group (n=12)



(c) SA+PT group (n=16)

Figure 25: **Functional connectivity graph measure, Eg, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Global efficiency (Eg), a graph theoretical measure of local network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p -value are provided. A p -value ≤ 0.05 was considered statistically significant.

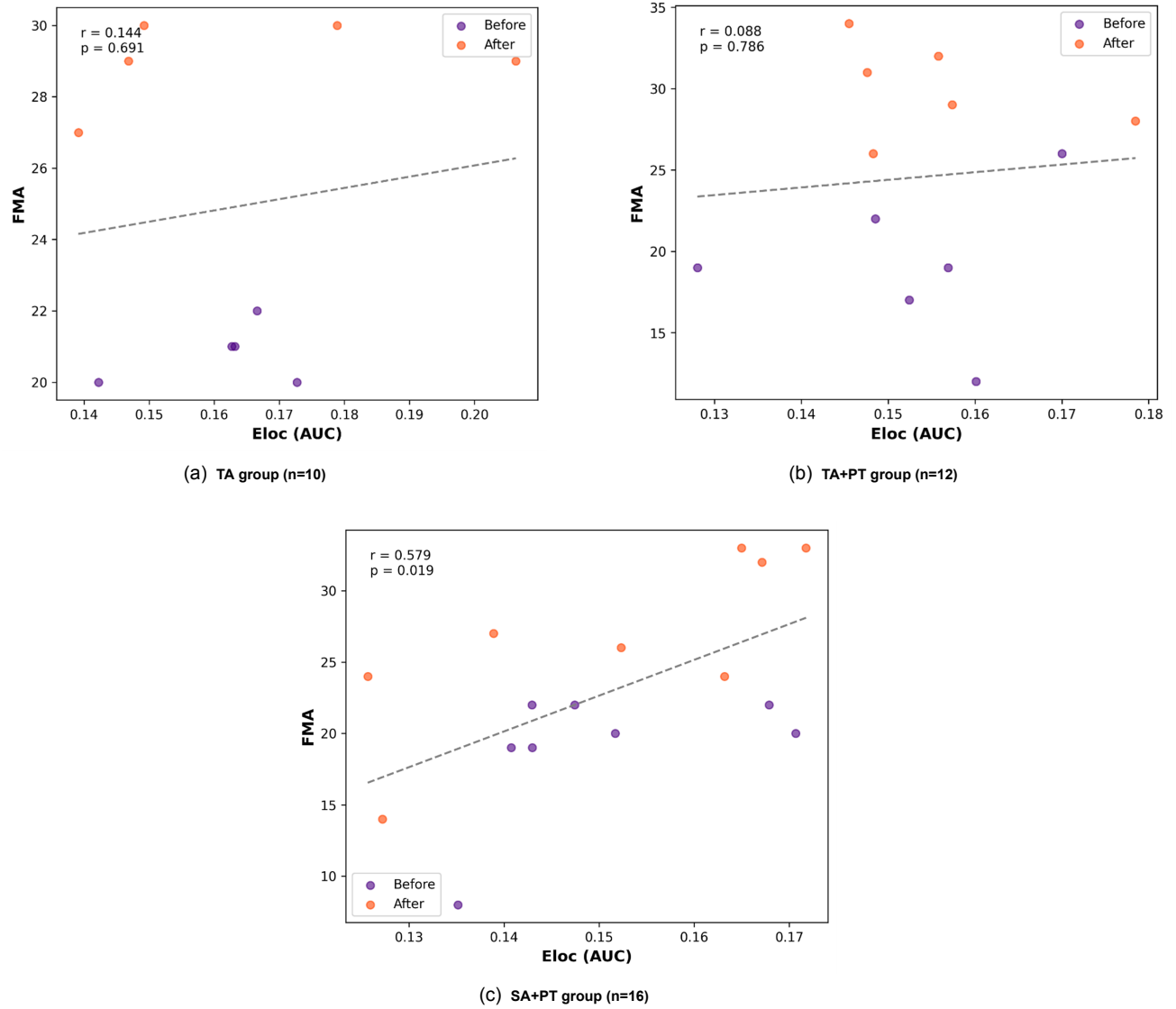
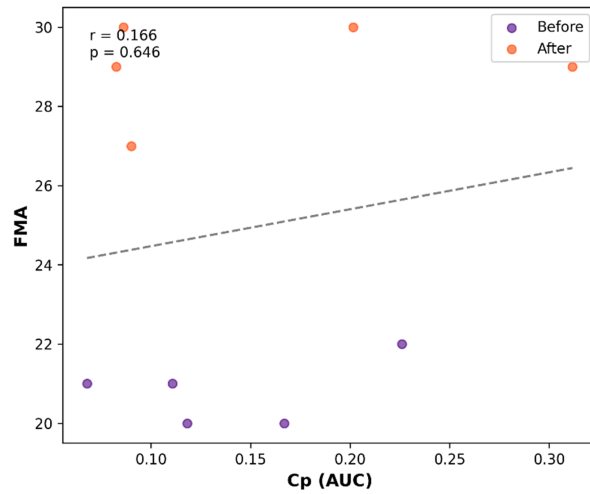
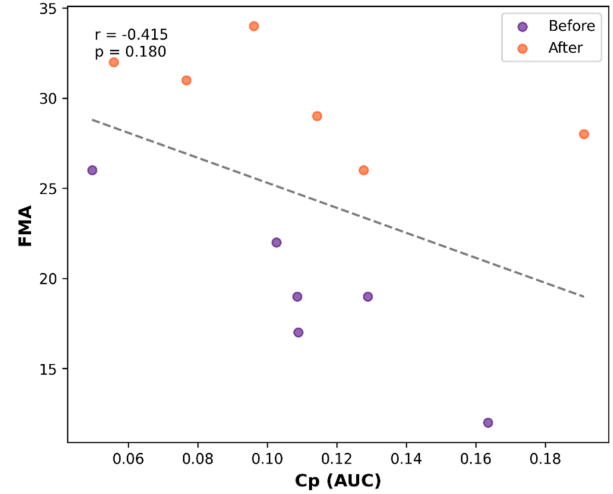


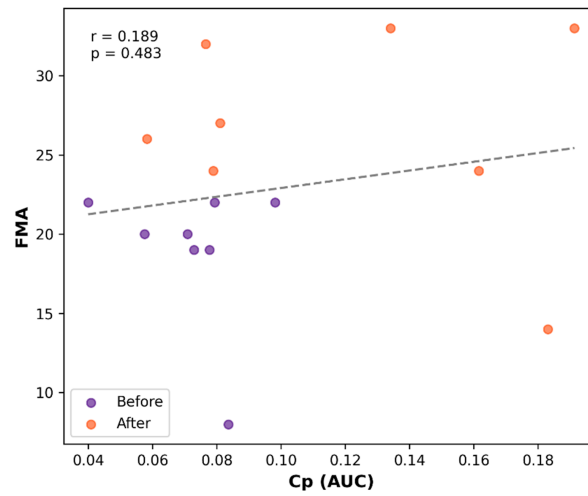
Figure 26: **Functional connectivity graph measure, Eloc, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Local Efficiency (Eloc), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant.



(a) TA group (n=10)

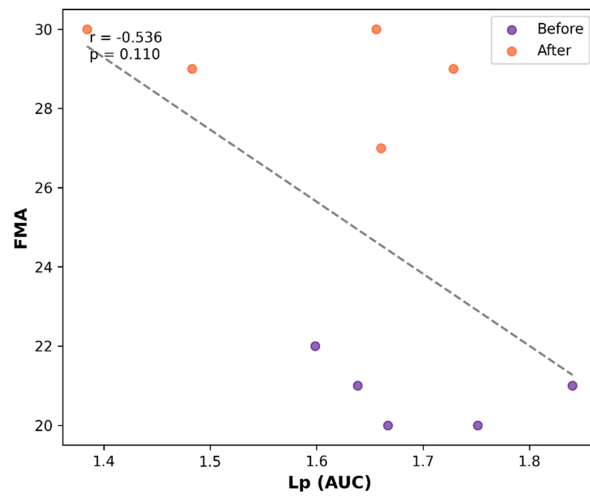


(b) TA+PT group (n=12)

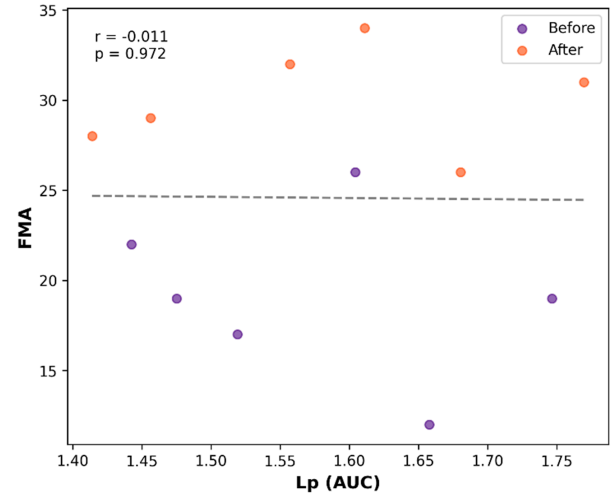


(c) SA+PT group (n=16)

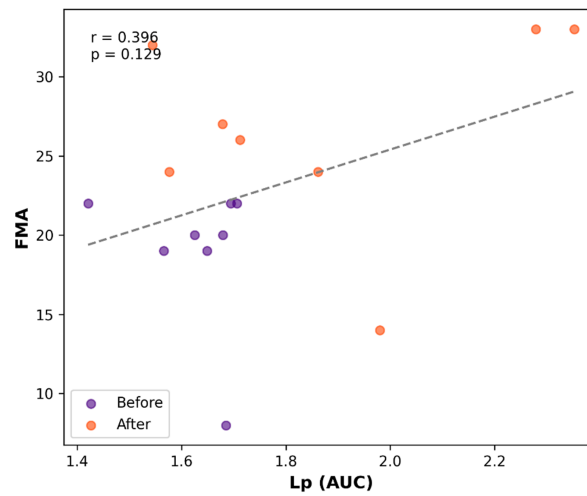
Figure 27: **Structural connectivity graph measure, Cp correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the Clustering Coefficient (Cp), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant.



(a) TA group (n=10)

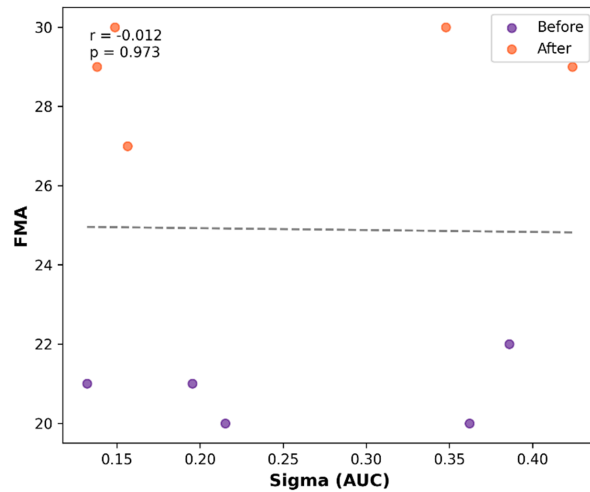


(b) TA+PT group (n=12)

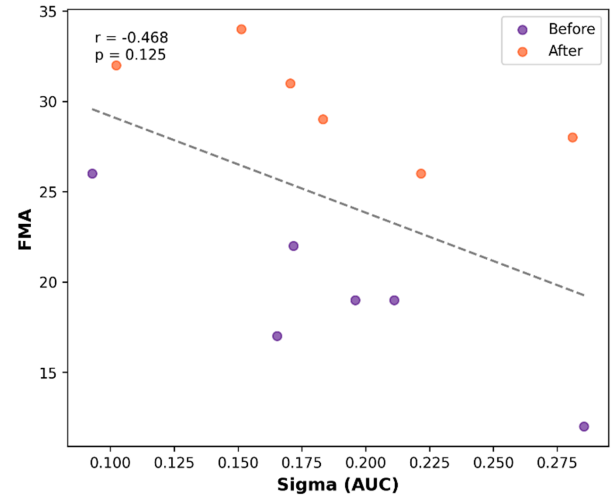


(c) SA+PT group (n=16)

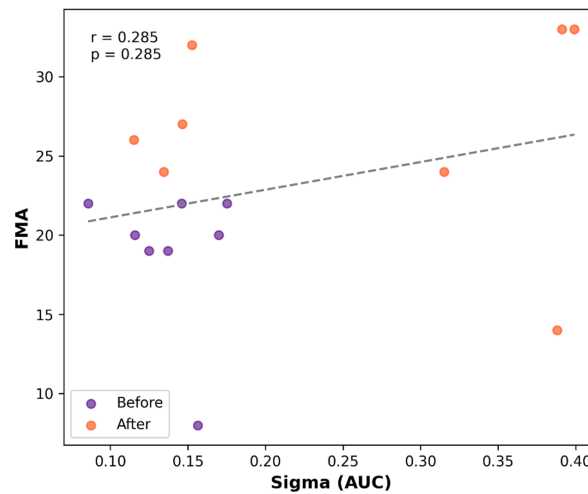
Figure 28: **Structural connectivity graph measure, Lp correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and the characteristic path length (Lp), a graph theoretical measure of network integration, across three treatment groups: (A) TA, B) TA+PT and C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p-value are provided. A p-value ≤ 0.05 was considered statistically significant.



(a) TA group (n=10)

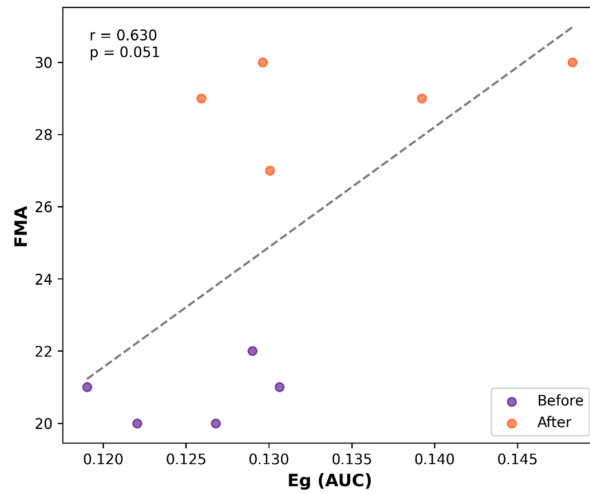


(b) TA+PT group (n=12)

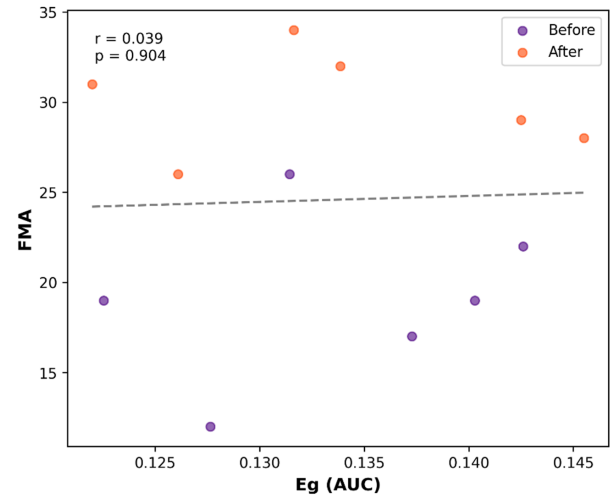


(c) SA+PT group (n=16)

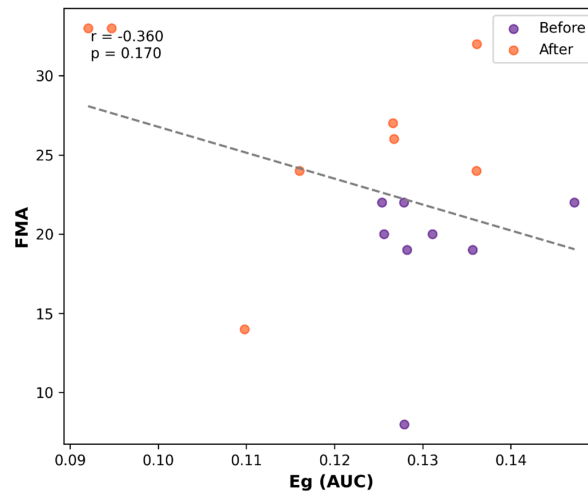
Figure 29: **Structural connectivity graph measure, Sigma, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Sigma, a graph theoretical measure of small-worldness, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p -value are provided. A p -value ≤ 0.05 was considered statistically significant.



(a) TA group (n=10)



(b) TA+PT group (n=12)



(c) SA+PT group (n=16)

Figure 30: **Structural connectivity graph measure, Eg, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and Global efficiency (Eg), a graph theoretical measure of network integration, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p -value are provided. A p -value ≤ 0.05 was considered statistically significant

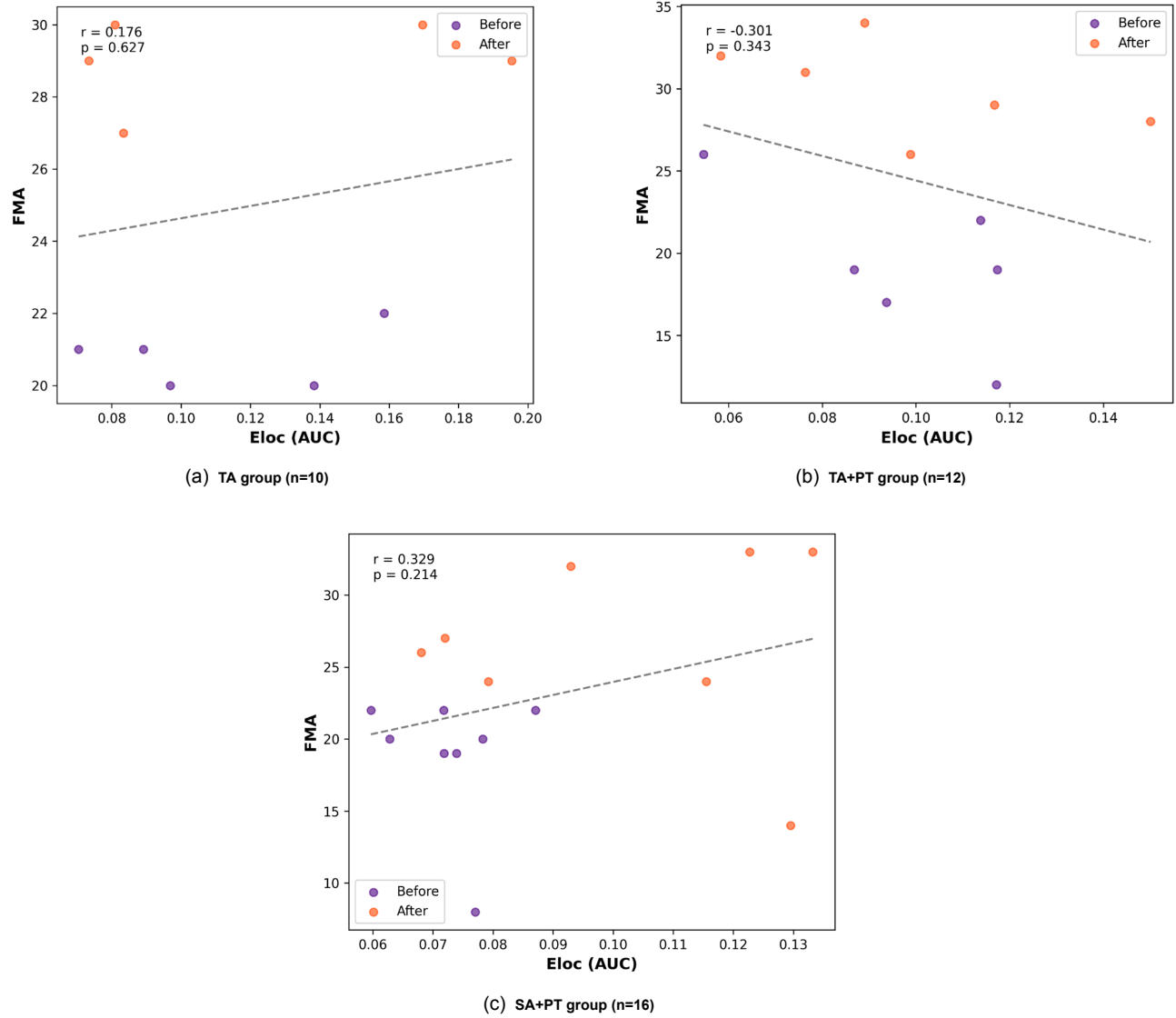


Figure 31: **Structural connectivity graph measure, Eloc, correlated with FMA.** Three scatterplots (A-C) illustrating the Pearson correlation between Fugl-Meyer Assessment (FMA) scores and local efficiency (Eloc), a graph theoretical measure of local network segregation, across three treatment groups: (A) TA, (B) TA+PT and (C) SA+PT. Each plot displays data points for individual patients, with pre-treatment ("before") values shown in purple and post-treatment ("after") values in orange. For each group, the Pearson correlation coefficient (r) and its corresponding p -value are provided. A p -value ≤ 0.05 was considered statistically significant.

The analysis within the three treatment groups showed significantly higher FMA scores after treatment compared to pre-treatment in all three groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8), with the p-values less than 0.01 (**Table 22 and Figure 32**). The very low p-values observed in the TA and SA+PT groups might be due to the outliers detected. For post-treatment data in the TA group, two outliers were detected, 21 and 27. For the pre-treatment data in the SA+PT group, the two FMA values, 12 and 8, were detected as outliers. These values can skew the data and therefore, the outliers were winsorized, and the statistical analysis was recorded in (**Table 23**). The winsorized data showed that the mean FMA increased compared to the non-winsorized data, reflected by the adjusted p-value (0.004), which is now higher than (p-value < 0.001) for the SA+PT group. Thus, replacing the outliers with the outlier bounds made the p-values larger but still significant. There were no significant differences between the different groups post-treatment ($F=1.02$, $\eta^2 = 0.11$, p-value = 0.38) (**Table 24**).

Table 22: **Within-group analysis of the FMA scores in the 3 groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8).** The mean $\pm$ SD and SEM are given. The p-value, effect size, absolute (Cohen's d), and T-statistic are shown. A p-value ≤ 0.05 was considered statistically significant.

Group	Before: Mean $\pm$ SD (SEM)	After: Mean $\pm$ SD (SEM)	p-value	Cohen's d	T-statistic
TA	20.80 $\pm$ 0.84 (0.37)	29.00 $\pm$ 1.22 (0.55)	< 0.001	6.29	-14.06
TA+PT	19.17 $\pm$ 4.71 (1.92)	30.00 $\pm$ 2.90 (1.18)	0.002	2.54	-6.23
SA+PT	19.00 $\pm$ 4.63 (1.64)	26.62 $\pm$ 6.37 (2.25)	< 0.001	2.20	-6.23

Table 23: **Within-group analysis of the FMA scores after winsorization in the 3 groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8).** The mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) are given. The p-value, effect size, absolute (Cohen's d), and T-statistic are shown. A p-value ≤ 0.05 was considered statistically significant.

Group	Before: Mean $\pm$ SD	After: Mean $\pm$ SD	p-value	Cohen's d	T-statistic
TA	20.80 $\pm$ 0.84	29.10 $\pm$ 1.02	0.0001	6.90	-15.41
TA+PT	19.17 $\pm$ 4.71	30.00 $\pm$ 2.90	0.002	2.54	-6.23
SA+PT	19.81 $\pm$ 2.51	26.62 $\pm$ 6.37	0.004	1.51	-4.28

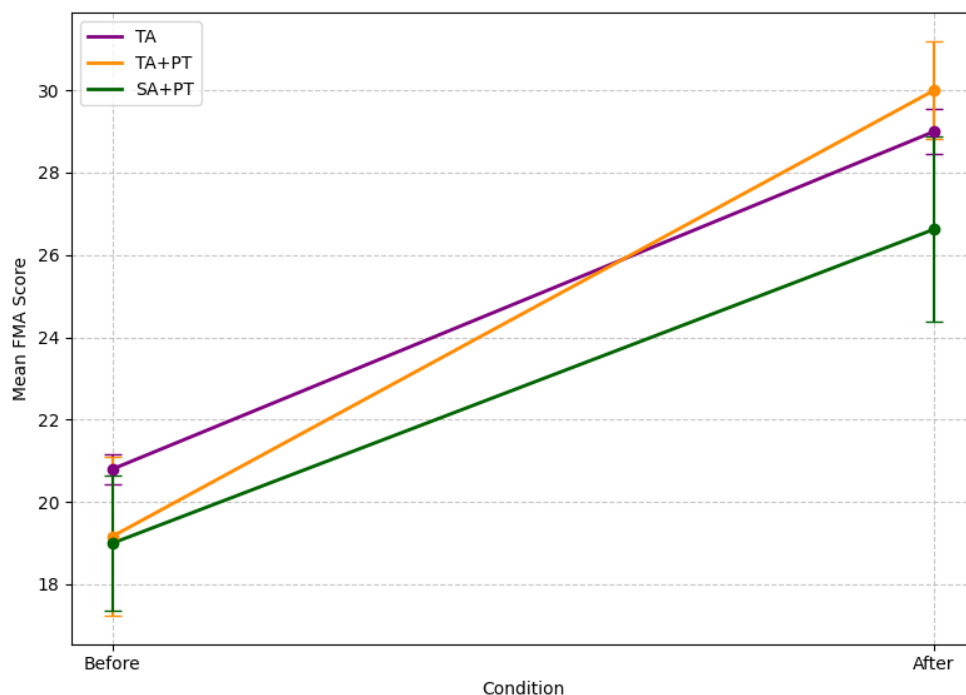


Figure 32: Line graphs illustrating the mean Fugl-Meyer Assessment (FMA) scores for three treatment groups: TA (n=5), TA+PT (n=6), and SA+PT (n=8), pre-treatment and post-treatment. Conditions are distinguished by a color-coded key. Each bar represents the group mean FMA score, with error bars indicating the standard error of the mean (SEM). A p-value ≤ 0.05 is considered statistically significant.

Table 24: Between-group analysis of the FMA scores in the 3 groups: TA (n=5), TA+PT (n=6) and SA+PT (n=8). The mean $\pm$ standard deviation (SD) and standard error of the mean (SEM) are given. The p-value, effect size (Eta-squared, η^2), and F-statistic are shown. A p-value ≤ 0.05 was considered statistically significant.

TA: Mean $\pm$ SD (SEM)	TA+PT: Mean $\pm$ SD (SEM)	SA+PT: Mean $\pm$ SD (SEM)	p-value	η^2	F-statistic
29.00 $\pm$ 1.22 (0.55)	30.00 $\pm$ 2.90 (1.18)	26.62 $\pm$ 6.37 (2.25)	0.38	0.11	1.02

Chapter 9

Discussion

9.1 Functional Connectivity: Within-Group Differences

Ischemic stroke not only damages brain regions adjacent to the infarct but also induces widespread alterations in global brain networks, leading to motor impairments, language deficits, and neuropsychiatric complications such as depression.^[231] Stroke compromises microstructural integrity, thereby disrupting communication between structural and functional networks. Neuroplasticity enables reorganisation of these networks, a process that can occur naturally in the form of spontaneous recovery.^[80] This raises a key question: Can therapeutic interventions be leveraged to act synergistically with spontaneous recovery to improve rehabilitation outcomes? Evidence suggests that acupuncture and physiotherapy promote neuroplasticity and improve motor outcomes in post-stroke patients.^[53] However, their specific impact on structural and functional connectivity in first-ever ischemic stroke patients remains unclear. To address this gap, neuroimaging studies that combine Diffusion-Weighted Imaging (DWI) and resting-state fMRI (rs-fMRI) to study whole-brain network changes are essential to determine whether structural and functional changes are isomorphic. Against this background, the present study examined the effect of adjunctive acupuncture on the whole-brain structural and functional connectivity in nineteen right-handed, male ischemic stroke patients from China, randomized into three treatment groups: 1) True Acupuncture (TA), 2) TA and Physiotherapy (PT), and 3) PT and Sham Acupuncture (SA, a placebo or non-acupoint acupuncture).

To investigate the functional connectivity in the ischemic stroke cohort, the CONN Toolbox was used to determine the significant brain connection changes. All patients in the cohort investigated in this study had a basal ganglia ischemic infarct, except for one, where the infarct occurred in the pons.

9.1.1 True Acupuncture (TA): Increased Functional Connections

The basal ganglia are a set of subcortical nuclei that shape complex motor control and also support non-motor functions such as reward and emotion.^[232] Anatomically, the caudate, putamen, and nucleus accumbens form the striatum, the principal afferent hub where cortical inputs converge.^[233] The globus pallidus externus/internus and the subthalamic nucleus further contribute as efferent elements that relay signals to other regions.^[234] Crucially, the basal ganglia and cerebellum communicate bidirectionally via disynaptic pathways.^[235;236]

Within this framework, our TA within-group analysis showed positive connections for Vermis_7 to Caudate_R, Vermis_7 to Thalamus_R, and Hippocampus_R to Temporal_Inf_R (**Figure 10.a and Table 5**). Converging evidence indicates that the basal ganglia and cerebellum jointly support motor timing:^[237–239] the cerebellum responds to timing irregularities, whereas the basal ganglia engage when task order is unpredictable.^[240] Vermis_7, in the posterior cerebellum, has been implicated in this timing role, and predictive motor timing appears to require coupled basal ganglia-cerebellar activation, observed also in healthy participants. Therefore, damage to the basal ganglia can impair motor timing.^[241;242]

The caudate participates in the cortico-thalamo-basal ganglia (CTBG) core timing circuit, a partially distributed, context-dependent mechanism.^[243;244] Consistent with this, the thalamus, an integrative relay, coactivated with Vermis_7. After true acupuncture (TA) treatment, a strengthened hippocampal connection also emerged. The hippocampus encodes temporal information via "time

cells” in rodents and humans, supporting episodic memory.^[245;246] Episodic memory is a type of long-term memory that allows for the conscious recall of past events, or experiences. Together, these patterns suggest that true acupuncture may upregulate CTBG, cerebellar, and hippocampal nodes to refine temporal integration and motor control, while facilitating retrieval of motor experiences. In contrast, the post-treatment decrease of the parahippocampal connection, important for spatial, visual, and episodic memory,^[247] in the SA+PT group may reflect sham acupuncture’s more limited engagement of episodic-memory circuits relative to TA (**Table 7**).

9.1.2 True Acupuncture (TA): Decreased Functional Connections

In the TA group, most post-treatment decreases involved the supplementary motor area (SMA) (**Figure 10.b; Table 5**), a core timing node.^[248] Globus pallidus internus inhibition of the SMA facilitates subsequent movements, consistent with basal ganglia suppression of competing motor circuits^[249] where the (pre-) SMA mediates response inhibition.^[250–252] Thus, reduced SMA connectivity may prevent excessive inhibition in already compromised motor pathways. In chronic stroke, Laplacian-EEG shows SMA overactivation post-response and reduced preparatory activity,^[253] suggesting impaired motor pre-planning. Together, our results imply that true acupuncture may prevent SMA overactivation by rebalancing activation and deactivation to optimize motor performance. However, another study where patients were treated with acupuncture showed an increased FC between the SMA and other brain regions.^[254] The contradictory results emphasise the need for critical evaluation of study design. Our study included manual and electroacupuncture (EA), whereas their study only included manual acupuncture (MA). EA was shown to be superior to MA in stroke recovery.^[255] Additionally, our results could support the notion previously introduced in which TA aids in inhibiting competing motor circuits, mediated by the increased SMA, specifically the pre-SMA. Further supporting our results is a study that showed a decrease in FC between the ipsilateral primary motor cortex and the ipsilateral SMA after conventional rehabilita-

tion and motor imagery therapy in ischemic stroke patients.^[256] However, their study did not use acupuncture, and instead of the primary motor cortex, our study showed the SMA connected to the rolandic operculum, which is anatomically close to the M1 (**Figure 10.b**). The rolandic operculum is near the central sulcus, where the temporal, parietal and frontal lobes converge and has multiple functions, with one notable one being processing integrated exteroceptive-interoceptive signals important for self-awareness and bodily self-consciousness (BSC).^[257] Interestingly, their fMRI results showed the activation of the bilateral rolandic operculum implicated in mental states associated with self-consciousness and interoceptive awareness. Furthermore, a study aiming to identify brain regions involved in body ownership showed bilateral activation of the rolandic operculum.^[258] Notably, this emphasizes the role of the BSC in alterations in limb ownership.^[259] Indeed, stroke patients can experience alterations in their subjective feeling of ownership for the affected limb and show decreased capability of detecting their real limb from a virtual limb.^[260] Alarming, this could limit motor recovery of the affected limb. The reduced functional connectivity between the SMA and bilateral rolandic operculum after acupuncture treatment in the current study (**Table 5**), might prevent distorted body perception post-stroke. However, whether decreased or increased functional connections involving this brain region prevent distortions in body ownership is presently unclear. A study where the TE5 acupoint was stimulated on the affected side, showed that brain regions involved in sensorimotor function as well as the medial frontal cortex was activated and enhanced lateralization to the contralateral (unaffected) hemisphere, consistent with compensatory takeover.^[261] In contrast, LI4/LI11 produced stronger somatosensory cortical activation,^[262] indicating acupoint-specific neural responses.

In this study, we needled GV26 (DU26), PC6, GB34, SP6, BL40, and GB40 (**Figure 7**); all were bilateral except BL40 and GV26, making it difficult to attribute specific neural effects, or lateralization, to a single acupoint. In the TA group, infarcts were evenly split left/right (one unknown) (**Appendix**

C.10.4.2). Post-treatment, all significantly decreased SMA connections were left-sided, linking to the bilateral rolandic operculum, bilateral superior temporal gyrus (STG), and bilateral Heschl gyrus (**Table 5**). These regions subserve sensory, auditory, and language processing, less central to motor recovery.^[263;264] Notably, the rolandic operculum and Heschl gyrus are hypoactivated in major depression,^[265] and rolandic operculum lesions relate to post-stroke depression.^[266] Because we did not administer depression questionnaires, we cannot determine whether the decreased connectivity reflects altered limb ownership or depressive symptoms. Moreover, the right cerebellar lobule VIIb (Cerebelum_7b_R) and the left cerebellar lobule VIII (Cerebelum_8_L) showed a significant decrease in connectivity post-treatment (**Table 5**). Thus, both hemispheres were engaged before treatment, but connectivity decreased following acupuncture, with the increased connections primarily originating from the right hemisphere. This could suggest dampening of excessive cross-talk between motor planning and sensory/auditory processing across both hemispheres, which could interfere with motor recovery. Thus, “pruning” away non-essential connections and allowing the SMA to dedicate more resources to brain regions involved in motor function.

9.1.3 True Acupuncture (TA) Combined with Physiotherapy

About one-third of patients develop neuropsychiatric complications (e.g., depression) within five years post-stroke,^[267;268] which can hinder motor recovery.^[269;270] Affected circuits link the prefrontal cortex, basal ganglia, and amygdala. The amygdala-basolateral complex (BLA) and central nucleus (CeA), mediates emotion, fear, reward^[271] and modulates pain, with activity tied to vulnerability and tolerance.^[272] The insula, with the anterior cingulate cortex (ACC), forms the salience network (SN) that prioritizes internal/external stimuli.^[273;274] Critically, the right anterior insula switches between the central executive network (CEN) and the default mode network (DMN), which often compete.^[210;275;276] Specifically, the right anterior insula (AI) upregulates the CEN and downregulates the DMN across modalities. In motor control, the insula operates in five stages,

from stimulus segregation (stage 1) to relaying to the ACC to select an appropriate motor response (stage 5).^[277] Two anti-correlated systems maintain balance under task demands: a task-positive network and the task-negative network.^[210;278] The task-negative network is called the limbic-paralimbic neocortical network (LPNN). The LPNN integrates limbic, paralimbic, and neocortical regions to coordinate emotion, interoception, salience, cognition, and autonomic control. In our data, the right insula appears to switch toward a task-positive pattern involving Vermis_1_2 (**Table 6**) while anti-correlating with LPNN nodes, the amygdala, Temporal_Pole_Mid (middle temporal gyrus (temporal pole)), and Frontal_Sup_Orb_L (orbital superior frontal gyrus), that might direct attention to motor goals (**Figure 11.b**).

Studies have shown that the DMN is deactivated during attention-demanding tasks.^[210;279;280] Consistent with this, the Frontal_Sup_Medial_R (medial superior frontal gyrus (SFG)), a DMN node, decreased post-treatment in the TA+PT group (**Table 6 and Figure 11.b**). The SFG supports self-related processing that is suppressed during intense sensorimotor demands.^[281] Alongside Vermis_1_2 (anterior cerebellum; sensorimotor/postural control) and the right insula, this pattern suggests adjunctive acupuncture may reallocate resources from self-reflection to sensorimotor processing, functionally “shutting off” nonessential circuits and lowering cognitive load. SFG coactivation with the amygdala, could imply a self-reinforcing anti-analgesic effect. Notably, multiple studies link acupuncture to neuroprotection, analgesia and energy balance via the endocannabinoid system (ECS).^[282–287]

The endocannabinoid system (ECS) is a likely mediator of acupuncture’s benefits. It comprises CB1R and CB2R, widely expressed in the CNS.^[288] In ischemic stroke, CB1R mitigates glutamate-driven excitotoxicity,^[289] where clinical and preclinical studies showed that electroacupuncture (EA) lowers serum glutamate and inhibits excessive release.^[290–292] EA also reduces NMDA mRNA in the amygdala, producing analgesia, consistent with high CB1R expression there,^[293] suggest-

ing TA+PT may attenuate glutamatergic excitotoxicity in amygdala circuits. CB1R further modulates GABAergic signaling, where its downregulation can increase GABA-mediated inhibition of glutamate.^[294;295] Furthermore, acupuncture's GABAergic analgesia is region-specific.^[296] Notably, activating GABA receptors can raise nociceptive thresholds in the spinal cord but lower them supraspinally. Thus, sham (non-acupoint) stimulation may have been less effective than TA at engaging supraspinal GABA/ECS pathways, potentially explaining the increased amygdala connectivity in SA+PT (**Figure 12.a; Table 7**), though, without molecular markers, this remains speculative.

9.1.4 Sham Acupuncture (SA) Combined with Physiotherapy

In contrast to the TA+PT group, connections involving the amygdala increased in the SA+PT group (**Table 7**). Some studies report sham acupuncture producing effects comparable to true acupuncture,^[297–299] yet concerns about bias and small samples persist.^[300] A meta-analysis study found no overall molecular differences and noted higher GABA with true acupuncture, supporting inhibitory effects on nociception.^[43] Interpretation is further complicated by heterogeneous sham protocols and incomplete blinding.^[301] Interestingly, acupuncture at GB34 can evoke stronger amygdala responses in females despite similar Deqi.^[302] Because Deqi has a pain component, and rehabilitation introduces joint pain, perception may be confounded; familiarity and expectation in our Chinese cohort may reduce perceived pain.^[303] Expectancy itself matters: believing one received true acupuncture lowers pain.^[304] Our randomized but unblinded design, with no exclusion of prior acupuncture users, could therefore amplify pain in SA+PT, helping explain its increased amygdala connectivity (**Figure 12.a; Table 7**).

Additionally, in contrast to the TA group, the functional connections involving the left Supp_Motor_Area_L (SMA) increased in the SA+PT group. Specifically, the amygdala coactivated with brain regions involved in the Prefrontal Cortex (PFC): Frontal_Sup_Orb_R (Right Superior Orbital

Frontal Gyrus), Frontal_Mid_Orb_R (Right Middle Orbital Frontal Gyrus), Frontal_Sup_R (Right Superior Frontal Gyrus), the Frontal_Sup_L (Left Superior Frontal Gyrus), and the Frontal_Sup_Medial_R (Right Superior Medial Frontal Gyrus) (**Figure 12**). All of these regions contribute to higher-order cognitive processes, including decision-making, executive control, working memory, social cognition, and emotional regulation, and they span across multiple functional networks. The prefrontal cortex (PFC) in particular is central to goal-directed behaviour, often referred to as "motivational control".^[305] The amygdala has been implicated in emotional conflict, which arises when emotionally incompatible stimuli are present, for example, during the interpretation of facial expressions or under conditions of uncertainty in decision-making. Evidence further suggests that the rostral anterior cingulate gyrus plays a regulatory role by inhibiting the amygdala to resolve such conflicts. Notably, this region was not significantly activated in the SA+PT group post-treatment, which may suggest that physiotherapy imposes additional cognitive and emotional demands on patients. These demands, coupled with the uncertainty inherent in motor performance tasks, might interact with patients' prior knowledge of the distinction between true and sham acupuncture. Thus, emotional conflict, mediated by the amygdala, could affect motor performance as seen by the significantly increased functional connection between the amygdala and the Left Cerebellar Crus II (Amygdala_R-Cerebellum_Crus2_L) (**Table 7**). Furthermore, the SMA coactivated with both the left and right fusiform, also significantly increased post-treatment in the SA+PT group. The fusiform gyrus plays a role in visual search and visual mental imagery, specifically the fusiform face area (FFA) activated when imagining faces and scenes.^[306–308] Mental imagery is the process by which a behaviour is rehearsed mentally, by imagining the actual scenario being played out, and has been shown by fMRI studies to overlap with the actual task being performed.^[309;310] An observational study investigating 39 ischemic stroke patients with upper limb dysfunction, found that there was a statistically significant correlation between the ability to visualize upper limb men-

tal motor images with motor performance.^[311] This is interesting, because it might suggest that sham acupuncture and physiotherapy could modulate the brain regions involved in mental motor imagery as seen by the involvement of SMA. A study showed that ischemic stroke patients receiving acupuncture at the GB34 acupoint had increased positive functional connectivity involving the fusiform gyrus and the parahippocampus.^[312] The parahippocampus was suggested to be involved in high-level cognitive functions and was significantly decreased in the FC for the SA+PT group in the present study. Again, showing differential effects of true acupuncture vs. sham acupuncture. The following brain regions also showed significant increases in our study: Frontal_Sup_R–Temporal_Pole_Mid_L (Left Middle Temporal Pole) and Frontal_Sup_R–Olfactory_L (Left Olfactory Cortex). The middle temporal pole functions as a semantic hub, supporting language processing and contributing to social–emotional integration,^[313] which may reflect the demands of interpreting instructions from the physiotherapist during motor tasks. The olfactory cortex, while primarily responsible for odor perception, is also a key substrate for the strong link between odors and emotional memory, influencing mood, learning, and the flexible use of information across social contexts.^[314] This pattern may therefore highlight the role of emotional modulation during sham acupuncture combined with physiotherapy.

9.1.5 Physiotherapy

Physiotherapy enhances post-stroke motor recovery, but its benefit depends on dose, timing, and task design, where effects can be beneficial, neutral, or detrimental.^[315] For example, VECTORS reported an inverse dose-response, with higher-dose Constraint-Induced Movement Therapy (CIMT) yielding less improvement at 90 days,^[316] while AMOBES found no FMA difference between "soft" and intensive PT.^[317] Notably, both trials intervened very early (within ≤ 11 for the former study and ≤ 3 days for the latter study), during the spontaneous-recovery window, when training may preferentially drive compensation rather than restoration of damaged networks.^[318]

This distinction matters: (1) true neurological recovery (impairment resolution with pre-stroke motor patterns), (2) functional recovery (goal achievement despite residual impairment), and (3) compensation (alternate strategies) are not equivalent. Overreliance on compensation, a “fundamentally inappropriate action”, can substitute for effective movement and cap long-term gains.^[319;320]

Mechanistically, patients with unilateral deficits often develop learned non-use of the paretic limb, where overuse of the unaffected limb and abnormal interhemispheric inhibition (excess suppression from the unaffected hemisphere) further impede recovery.^[321] Intrahemispheric competition and disinhibition of residual engrams can also hinder relearning. Critically, apparent activation from disinhibition may reflect compensation, not true recovery. These dynamics help explain the maladaptive patterns resulting when therapies are not administered at the appropriate time or dose. To promote genuine recovery, PT should be engineered to counter learned non-use and rebalance hemispheric interactions, favoring challenging, novel, and meaningful practice (not mere repetition), at an appropriate dose and time point, to drive true neurological recovery rather than compensatory shortcuts.

Nociception disrupts afferent (including proprioceptive) and efferent pathways,^[322] thereby impairing somatosensory input and inhibiting motor performance in ischemic stroke, potentially creating a vicious cycle.

9.2 Functional Connectivity: Between-Group Differences

Post-treatment comparisons between the SA+PT and TA groups revealed only decreased functional connections (**Figure 13**), indicating that these connections were significantly stronger in the TA group. The affected connections primarily involved brain regions associated with motor function, including the cerebellum. Specifically, the connections between the cerebellum and the

left parietal lobule, as well as between the cerebellum and the left rectus gyrus, suggested that TA modulated regions implicated in spatial awareness and decision-making, respectively. There were substantially more significantly increased connections in the TA+PT group compared to the SA+PT group post-treatment (**Figure 14**). Most of these connections involved brain regions responsible for motor control, such as the vermis and the cerebellum. Conversely, the connection between the left olfactory cortex and the left angular gyrus significantly decreased in the TA+PT group, supporting the within-group findings that TA combined with physiotherapy dampened activity in non-motor networks, such as the default mode network (DMN), that might otherwise interfere with recovery.

Compared to the SA+PT group, the TA+PT group exhibited fewer significantly increased connections than the TA group, potentially indicating similar patterns of neuromodulation between the two intervention groups (**Figure 15**). The TA+PT group also showed significantly increased connectivity between the cerebellum and the left inferior frontal gyrus, including both the opercular and triangular parts. This pattern further supported the involvement of brain regions required for comprehending and responding to verbal instructions during physiotherapy, as the inferior frontal gyrus is implicated in motor planning for speech, response inhibition, and language comprehension. In contrast, the significantly decreased connections in the TA+PT group relative to the TA group predominantly involved the cerebellum, suggesting that enhanced activity within the inferior frontal gyrus may have inhibited motor-related responses and thus reduced activation in regions underlying motor execution, possibly reflecting interference from cognitive and emotional regulatory processes.

9.3 Structural Connectivity

The TA group showed decreased mean FA in the Supp_Motor_Area_L-Rolandic_Oper_L, Supp_Motor_Area_L-Rolandic_Oper_R, and Amygdala_L-Frontal_Mid_Orb_R connections post-treatment (**Figure 16.b**) compared to pre-treatment (**Figure 16.a**), mirroring the functional connectivity results (**Table 5**). In particular, the reduced FA between the right rolandic operculum and the left SMA suggests that true acupuncture may dampen interhemispheric cross-talk.

In contrast to the FC results, interhemispheric cross-talk appeared to increase in the TA+PT group, as reflected by higher mean FA between the Cerebelum_7b_R and Cerebelum_8_L post-treatment (**Figure 17**). Without tract-tracing, the exact white matter pathway cannot be identified. However, possible explanations include indirect routes via the ascending cerebello-thalamo-cortical and descending cortico-ponto-cerebellar tracts, or direct connections through commissural fibers and the cerebellar vermis.^[323] The SA+PT group exhibited increased FA in the Supp_Motor_Area_L-Rolandic_Oper_L, Amygdala_L-Frontal_Sup_Orb_R, and Amygdala_R-Frontal_Sup_Orb_L connections (**Figure 18**).

Since structural connectivity was only qualitatively assessed, the significance of these FA changes remains uncertain. Nevertheless, the results indicate distinct microstructural effects across the three treatment groups. A longitudinal study showed progressive FA reductions in the superior corona radiata, cerebral peduncle, and corticospinal tract (CST) in ischemic stroke patients with basal ganglia infarcts.^[324] Supporting this, another study found widespread functional and structural disruptions in patients with basal ganglia lesions.^[325] Notably, FA reductions often occur in regions remote from the infarct, consistent with secondary white matter degeneration, the breakdown of axonal fibers distant from the lesion, either retrogradely toward the cell body or anterogradely (Wallerian degeneration) toward the axon terminals.^[326] Despite all patients in the present

study having basal ganglia infarcts, these findings suggest widespread structural network-level reorganization extending beyond the lesion site.

9.4 Structural-Functional Connectivity Coupling

Although functional networks are expected to become isomorphic with structural networks over time, this was not observed in the current study (**Figure 19**). Functional changes can occur in structurally intact regions distant from the infarct.^[174] While we hypothesized that functional connectivity reflects structural connectivity, the reverse may also hold, with functional connectivity existing without direct structural links.^[327] This is supported by weak SC-FC coupling across all groups (**Figure 19.a-c**), where highly correlated time series sometimes coincided with lower FA. The highest absolute Fisher-Z values were expected to correlate with the highest FA values, but this was not seen. The very weak, negative post-treatment coupling may reflect increased flexibility for network reorganization. A study reported altered SC-FC coupling in stroke patients, which was lower than in controls but weakly positive, whereas ours was negative.^[328] This pattern suggests acupuncture could further promote decoupling, creating a permissive baseline for new structural and functional connections, rather than the constraints of tightly coupled networks. Thus emphasizing the weak evidence for a linear relationship between functional and structural connectivity after lesions to anatomical structures and that functional connectivity networks might rely on indirect structural pathways, potentially reflecting a mechanism for neuroplasticity. Another study found negative coupling in the right middle cingulate gyrus (MCG) and paracentral lobule (PCL), and positive coupling in the right supramarginal gyrus (SMG) and middle temporal gyrus (MTG), indicating that individual brain regions may exhibit different coupling strengths compared to when brain regions are grouped together.^[329]

Beyond region-specific analyses, future work should also assess SC-FC coupling at multiple time points (e.g., 3 and 6 months) to test for a positive shift. The 3-5 week post-stroke window in this study may have been too short for substantial structural remodeling, which takes weeks to months,^[330] whereas functional remodeling occurs within hours to days through redundant connections^[331] or unmasking latent pathways.^[332] This interpretation is reinforced by significant correlations between FMA and FC graph measures (**Figures 22-26**), but not SC measures (**Figures 27-31**), with effects limited to the SA+PT group.

9.5 Functional Graph Measures

The human brain is a complex system characterized by a small-world network topology, which has been shown in both structural and functional networks.^[147] A small-world network, is a combination of a high clustering coefficient (C_p) and short characteristic path length (L_p), defined as the number of edges that exist between nearest neighbors (nodes) as a proportion of the maximum number of possible connections and the minimum number of edges that need to be traversed to go from one node to another, respectively.^[157] This network topology is different from a random network, classified by low C_p and short L_p , and regular networks classified by high C_p and long L_p .^[333] Therefore, the small-world network is a combination of a random and regular network, improving the high efficiency of information transfer while minimizing wiring cost. Thus, small-world networks maintain complex dynamics by optimizing a balance between segregation and integration.^[157;334;335] Segregation is the statistical independence of neuronal groups (collective neurons sharing inputs, outputs, and responses) or intra-domain connectivity, whereas integration is the statistical dependence of these neuronal groups or inter-domain connectivity.^[335]

Neurological insult results in disruption of this balance, which can result in the small-world network shifting to a more random or regular network.^[336;337] However, some studies showed that acute

ischemic stroke patients and control subjects exhibited small-world network organization.^[338;339] The graph theoretical measurement, Sigma, reflects a small-world network topology when it is more than 1, a random network topology when equal to 1, and a regular network when less than 1. In the current study, Sigma for both structural and functional data was less than 1 at baseline and post-treatment, indicating a regular network (**Table 11 and Table 15**). A regular network topology is metabolically costly as it has a high Cp and a long Lp.

Evaluating the functional connectivity graph measures in this study (**Figure 20**), only Lp showed a significant post-treatment decrease in the SA+PT group after adjusting for motion (**Table 11**), contrasting with the structural data for the same group. No other graph measures changed significantly. This is consistent with what was observed in a previous study,^[328] where no statistically significant differences were observed for global network properties (Cp, Lp, Eg, Eloc) for the functional data and thus the only difference in our study was Lp. Interestingly, their study^[328] reported a Sigma > 1 for both structural and functional networks, whereas in our study, Sigma was < 1 for both. Their cohort included males and females, while ours included only males. Interestingly, a study found that males had stronger inter-network connectivity, while females showed enhanced intra-network connectivity, with age contributing to a decline in intra-connectivity.^[340] Notably, the females show more pronounced changes.

Another study found lower Lp and higher Eg in acute stroke patients compared to controls, consistent with our findings (**Figure 20**), and suggested a shift from small-world to random networks.^[339] In our data, mean Sigma values for FC (**Table 8**) were closer to 1 (0.70–0.81) than for SC that showed a Sigma less than 0.26 (**Table 15**). Sigma increased in TA and TA+PT but not SA+PT, the opposite of the SC results (**Table 11**). A study reported higher Cp and Lp but lower Eg and Sigma in both left- and right-hemisphere infarcts compared to controls.^[338] This pattern contrasted with our findings, except in the SA+PT group, where non-significant trends in Cp (increasing) and

Sigma (decreasing) aligned with their results. Moreover, Cp in the TA group increased significantly post-treatment compared to baseline. At baseline, only Lp differed significantly across groups (**Table 12**), with the SA+PT group showing higher Lp than both the TA and TA+PT groups (**Figure 14**).

9.6 Structural Graph Measures

A study that investigated structural connectivity showed that Lp significantly increased in the stroke patients compared to the control subjects,^[328] which is similar to the Lp in the SA+PT and TA+PT group in our study, which increased post-treatment compared to pre-treatment, but not significantly (**Figure 21 and Table 15**). On the other hand, in their study stroke patients showed a significant decrease in global efficiency (Eg). In our study, the SA+PT group demonstrated a similar trend toward reduced Eg, although this did not reach statistical significance. This might suggest a decrease in integration. Additionally, the TA group showed an opposite trend to the SA+PT group for both the Lp and Eg graph measures in our study, decreasing and increasing, respectively.

The significantly increased Lp in the SA+PT group compared to the TA and TA+PT groups (**Table 17**) might indicate a shift towards a highly integrated network, which is metabolically costly. This is in contrast to the significantly decreased Lp for the same group in the FC results (**Table 11**). This suggests that FC might reflect network segregation compared to integration in the SC network. Additionally, the increased Lp and Cp in this group are characteristic of a regular network, further suggesting abnormal network topology (**Table 15**). The decreased Lp and significantly increased Cp in the TA group might suggest that true acupuncture could balance segregation and integration, by increasing communication between local neuronal groups and reducing wiring cost, respectively. The significantly increased Eloc in the TA group after adjusting for motion further sup-

ports this, by indicating a more segregated network of intra-domain connectivity. This could occur by using alternative white matter pathways still intact after the ischemic infarct.^[341]

Unexpectedly, Sigma significantly decreased post-treatment for the TA group after correcting for motion (**Figure 21**), which was expected to increase due to increased Cp and decreased Lp. Sigma is calculated as the ratio of the normalized Cp to the normalized Lp.^[342] However, the changes might not have been sufficient for an increase in Sigma. Importantly, the normalized graph measures were not investigated separately in this study, and thus, the influence of Cp and Lp on sigma can only partially be explained. Interestingly, the significantly increased sigma post-treatment in the SA+PT group (**Table 17**) was still lower compared to the sigma pre-treatment in the TA group (**Table 16**). Even though patients were randomized into treatment arms and had moderate stroke, the patients in the SA+PT group could have been more susceptible to ischemic stroke as a result of their different physiology, which would require broader network changes to return to homeostasis.^[144;343] The significantly lower baseline levels of Cp, Sigma and Eloc in the SA+PT group could explain the greater changes observed post-treatment, compared to the TA and TA+PT groups (**Table 16+18**). Additionally, SC changes may reflect the vulnerability of white matter to damage and the extensive reorganization after treatment.^[344–346] Alternatively, the larger sample size in the SA+PT group (n = 8), compared with the TA (n = 5) and TA+PT (n = 6) groups, may have provided greater statistical power to detect these changes. In contrast, the TA+PT group showed little to no post-treatment differences across graph measures (**Table 15**). At baseline, Eg was significantly higher in the TA+PT group than in the TA group after adjusting for motion, which may explain the relative stability of post-treatment values (**Tables 16 and 18**). Additionally, it could indicate a ceiling effect.^[347] Because neuroplasticity can extend beyond 6 months,^[348] any “ceiling” here likely reflects statistical limits (e.g., small sample) rather than physiology. The FMA gains (**Figure 32**) further argue against a physiological ceiling.

By contrast, the smaller FC changes may indicate greater flexibility, as indirect white matter pathways can compensate for damaged ones, allowing adaptation without major functional alterations. Previous studies focusing on the acute stage, likely captured transitions from small-world to random or regular networks, whereas our subacute/chronic cohort suggests patients had already shifted to a regular network, no longer reflecting small-worldness. This raises the possibility that brain reorganization during neuroplasticity involves transitioning from small-world to random, then to regular networks, and eventually back to small-world. Acupuncture may modulate this process, as suggested by Han et al,^[174] who reported increased Cp and Eloc, also seen for both FC (**Figure 20.a+e**) and SC networks (**Figure 21.a+e**) across all treatment groups in our data, though not significantly. Their subacute cohort (all right-hemispheric infarcts) showed a shift toward random networks, which acupuncture might help reorganize, though again not significantly. Additionally, the increased Cp and Eloc might indicate that these networks are slowly becoming more segregated, favouring intra-domain communication.

Overall, SC shows a compromised segregation-integration balance, as evidenced by a very low Sigma. In contrast, FC Sigma values near 1 suggest that acupuncture may better improve this balance and promote a shift from regular to random organization. Random networks are preferred, as regular networks exhibit longer characteristic path lengths and greater metabolic costs; moving Sigma closer to 1 enhances the efficiency of information transfer. Given the 3-week regimen, longer interventions with longitudinal assessments are needed to test a sustained change.

9.7 Correlation Between FMA and Graph Measures

The significant associations between functional graph theoretical measures and FMA, but not structural measures, suggest that FC may serve as a more sensitive biomarker of motor recovery

in ischemic stroke patients (**Figures 22–26**). In particular, Cp, Lp, Eg, and Eloc were significantly correlated with FMA, though only within the SA+PT group (n=16). This finding implies that functional graph measures may have predictive value for motor outcome gains. Specifically, positive correlations were observed for Cp, Eg, and Eloc, consistent with a network becoming more efficient and balancing integration and segregation. The expected inverse relationship seen for Lp further supports this. The absence of a significant association with Sigma further supports the interpretation that the network is shifting from a more regular to a more random topology. The restriction of significant results to the SA+PT group may reflect the larger sample size compared to TA (n=10) and TA+PT (n=12).

9.7.1 Fugl-Meyer Assessment (FMA) Changes

Motor impairment is the most common complication after ischemic stroke and rehabilitation is performed to improve motor outcomes.^[349] Fugl-Meyer Assessment (FMA) is the first quantitative method to measure sensorimotor stroke recovery in hemiplegic patients.^[187]

The FMA for Upper Extremity (FMA-UE) and Lower Extremity (FMA-LE) is widely recommended as a primary outcome measure of motor recovery in stroke patients.^[350] The FMA-UE evaluates motor function across 33 tasks involving the shoulder and upper arm to wrist and hand. In contrast, the FMA-LE assesses movement across major lower-limb joints, including the hip, knee, ankle, and associated reflexes, through 16 tasks, each rated on a 3-point scale ranging from 0 (no active motion) to 2 (normal motion). In this study, the FMA was analyzed as a total motor score rather than separating upper and lower extremity subscores. Previous work has shown no significant difference in the rate of recovery between upper and lower extremities.^[351] Furthermore, FMA is widely regarded as the gold standard for quantifying motor recovery and determining stroke severity.^[352] Severity is often categorized as severe (FMA-UE < 30), moderate ($30 \leq \text{FMA-UE} \leq 45$), and mild (FMA-UE > 45).^[353] Applying these thresholds, all patients in the present study would

fall into the severe category, as the mean FMA scores did not exceed 30, with the lowest mean score observed before treatment (19.17). However, because this study reported only the total FMA rather than separate extremity scores, direct comparison with these categories is limited.

The total FMA scores significantly increased post-treatment for all three treatment groups (**Figure 32**). Despite no significant differences between the groups post-treatment, the TA+PT group had the greatest change in FMA. This pattern is reflected in the group that began with a mean FMA comparable to the SA+PT group but, after three weeks, achieved higher mean scores than both the TA and SA+PT groups. These findings suggest a potential additive benefit of combining true acupuncture with physiotherapy.

In another study of first-ever ischemic stroke, adding electroacupuncture (EA) to physiotherapy (PT) produced significantly larger FMA gains than physiotherapy alone at 2 weeks, 4 weeks, and 3 months, but not at 6 months.^[354] The total FMA (combined FMA-UE and FMA-LE) in their EA+PT arm rose from 16.2 at 2 weeks to 34.7 at 3 months. In our cohort, the total post-treatment mean FMA after 3 weeks was >25; the TA+PT group averaged 30, exceeding their EA+PT group's 27.4 at 4 weeks. A likely contributor is protocol intensity: we delivered 5 sessions/week for 3 weeks (acupuncture + physiotherapy), whereas they provided 2 sessions/week (8 sessions over a month). They also investigated Chinese patients with older mean ages (EA+PT: 68.8; PT-only: 70.7) compared with our sample (TA: 52.40; TA+PT: 63.83; SA+PT: 54.75; all <65). Thus, we can speculate that combining EA with manual acupuncture (MA) and using six acupoints (vs five in their protocol, with GB34 in common), along with a higher treatment dose and a younger sample, may underlie our larger early FMA gains.

Notably, we did not assess differences by education level, pre-morbid social activity, or smoking, which could influence outcomes.^[355;356] A notable observation was that the Functional In-

dependence Measure (FIM) did not show a significant improvement when comparing EA+PT to PT alone.^[354] The FIM assesses functional domains such as self-care, mobility, locomotion, and social cognition. Accordingly, improvement in motor function as measured by the FMA does not necessarily translate to improved functionality. This highlights a limitation of our study, as no assessment for functional independence was performed. Nevertheless, the improved FMA scores and the distinct neural correlates observed across the three treatment groups represent a first step toward reducing reliance on daily assistance and returning to pre-stroke conditions.

Chapter 10

Conclusion

Overall, this study successfully showed differential neural correlates across first-ever male ischemic stroke patients, randomized into three treatment groups: 1) True Acupuncture (TA), 2) TA and Physiotherapy (PT), and 3) PT and Sham Acupuncture (SA, a placebo or non-acupoint acupuncture). True acupuncture (TA) alone may preferentially allocate resources to motor pathways, without the sensory competition introduced by physiotherapy. Delivered during spontaneous recovery, TA could synergize with endogenous plasticity, potentially modulating corticothalamic-basal ganglia circuits involving the thalamus, caudate, and hippocampus. The increased SMA with its role in motor timing and inhibition, might prevent the inhibition of already compromised motor circuits. In contrast, adjunctive acupuncture compared to TA alone may impose greater cognitive, sensory, and psychological load, as well as nociception, common post-stroke, which can further hinder motor recovery. These additional demands likely stem from the need to process instructions, engage higher-order cognitive functions, and execute motor decisions.

When combined, TA+PT appears to suppress competing neural circuits characteristic of emotional conflict, such as the LPNN involved in nociception and depression-like states, thereby redirecting resources toward sensorimotor processing and motor learning. This may explain why the TA+PT group achieved the greatest change in FMA values. Integrated networks, while useful for complex cognitive tasks, are metabolically costly and may induce cognitive fatigue. The increased Cp and Eloc, might suggest that adjunctive acupuncture mediates the balance between segregation and integration. Additionally, increased Sigma values for FC compared to SC, significant correlations

between the FC graph measures and FMA as well as interhemispheric connections, highlight functional reorganization beyond structural constraints, possibly involving indirect pathways or disinhibition mechanisms.

By contrast, SA+PT may lack sufficient neuromodulatory effects to inhibit nociceptive circuits or engage the endocannabinoid system, seen by the increased connections with the amygdala. However, this remains speculative as molecular markers were not studied. In contrast to the TA group, connections in the SA+PT group involved the SMA, with the fusiform gyrus, implicating the role of sham acupuncture combined with physiotherapy in mental motor imagery. The involvement of brain regions in the prefrontal cortex such as the superior frontal gyrus, might further support the emotional and cognitive demands of physiotherapy. Consequently, recovery in this group may be limited compared to TA and TA+PT. Nevertheless, all groups exhibited FMA gains, indicating convergent behavioral improvements despite divergent neural circuit engagement. Whether these gains reflect true neurological recovery, functional recovery without structural repair, or compensatory mechanisms remains uncertain. Function can improve without reduced impairment, and lesion repair does not always translate to restored function.^[357;358]

Importantly, psychosomatic responses to acupuncture could not be fully ruled out. Still, our findings demonstrate distinct neural substrates modulated by TA, TA+PT, and SA+PT. This differentiation has important implications for clinical decision-making, suggesting that integrating acupuncture with physiotherapy may optimize rehabilitation outcomes in ischemic stroke patients by leveraging both endogenous plasticity and circuit-specific neuromodulation.

Chapter 11

Limitations and Recommendations

The current study have multiple limitations, with an important one being the small sample size, which could increase the risk of type I and type II errors, resulting in false positives and false negatives, respectively. The small sample size also reduced the statistical power of ANCOVA, reducing the ability to detect true effects when they exist and thus might fail to reject false null hypotheses. Correcting for multiple covariates in a small sample size can result in overfitting, where the model can fit to noise and therefore, no more than one covariate was corrected at a given time. However, excluding covariates can result in biased estimates, where the observed effect is attributed to the independent variable instead of the uncorrected cofounder.

The assumptions of the ANCOVA test were: normality, linearity, homogeneity of regression slopes, homogeneity of variance, and multicollinearity. The assumption of homogeneity of regression slopes was violated for the graph theoretical measures. However, a study found that ANCOVA is robust and can obviate violations such as homogeneity of variance, normality and even homogeneity of regression slopes.^[359] Nevertheless, the small and unequal sample sizes in this study could make the ANCOVA less robust. Indeed, another study found that ANCOVA does not maintain its robustness under violation of normality and homogeneity of regression slopes when the sample sizes are unequal.^[360] Thus, emphasising the caution when interpreting the results in this study. Moreover, outliers detected were not removed but rather winsorized to preserve the sample size. Winsorizing is a robust procedure to reduce the influence of outliers on the mean and the variance without reducing the degrees of freedom.^[361] Future studies should include larger

sample sizes to increase statistical power. A depression score was not used in this study, which could aid in interpreting the result, as poststroke depression has been shown to influence motor recovery.^[269;362] A future study could use the 24-item Hamilton depression rating scale to distinguish patients with mild depression from those with moderate depression. Cognitive tests were not done, and thus we can only infer that the physiotherapy was cognitively more demanding than TA alone, by the increased brain regions involved in higher cognitive processes. Future studies can make use of the NASA Task Load Index (NASA-TLX) to determine the cognitive, physical, and emotional demands during a task to rate their experience.^[363] The total FMA was a combination of upper and lower motor improvement, but future studies could include both to better understand the neural mechanisms involved. The brain regions investigated to understand the structural connectivity relied on the brain regions involved in significant functional connections, which might have excluded white matter changes. Future studies should do structural connectivity analysis independent of functional connectivity analysis. Alternatively, structural connectivity should be used as a confounder in functional data to control for white matter damage caused by stroke and determine the unique contributions of FC to motor recovery.^[364] Importantly, a control group was not used for the structural and functional data, which could help to understand how graph measures differ in ischemic stroke patients compared to non-ischemic stroke patients.

FA was used as an indirect measure of white matter injury or repair. White matter voxels contain multiple fibers with complex geometries and different orientations. FA makes use of the principal eigenvector and assumes dominant fiber orientation. However, the diffusion profile of crossing fibers cannot be captured by a single eigenvector, and the FA value would be lower as the combination of fibers in different orientations would make diffusion of water molecules seem more isotropic.^[365] Thus making the FA an underestimation of the actual microenvironment. Future studies could use MD, which is the mean apparent diffusion along three tensor dimensions and

is more robust in capturing complex fiber geometries. Alternatively, diffusion kurtosis imaging (DKI)^[366] or neurite orientation dispersion and density imaging (NODDI)^[367] can be used, which can better resolve crossing fibers and estimate angular variability between neurites, respectively. DWI are acquired using Echo-planar imaging (EPI), which results in geometric distortions along the phase encoding directions caused by b0 field inhomogeneities.^[214] These distortions manifest as “signal pile-up” due to compressions, which results in dilutions in other areas of the image. Diffeomorphic Registration for Blip-Up blip-Down Diffusion Imaging (DR-BUDDI) is a robust method to correct these distortions; however, it requires reverse phase encoding gradients. Unfortunately, we did not have these gradients and could not use DR-BUDDI. This is a major limitation and could severely affect the structural connectivity data. It has been proposed that field maps be used, which we also did not have. Additionally, it has been proposed to use T1 and T2 weighted images;^[368;369] however, this is advised against as it resulted in abnormal anatomy of the white matter pathways.^[214] Future studies could ensure acquisition of phase encoding gradients in the opposite direction or use deep learning approaches such as 3D U-nets to synthesize undistorted field maps that match the geometry of T1w images and intensity of the contrast in diffusion images.^[370] Similarly, field maps were not acquired for the rs-fMRI data, preventing correction for susceptibility-induced distortions.^[371] As a result, geometric misalignment may occur, leading to biased region-specific analyses and reduced sensitivity in detecting functional connectivity. Only male subjects’ data were analyzed, and thus the results can not be generalized to the population.^[372] Sex differences are potentially due to differential endocrine systems and the influence of different hormones on pain modulation.^[373] Additionally, manual and electroacupuncture have shown different effects in males compared to females.^[374] Previous studies have separated ischemic stroke patients into left- and right-hemisphere infarct groups to distinguish ipsilateral from contralateral neural repair.^[338] In the present study, however, the small sample size precluded fur-

ther subdivision of participants. Patients with a left hemispheric infarct may demonstrate different recovery patterns compared to those with right-sided infarcts, particularly since all participants in this study were right-handed.^[375] Thus, lesion side could represent a potential confounder. However, because this analysis was conducted at the whole-brain network level rather than using a voxel-wise approach, lesion side was not explicitly controlled for. Future studies with larger sample sizes should stratify patients by hemisphere of infarct and examine how handedness and lesion laterality interact to influence motor recovery.

Additionally, pain scores were not recorded, and even though they might be subjective would aid in understanding the effect of expectations as the patients and acupuncturists were not blinded in this study, which could result in bias.^[303] The Numeric Rating Scale (NRS) or the McGill Pain Questionnaire can be used to assess the level of pain.^[376;377] Prior experience with acupuncture may influence rehabilitation outcomes, as patients familiar with the practice, such as many Chinese patients, are more likely to distinguish true acupuncture from sham procedures and may hold specific expectations regarding its effects.^[378] While this concern may be less pronounced in Western populations, where acupuncture is not part of routine care and general knowledge of the practice is limited, rigorous blinding of both patients and practitioners should nonetheless be prioritized to minimize expectancy effects and performance bias. Lastly, the so-called “smoking paradox” has been reported in clinical studies, suggesting that stroke patients who smoke may experience better outcomes following thrombolytic therapy compared to non-smokers.^[379] However, more recent evidence indicates that smoking is not associated with neurological improvement during hospitalization or with favorable functional outcomes at 3 months after acute ischemic stroke.^[380] Data on smoking status were not available in the present study, and future work should incorporate this information alongside other important social determinants, such as socioeconomic status and education level.^[381]

Bibliography

- [1] Valery L Feigin, Bo Norrving, and George A Mensah. Global burden of stroke. *Circulation research*, 120(3): 439–448, 2017.
- [2] Yang Liu, Wenxin Wang, Xuewei Huang, Xingyuan Zhang, Lijin Lin, Juan-Juan Qin, Fang Lei, Jingjing Cai, and Bo Cheng. Global disease burden of stroke attributable to high fasting plasma glucose in 204 countries and territories from 1990 to 2019: An analysis of the global burden of disease study. *Journal of Diabetes*, 14(8): 495–513, 2022.
- [3] Ajaya Kumar A Unnithan, Parth Mehta, et al. Hemorrhagic stroke. 2020.
- [4] Matthew B Bevers and W Taylor Kimberly. Critical care management of acute ischemic stroke. *Current treatment options in cardiovascular medicine*, 19:1–14, 2017.
- [5] Hooman Kamel, Gino Gialdini, Hedyeh Baradaran, Ashley E Giambrone, Babak B Navi, Michael P Lerario, James K Min, Costantino Iadecola, and Ajay Gupta. Cryptogenic stroke and nonstenosing intracranial calcified atherosclerosis. *Journal of Stroke and Cerebrovascular Diseases*, 26(4):863–870, 2017.
- [6] Jiahui Fan, Xiaoguang Li, Xueying Yu, Zhenqiu Liu, Yanfeng Jiang, Yibin Fang, Ming Zong, Chen Suo, Qihong Man, and Lize Xiong. Global burden, risk factor analysis, and prediction study of ischemic stroke, 1990–2030. *Neurology*, 101(2):e137–e150, 2023.
- [7] Anna Johansson, Isabel Drake, Gunnar Engström, and Stefan Acosta. Modifiable and non-modifiable risk factors for atherothrombotic ischemic stroke among subjects in the malmö diet and cancer study. *Nutrients*, 13(6):1952, 2021.
- [8] Neema W Minja, Doreen Nakagaayi, Twalib Aliku, Wanzhu Zhang, Isaac Ssinabulya, Juliet Nabaale, Willington Amutuhaire, Sarah R de Loizaga, Emma Ndagire, Joselyn Rwebembera, et al. Cardiovascular diseases in africa in the twenty-first century: Gaps and priorities going forward. *Frontiers in Cardiovascular Medicine*, 9:1008335, 2022.
- [9] Bertha Chioma Ekeh. Challenges of the management of stroke in sub saharan africa: evaluating awareness, access and action. *J Pediatr Neurol Med*, 2(128):10–4172, 2017.
- [10] Nassib Tawa, Anthea Rhoda, Yolandi Brink, Gerard Urimubenshi, Marlie Giljam-Enright, Maria Y Charumbira, SM Niekerk, and Quinette Louw. Stroke rehabilitation services in africa—challenges and opportunities: A scoping review of the literature. 2021.
- [11] Walter Johnson, Oyere Onuma, Mayowa Owolabi, and Sonal Sachdev. Stroke: a global response is needed. *Bulletin of the World Health Organization*, 94(9):634, 2016.
- [12] Mohammad H Rahbar, Martin Medrano, Franck Diaz-Garelli, Cosme Gonzalez Villaman, Sepideh Saroukhani, Sori Kim, Amirali Tahanan, Yahaira Franco, Gelanys Castro-Tejada, Sarah A Diaz, et al. Younger age of stroke in low-middle income countries is related to healthcare access and quality. *Annals of Clinical and Translational Neurology*, 9(3):415–427, 2022.
- [13] Mayowa O Owolabi, Oyedunni Arulogun, Sylvia Melikam, Abiodun M Adeoye, Sally Akarolo-Anthony, Rufus Akinyemi, Donna Arnett, Hemant Tiwari, Mulugeta Gebregziabher, Carolyn Jenkins, et al. The burden of stroke in africa: a glance at the present and a glimpse into the future. *Cardiovascular journal of Africa*, 26(2 H3Africa Suppl):S27, 2015.

- [14] Lyness Matizirofa and Delson Chikobvu. Analysing and quantifying the effect of predictors of stroke direct costs in south africa using quantile regression. *BMC Public Health*, 21:1–9, 2021.
- [15] Kezia Kodawa Tessua, Patricia Munseri, and Sarah Shali Matuja. Outcomes within a year following first ever stroke in tanzania. *PloS one*, 16(2):e0246492, 2021.
- [16] TH Jafar. Blood pressure, diabetes, and increased dietary salt associated with stroke—results from a community-based study in pakistan. *Journal of human hypertension*, 20(1):83–85, 2006.
- [17] Tracey Smythe, Gakeemah Inglis-Jassiem, T Conradie, S Kamalakannan, S Fernandes, SM Van-Niekerk, R English, J Webster, S Hameed, and QA Louw. Access to health care for people with stroke in south africa: a qualitative study of community perspectives. *BMC health services research*, 22(1):464, 2022.
- [18] Mokgobadibe V Ntsiea. Current stroke rehabilitation services and physiotherapy research in south africa. *South African Journal of Physiotherapy*, 75(1):1–10, 2019.
- [19] Meeka Khanna, Dhinla Sivadas, Anupam Gupta, Partha Haldar, and Navin B Prakash. Impact of inpatient rehabilitation on quality of life among stroke patients. *Journal of Neurosciences in Rural Practice*, 2022.
- [20] Gerard Urimubenshi, Dominique A Cadilhac, Jeanne N Kagwiza, Olivia Wu, and Peter Langhorne. Stroke care in africa: a systematic review of the literature. *International Journal of Stroke*, 13(8):797–805, 2018.
- [21] Babawale Arabambi, Olajumoke Oshinaike, Shamsideen Abayomi Ogun, Chukwuemeka Eze, Abiodun Hamzat Bello, Steven Igetei, Yakub Yusuf, Rashidat Amoke Olanigan, and Sikirat Yetunde Ashiru. Stroke units in nigeria: a report from a nationwide organizational cross-sectional survey. *The Pan African Medical Journal*, 42, 2022.
- [22] Jane Hart. Poststroke recovery: emerging complementary therapies. *Alternative and Complementary Therapies*, 16(5):277–280, 2010.
- [23] Jie-Dan Mu, Liang-Xiao Ma, Zhou Zhang, Xu Qian, Qin-Yong Zhang, Ling-Hui Ma, and Tian-Yi Sun. The factors affecting neurogenesis after stroke and the role of acupuncture. *Frontiers in Neurology*, 14:1082625, 2023.
- [24] Hai-Ming Zhang, Dan Luo, Rui Chen, Shu-Han Wang, Ya-Juan Zhao, Jin-Xiao Li, Min-Feng Zhou, Zhao-Min Yu, Jun-Li Zhang, and Feng-Xia Liang. Research progress on acupuncture treatment in central nervous system diseases based on nlrp3 inflammasome in animal models. *Frontiers in Neuroscience*, 17:1118508, 2023.
- [25] Yun-Yun Wang, Shao-Fu Yu, Hong-Yang Xue, Yang Li, Chen Zhao, and Ying-Hui Jin. Effectiveness and safety of acupuncture for the treatment of alzheimer's disease: a systematic review and meta-analysis. *Frontiers in Aging Neuroscience*, 12:98, 2020.
- [26] Liping Liu, Weiqi Chen, Hongyu Zhou, Wanying Duan, Shujuan Li, Xiaochuan Huo, Weihai Xu, Huaguang Zheng, Jingyi Liu, Hui Liu, et al. Chinese stroke association guidelines for clinical management of cerebrovascular disorders: executive summary and 2019 update of clinical management of ischaemic cerebrovascular diseases. *Stroke and vascular neurology*, 5(2), 2020.
- [27] Janita Pak Chun Chau, Suzanne Hoi Shan Lo, Laveeza Butt, and Surui Liang. Post-stroke experiences and rehabilitation needs of community-dwelling chinese stroke survivors: A qualitative study. *International Journal of Environmental Research and Public Health*, 19(23):16345, 2022.
- [28] Tetsuya Asakawa, Liang Zong, Liang Wang, Ying Xia, and Hiroki Namba. Unmet challenges for rehabilitation after stroke in china. *The Lancet*, 390(10090):121–122, 2017.
- [29] Liping Liu, Jingyi Liu, Yilong Wang, David Wang, and Yongjun Wang. Substantial improvement of stroke care in china. *Stroke*, 49(12):3085–3091, 2018.

- [30] Wen Wen Zhang, Sally Speare, Leonid Churilov, Matthew Thuy, Geoffrey Donnan, and Julie Bernhardt. Stroke rehabilitation in china: a systematic review and meta-analysis. *International Journal of Stroke*, 9(4):494–502, 2014.
- [31] Yuan Hongwen, Wang Pei, Hu Nijuan, Zhang Peng, Li Chunhua, Liu Yuqi, Ma Liangxiao, and Zhu Jiang. A review of the methods used for subjective evaluation of de qi. *Journal of Traditional Chinese Medicine*, 38(2):309–314, 2018.
- [32] Xing-Yue Yang, Guang-Xia Shi, Qian-Qian Li, Zhen-Hua Zhang, Qian Xu, Cun-Zhi Liu, et al. Characterization of deqi sensation and acupuncture effect. *Evidence-based complementary and alternative medicine*, 2013, 2013.
- [33] Xiaopeng Si, Shaoxin Xiang, Ludan Zhang, Sicheng Li, Kuo Zhang, and Dong Ming. Acupuncture with deqi modulates the hemodynamic response and functional connectivity of the prefrontal-motor cortical network. *Frontiers in Neuroscience*, 15:693623, 2021.
- [34] Fang Li, Tian He, Qian Xu, Li-Ting Lin, Hui Li, Yan Liu, Guang-Xia Shi, and Cun-Zhi Liu. What is the acupoint? a preliminary review of acupoints. *Pain Medicine*, 16(10):1905–1915, 2015.
- [35] Won-Mo Jung, Woosun Shim, Taehyung Lee, Hi-Joon Park, Yeonhee Ryu, Florian Beissner, and Younbyoung Chae. More than deqi: spatial patterns of acupuncture-induced bodily sensations. *Frontiers in neuroscience*, 10: 462, 2016.
- [36] Sha-bei Xu, Bo Huang, Chen-yan Zhang, Peng Du, Qi Yuan, Gui-juan Bi, Gui-bin Zhang, Min-jie Xie, Xiang Luo, Guang-ying Huang, et al. Effectiveness of strengthened stimulation during acupuncture for the treatment of bell palsy: a randomized controlled trial. *Cmaj*, 185(6):473–478, 2013.
- [37] Y Chen. A study on the therapeutic effectiveness of acupuncture to cervical spondylosis: quality of life research on cervical spondylosis patients [ms thesis]. *China: Guangzhou University of Chinese Medicine*, 2011.
- [38] J Xiong, F Liu, W Wang, and G Huang. The effects of deqi and manual acupuncture needle manipulation on the clinical outcomes of acupuncture analgesia. *Liaoning J Tradit Chin Med*, 38(8):1482–5, 2011.
- [39] Huanqin Li, Huilin Liu, Cunzhi Liu, Guangxia Shi, Wei Zhou, Chengmei Zhao, Tao Zhang, Xuefei Wang, Guiling Wang, Yin Zhao, et al. Effect of “deqi” during the study of needling “wang’s jiaji” acupoints treating spasticity after stroke. *Evidence-Based Complementary and Alternative Medicine*, 2014, 2014.
- [40] Cássia Maria Grillo, Vera Lúcia Rasera Zotelli, Maria Lúcia Bressiani Gil, and Maria da Luz Rosário de Sousa. Would a placebo acupuncture needle be able to induce deqi? *Journal of acupuncture and meridian studies*, 11 (5):273–279, 2018.
- [41] Stephen Birch, Myeong Soo Lee, Tae-Hun Kim, and Terje Alraek. Historical perspectives on using sham acupuncture in acupuncture clinical trials. *Integrative Medicine Research*, 11(1):100725, 2022.
- [42] Yixuan Xie, Xiaoyu Liu, Tinglan Liu, Chiyun Sun, Zeyin Xin, Yuzhi Hu, Yue Wang, Cheng Zhang, and Shiyan Yan. Descriptions of sham acupuncture in randomised controlled trials: a critical review of the literature. *BMC Complementary Medicine and Therapies*, 23(1):173, 2023.
- [43] Tae-Hun Kim, Myeong Soo Lee, Stephen Birch, and Terje Alraek. Plausible mechanism of sham acupuncture based on biomarkers: A systematic review of randomized controlled trials. *Frontiers in Neuroscience*, 16:8, 2022.
- [44] AS Habib. Do we really understand what constitutes an acupuncture point. *Eur J Pain*, 16(9):1207–1208, 2012.
- [45] Edzard Ernst. Acupuncture—a critical analysis. *Journal of internal medicine*, 259(2):125–137, 2006.
- [46] Yong-Chen Zheng, Ting-Ting Yuan, and Tao Liu. Is acupuncture a placebo therapy? *Complementary therapies in medicine*, 22(4):724–730, 2014.

- [60] Hyeonsoo Kwon, Jimin Kim, Minjun Bang, Jaewon Bae, Byunghyun Oh, Hongik Kim, Sehun Jung, and Myungseok Ryu. Anatomical structure and needling methods of acupoint bl40. *Journal of Korean Medical Society of Soft Tissue*, 7(2):131–143, 2023.
- [61] Shihong Zhang, Bo Wu, Ming Liu, Ning Li, Xianrong Zeng, Hua Liu, Qingcheng Yang, Zhao Han, Ping Rao, and Dong Wang. Acupuncture efficacy on ischemic stroke recovery: multicenter randomized controlled trial in china. *Stroke*, 46(5):1301–1306, 2015.
- [62] Zhi-xin Yang, Jia-hong Xie, and Ding-ding Liu. Xingnao kaiqiao needling method for acute ischemic stroke: a meta-analysis of safety and efficacy. *Neural Regeneration Research*, 12(8):1308, 2017.
- [63] Xin Mu, Lan Guo, Ying Yang, Yu-xi Qin, Xing-wen Jing, and Min Mao. A randomized controlled clinical trial of xingnao kaiqiao acupuncture combined with modern rehabilitation therapy in the treatment of cognitive dysfunction after acute ischemic stroke. *Topics in Geriatric Rehabilitation*, 40(3):196–202, 2024.
- [64] Chung-Yen Lu, Shu-Ru Lee, Chee-Jen Chang, and Pei-Chun Chen. Adjuvant therapy with traditional chinese medicine and long-term mortality in patients with stroke: A nationwide population-based cohort study in taiwan. *Maturitas*, 158:47–54, 2022.
- [65] Chuen-Chau Chang, Ta-Liang Chen, Hsienhsueh Elley Chiu, Chaur-Jong Hu, Chun-Chieh Yeh, Chin-Chuan Tsai, Hsin-Long Lane, Mao-Feng Sun, Fung-Chang Sung, Chien-Chang Liao, et al. Outcomes after stroke in patients receiving adjuvant therapy with traditional chinese medicine: a nationwide matched interventional cohort study. *Journal of ethnopharmacology*, 177:46–52, 2016.
- [66] Jawaria Shahid, Ayesha Kashif, and Muhammad Kashif Shahid. A comprehensive review of physical therapy interventions for stroke rehabilitation: impairment-based approaches and functional goals. *Brain Sciences*, 13(5):717, 2023.
- [67] Stephanie Goncalves, Morgane Le Bourvellec, Stephane Mandigout, and Noemie C Duclos. Impact of active physiotherapy on physical activity level in stroke survivors: a systematic review and meta-analysis. *Stroke*, 54(12):3097–3106, 2023.
- [68] Yu-Long Bai, Li Li, Yong-Shan Hu, Yi Wu, Pei-Jing Xie, Song-Wei Wang, Ming Yang, Yi-Ming Xu, and Bing Zhu. Prospective, randomized controlled trial of physiotherapy and acupuncture on motor function and daily activities in patients with ischemic stroke. *The Journal of Alternative and Complementary Medicine*, 19(8):684–689, 2013.
- [69] LX Zhuang CMD, SF Xu CMD, C Jia CMD, J He CMD, and DX Han CMD. An effectiveness study comparing acupuncture, physiotherapy, and their combination in poststroke rehabilitation: a multicentered, randomized, controlled clinical trial. *Alternative therapies in health and medicine*, 18(3):8, 2012.
- [70] Lifang Chen, Jianqiao Fang, Ruijie Ma, Xudong Gu, Lina Chen, Jianhua Li, and Shouyu Xu. Additional effects of acupuncture on early comprehensive rehabilitation in patients with mild to moderate acute ischemic stroke: a multicenter randomized controlled trial. *BMC complementary and alternative medicine*, 16(1):226, 2016.
- [71] Xufang Wei, Shengtong Sun, Manyu Zhang, and Zhenqiang Zhao. A systematic review and meta-analysis of clinical efficacy of early and late rehabilitation interventions for ischemic stroke. *BMC neurology*, 24(1):91, 2024.
- [72] Carla Cirillo, Nabila Brihmat, Evelyne Castel-Lacanal, Alice Le Friec, Marianne Barbieux-Guillot, Nicolas Raposo, Jérémie Pariente, Alain Viguié, Marion Simonetta-Moreau, Jean-Francois Albucher, et al. Post-stroke remodeling processes in animal models and humans. *Journal of Cerebral Blood Flow & Metabolism*, 40(1):3–22, 2020.

- [73] Steven R Zeiler, Robert Hubbard, Ellen M Gibson, Tony Zheng, Kwan Ng, Richard O'Brien, and John W Krakauer. Paradoxical motor recovery from a first stroke after induction of a second stroke: reopening a postischemic sensitive period. *Neurorehabilitation and neural repair*, 30(8):794–800, 2016.
- [74] Alexander W Dromerick, Matthew A Edwardson, Dorothy F Edwards, Margot L Giannetti, Jessica Barth, Kathaleen P Brady, Evan Chan, Ming T Tan, Irfan Tamboli, Ruth Chia, et al. Critical periods after stroke study: translating animal stroke recovery experiments into a clinical trial. *Frontiers in human neuroscience*, 9:231, 2015.
- [75] Sheng Li. Stroke recovery is a journey: Prediction and potentials of motor recovery after a stroke from a practical perspective. *Life*, 13(10):2061, 2023.
- [76] Yao Sun, Joanne Boots, and E Paul Zehr. The lingering effects of a busted myth—false time limits in stroke rehabilitation. *Applied Physiology, Nutrition, and Metabolism*, 40(8):858–861, 2015.
- [77] Belén Rubio Ballester, Martina Maier, Armin Duff, Mónica Cameirão, Sergi Bermúdez, Esther Duarte, Ampar Cuxart, Susana Rodríguez, Rosa María San Segundo Mozo, and Paul FMJ Verschure. A critical time window for recovery extends beyond one-year post-stroke. *Journal of neurophysiology*, 2019.
- [78] Jinhuan Zhang, Chunjian Lu, Xiaoxiong Wu, Dehui Nie, and Haibo Yu. Neuroplasticity of acupuncture for stroke: an evidence-based review of mri. *Neural plasticity*, 2021, 2021.
- [79] Cheng-Yu Tseng, Pei-Shan Hsu, Chang-Ti Lee, Hui-Fen Huang, Chou-Chin Lan, Tsung-Han Hsieh, Guan-Ting Liu, Chan-Yen Kuo, Ming-Chieh Wang, and Po-Chun Hsieh. Acupuncture and traditional chinese herbal medicine integrated with conventional rehabilitation for post-stroke functional recovery: a retrospective cohort study. *Frontiers in Neuroscience*, 16:851333, 2022.
- [80] Douwe P Bergsma, Joris A Elshout, and Albert V Van den Berg. Segregation of spontaneous and training induced recovery from visual field defects in subacute stroke patients. *Frontiers in Neurology*, 8:681, 2017.
- [81] Anson CM Chau, Raymond Tak Fai Cheung, Xianyong Jiang, Paul KM Au-Yeung, and Leonard SW Li. An fmri study showing the effect of acupuncture in chronic stage stroke patients with aphasia. *Journal of acupuncture and meridian studies*, 3(1):53–57, 2010.
- [82] Sauson Soldozy, Christina Dalzell, Anthony Skaff, Yusuf Ali, Pedro Norat, Kaan Yagmurlu, Min S Park, and M Yashar S Kalani. Reperfusion injury in acute ischemic stroke: tackling the irony of revascularization. *Clinical Neurology and Neurosurgery*, page 107574, 2023.
- [83] Camilo R Gomez. Time is brain: the stroke theory of relativity. *Journal of Stroke and Cerebrovascular Diseases*, 27(8):2214–2227, 2018.
- [84] Qing-zhang Tuo, Shu-ting Zhang, and Peng Lei. Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. *Medicinal research reviews*, 42(1):259–305, 2022.
- [85] Keqing Nian, Ian C Harding, Ira M Herman, and Eno E Ebong. Blood-brain barrier damage in ischemic stroke and its regulation by endothelial mechanotransduction. *Frontiers in Physiology*, 11:605398, 2020.
- [86] Elizabeth M Rhea and William A Banks. Role of the blood-brain barrier in central nervous system insulin resistance. *Frontiers in neuroscience*, 13:521, 2019.
- [87] Hossam Kadry, Behnam Noorani, and Luca Cucullo. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids and Barriers of the CNS*, 17(1):1–24, 2020.
- [88] Shaheen E Lakhan, Annette Kirchgessner, Deborah Tepper, and Aidan Leonard. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Frontiers in neurology*, 4:32, 2013.

- [89] Rakesh Khatri, Alexander M McKinney, Barbara Swenson, and Vallabh Janardhan. Blood–brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. *Neurology*, 79(13 Supplement 1):S52–S57, 2012.
- [90] Yue Zhuo, Ming Xu, Shifeng Deng, Yuchen Zhang, Xiaoye Lu, Boyu Wu, and Hong Zhang. Efficacy and safety of dissimilar acupuncture intervention time-points in treating stroke: a systematic review and network meta-analysis. *Ann Palliat Med*, 10(10):10196–10212, 2021.
- [91] Bangqi Wu, Yi Ding, Maohan Peng, Xuhui Wang, Yibing Li, and Xinyue Cheng. Influence of acupuncture and other clinical factors on the recovery of limb motor function in patients after stroke: A retrospective study. *Journal of Multidisciplinary Healthcare*, pages 463–474, 2023.
- [92] Yang-Zi He, Bing Han, Shi-Fu Zheng, Li-Na Wang, Zhuo-Ming Chen, Jing Hu, Jing-Ming Li, and Ju-Xiu Peng. Effect of different acupuncture needle-retaining time on hemorheology in ischemic stroke patients. *Zhen ci yan jiu= Acupuncture Research*, 32(5):338–341, 2007.
- [93] Hai-zhou Li, Kai Xie, Li-feng Zhou, Qing-lan Fu, and Pei-hong Zheng. Effect of needle-retaining time on therapeutic efficacy for post-stroke hemiplegia. *Journal of Acupuncture and Tuina Science*, 11:222–225, 2013.
- [94] Helene M Langevin, Rosa Schnyer, Hugh MacPherson, Robert Davis, Richard E Harris, Vitaly Napadow, Peter M Wayne, Ryan J Milley, Lixing Lao, Elisabet Stener-Victorin, et al. Manual and electrical needle stimulation in acupuncture research: pitfalls and challenges of heterogeneity. *The Journal of Alternative and Complementary Medicine*, 21(3):113–128, 2015.
- [95] Vitaly Napadow, Nikos Makris, Jing Liu, Norman W Kettner, Kenneth K Kwong, and Kathleen KS Hui. Effects of electroacupuncture versus manual acupuncture on the human brain as measured by fmri. *Human brain mapping*, 24(3):193–205, 2005.
- [96] Chaobo Zheng, Yaguo Lee, Hu Bin, and Wu Jiong. A randomized controlled trial comparing electroacupuncture with manual acupuncture for motor function recovery after ischemic stroke. *European Journal of Integrative Medicine*, 22:76–80, 2018.
- [97] Poonam Chaturvedi, Ajai Kumar Singh, Vandana Tiwari, and Anup Kumar Thacker. Brain-derived neurotrophic factor levels in acute stroke and its clinical implications. *Brain circulation*, 6(3):185, 2020.
- [98] Wei Liu, Xiaohui Wang, Margaret O'Connor, Guan Wang, Fang Han, et al. Brain-derived neurotrophic factor and its potential therapeutic role in stroke comorbidities. *Neural plasticity*, 2020, 2020.
- [99] Meng Xu, Chaoqun Yang, Tao Nian, Chen Tian, Liying Zhou, Yanan Wu, Yanfei Li, Xinxin Deng, Xiuxia Li, and Kehu Yang. Adverse effects associated with acupuncture therapies: An evidence mapping from 535 systematic reviews. *Chinese Medicine*, 18(1):1–10, 2023.
- [100] Min-Wook Kim, You Chul Chung, Hee Chan Jung, Moon-Seo Park, Young-Min Han, Yong-An Chung, Lee-So Maeng, Sang-In Park, Jiyeon Lim, Woo-Seok Im, et al. Electroacupuncture enhances motor recovery performance with brain-derived neurotrophic factor expression in rats with cerebral infarction. *Acupuncture in Medicine*, 30(3): 222–226, 2012.
- [101] Si-Si Li, Xu-Yun Hua, Mou-Xiong Zheng, Jia-Jia Wu, Zhen-Zhen Ma, Xiang-Xin Xing, Jie Ma, Chun-Lei Shan, and Jian-Guang Xu. Electroacupuncture treatment improves motor function and neurological outcomes after cerebral ischemia/reperfusion injury. *Neural regeneration research*, 17(7):1545, 2022.

- [102] Xiaoqian Luan, Huihua Qiu, Xianchai Hong, Chaowen Wu, Kai Zhao, Huijun Chen, Zhuoying Zhu, Xianmei Li, Huiping Shen, and Jincai He. High serum nerve growth factor concentrations are associated with good functional outcome at 3 months following acute ischemic stroke. *Clinica Chimica Acta*, 488:20–24, 2019.
- [103] Luigi Aloe, Maria Luisa Rocco, Bijorn Omar Balzamino, and Alessandra Micera. Nerve growth factor: a focus on neuroscience and therapy. *Current neuropharmacology*, 13(3):294–303, 2015.
- [104] Yibin Zhao, Xuqing Mao, Hao Wang, Lin Gan, Shanshan Zhang, Peng Gong, and Xianming Lin. The influence of electronic acupuncture at a specific frequency in facilitating the passage of ngf through the blood-brain barrier and its effect on learning and memory in mcao/r rats. 2021.
- [105] Patrice Voss, Maryse E Thomas, J Miguel Cisneros-Franco, and Étienne de Villers-Sidani. Dynamic brains and the changing rules of neuroplasticity: implications for learning and recovery. *Frontiers in psychology*, 8:1657, 2017.
- [106] Greg J Duncan, Tyrell J Simkins, and Ben Emery. Neuron-oligodendrocyte interactions in the structure and integrity of axons. *Frontiers in Cell and Developmental Biology*, 9:653101, 2021.
- [107] Natalia Egorova, Thijs Dhollander, Mohamed Salah Khelif, Wasim Khan, Emilio Werden, and Amy Brodtmann. Pervasive white matter fiber degeneration in ischemic stroke. *Stroke*, 51(5):1507–1513, 2020.
- [108] CHB van Niftrik, Martina Sebök, Giovanni Muscas, S Wegener, AR Luft, C Stippich, Luca Regli, and Jorn Fierstra. Investigating the association of wallerian degeneration and diaschisis after ischemic stroke with bold cerebrovascular reactivity. *Frontiers in Physiology*, 12:645157, 2021.
- [109] Chunmei Ma, Ying Zhou, Wei Yi, Xianxi Zhou, Wenping Guo, Xiaowu Xu, Jing Luo, Ziwei Luo, Aijun Liu, and Dongfeng Chen. Electroacupuncture of the baihui and shenting acupoints for vascular dementia in rats through the mir-81/il-16/psd-95 pathway. *Annals of Translational Medicine*, 10(10), 2022.
- [110] Siru Qin, Zichen Zhang, Yadan Zhao, Jingyi Liu, Jiwen Qiu, Yinan Gong, Wen Fan, Yongming Guo, Yi Guo, Zhifang Xu, et al. The impact of acupuncture on neuroplasticity after ischemic stroke: a literature review and perspectives. *Frontiers in Cellular Neuroscience*, 16:817732, 2022.
- [111] Magdalena Ziółkowska, Małgorzata Borczyk, Anna Cały, Kamil F Tomaszewski, Agata Nowacka, Maria Nalberczak-Skóra, Małgorzata Alicja Śliwińska, Kacper Łukasiewicz, Edyta Skonieczna, Tomasz Wójtowicz, et al. Phosphorylation of psd-95 at serine 73 in dca1 is required for extinction of contextual fear. *Plos Biology*, 21(5): e3002106, 2023.
- [112] Christos Galanis and Andreas Vlachos. Hebbian and homeostatic synaptic plasticity—do alterations of one reflect enhancement of the other? *Frontiers in Cellular Neuroscience*, 14:50, 2020.
- [113] Alberto Lazari, Piergiorgio Salvan, Michiel Cottaar, Daniel Papp, Matthew FS Rushworth, and Heidi Johansen-Berg. Hebbian activity-dependent plasticity in white matter. *Cell Reports*, 39(11), 2022.
- [114] Kevin Fox and Michael Stryker. Integrating hebbian and homeostatic plasticity: introduction, 2017.
- [115] Sue Peters, Katie P Wadden, Kathryn S Hayward, Jason L Neva, Angela M Auriat, and Lara A Boyd. A structural motor network correlates with motor function and not impairment post stroke. *Neuroscience letters*, 658:155–160, 2017.
- [116] Sinisa Pajevic, Dietmar Plenz, Peter J Basser, and R Douglas Fields. Oligodendrocyte-mediated myelin plasticity and its role in neural synchronization. *Elife*, 12:e81982, 2023.

- [117] Daniel N Bullock, Elena A Hayday, Mark D Grier, Wei Tang, Franco Pestilli, and Sarah R Heilbronner. A taxonomy of the brain's white matter: twenty-one major tracts for the 21st century. *Cerebral Cortex*, 32(20):4524–4548, 2022.
- [118] Shao-Hua Yang and Ran Liu. Four decades of ischemic penumbra and its implication for ischemic stroke. *Translational stroke research*, 12:937–945, 2021.
- [119] Lisa D Alexander, Sandra E Black, Fuqiang Gao, Gregory Szilagyi, Cynthia J Danells, and William E McIlroy. Correlating lesion size and location to deficits after ischemic stroke: the influence of accounting for altered perinecrotic tissue and incidental silent infarcts. *Behavioral and brain functions*, 6:1–10, 2010.
- [120] Chiara Begliomini, Francesco Ceccarini, Veronica Pinuccia Dell'Acqua, Sanja Budisavljevic, and Umberto Castiello. Structure of the motor descending pathways correlates with the temporal kinematics of hand movements. *Biology*, 11(10):1482, 2022.
- [121] SJ Kashalikar. An explanation for the development of decussations in the central nervous system. *Medical Hypotheses*, 26(1):1–8, 1988.
- [122] Saad Jbabdi and Heidi Johansen-Berg. Tractography: where do we go from here? *Brain connectivity*, 1(3):169–183, 2011.
- [123] Lauren J O'Donnell and Carl-Fredrik Westin. An introduction to diffusion tensor image analysis. *Neurosurgery Clinics*, 22(2):185–196, 2011.
- [124] Patric Hagmann, Leila Cammoun, Xavier Gigandet, Stephan Gerhard, P Ellen Grant, Van Wedeen, Reto Meuli, Jean-Philippe Thiran, Christopher J Honey, and Olaf Sporns. Mr connectomics: principles and challenges. *Journal of neuroscience methods*, 194(1):34–45, 2010.
- [125] José M Soares, Paulo Marques, Victor Alves, and Nuno Sousa. A hitchhiker's guide to diffusion tensor imaging. *Frontiers in neuroscience*, 7:31, 2013.
- [126] A Pena, HAL Green, TA Carpenter, SJ Price, JD Pickard, and JH Gillard. Enhanced visualization and quantification of magnetic resonance diffusion tensor imaging using the p: q tensor decomposition. *The British journal of radiology*, 79(938):101–109, 2006.
- [127] Xiao Han, Lijun Bai, Chuazhu Sun, Xuan Niu, Yanzhe Ning, Zhen Chen, Yingying Li, Kuangshi Li, Diyang Lyu, Caihong Fu, et al. Acupuncture enhances communication between cortices with damaged white matters in poststroke motor impairment. *Evidence-Based Complementary and Alternative Medicine*, 2019, 2019.
- [128] Bahaa Eldin Mahmoud, Mohammad Edrees Mohammad, and Dalia K Serour. What can dti add in acute ischemic stroke patients? *Egyptian Journal of Radiology and Nuclear Medicine*, 50:1–8, 2019.
- [129] Maurizio Bergamino, Simona Schiavi, Alessandro Daducci, Ryan R Walsh, and Ashley M Stokes. Analysis of brain structural connectivity networks and white matter integrity in patients with mild cognitive impairment. *Frontiers in Aging Neuroscience*, 14:793991, 2022.
- [130] Min Su Kim, Byung Soon Moon, Jae-yoon Ahn, Sang-song Shim, Jong-Min Yun, and Min Cheol Joo. Elucidating the mechanisms of post-stroke motor recovery mediated by electroacupuncture using diffusion tensor tractography. *Frontiers in Neurology*, 13:888165, 2022.
- [131] Nicholas Aderinto, Muili O AbdulBasit, Gbolahan Olatunji, and Temilade Adejumo. Exploring the transformative influence of neuroplasticity on stroke rehabilitation: a narrative review of current evidence. *Annals of Medicine and Surgery*, 85(9):4425–4432, 2023.

- [132] Cinzia Calautti and Jean-Claude Baron. Functional neuroimaging studies of motor recovery after stroke in adults: a review. *Stroke*, 34(6):1553–1566, 2003.
- [133] Elizabeth MC Hillman. Coupling mechanism and significance of the bold signal: a status report. *Annual review of neuroscience*, 37:161–181, 2014.
- [134] Phillip GD Ward, Edwina R Orchard, Stuart Oldham, Aurina Arnatkevičiūtė, Francesco Sforazzini, Alex Fornito, Elsdon Storey, Gary F Egan, and Sharna D Jamadar. Individual differences in haemoglobin concentration influence bold fmri functional connectivity and its correlation with cognition. *NeuroImage*, 221:117196, 2020.
- [135] Susan T Francis and Sally Eldeghaidy. Imaging methodologies and applications for nutrition research: what can functional mri offer? *Proceedings of the Nutrition Society*, 74(2):89–98, 2015.
- [136] Richard D Hoge, Jeff Atkinson, Brad Gill, Gérard R Crelier, Sean Marrett, and G Bruce Pike. Linear coupling between cerebral blood flow and oxygen consumption in activated human cortex. *Proceedings of the National Academy of Sciences*, 96(16):9403–9408, 1999.
- [137] Kenneth K Kwong, John W Belliveau, David A Chesler, Inna E Goldberg, Robert M Weisskoff, Brigitte P Poncelet, David N Kennedy, Bernice E Hoppel, Mark S Cohen, and Robert Turner. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proceedings of the National Academy of Sciences*, 89(12):5675–5679, 1992.
- [138] Seiji Ogawa, Tso-Ming Lee, Alan R Kay, and David W Tank. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *proceedings of the National Academy of Sciences*, 87(24):9868–9872, 1990.
- [139] Richard B Buxton, Valerie EM Griffeth, Aaron B Simon, and Farshad Moradi. Variability of the coupling of blood flow and oxygen metabolism responses in the brain: a problem for interpreting bold studies but potentially a new window on the underlying neural activity. *Frontiers in neuroscience*, 8:139, 2014.
- [140] Joshua S Siegel, Abraham Z Snyder, Lenny Ramsey, Gordon L Shulman, and Maurizio Corbetta. The effects of hemodynamic lag on functional connectivity and behavior after stroke. *Journal of Cerebral Blood Flow & Metabolism*, 36(12):2162–2176, 2016.
- [141] Luoyu Wang, Xiumei Wu, Jinyi Song, Yanhui Fu, Zhenqiang Ma, Xiaoyan Wu, Yiyang Wang, Yulin Song, Fenyang Chen, Zhongxiang Ding, et al. Unraveling the influences of hemodynamic lag and intrinsic cerebrovascular reactivity on functional metrics in ischemic stroke. *NeuroImage*, 303:120920, 2024.
- [142] Martijn P Van Den Heuvel and Hilleke E Hulshoff Pol. Exploring the brain network: a review on resting-state fmri functional connectivity. *European neuropsychopharmacology*, 20(8):519–534, 2010.
- [143] Foteini Christidi, Ilias Orgianelis, Ermis Merkouris, Christos Koutsokostas, Dimitrios Tsiptsios, Efstratios Karavasilis, Evlampia A Psatha, Anna Tsiakiri, Aspasia Serdari, Nikolaos Aggelousis, et al. A comprehensive review on the role of resting-state functional magnetic resonance imaging in predicting post-stroke motor and sensory outcomes. *Neurology International*, 16(1):189–201, 2024.
- [144] Christian Grefkes and Gereon R Fink. Reorganization of cerebral networks after stroke: new insights from neuroimaging with connectivity approaches. *Brain*, 134(5):1264–1276, 2011.
- [145] Megan H Lee, Christopher D Smyser, and Joshua S Shimony. Resting-state fmri: a review of methods and clinical applications. *American Journal of neuroradiology*, 34(10):1866–1872, 2013.
- [146] Christian F Beckmann, Marilena DeLuca, Joseph T Devlin, and Stephen M Smith. Investigations into resting-state connectivity using independent component analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360(1457):1001–1013, 2005.

- [147] Ed Bullmore and Olaf Sporns. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature reviews neuroscience*, 10(3):186–198, 2009.
- [148] Tracy Warbrick, Jessica Rosenberg, and N Joni Shah. The relationship between bold fmri response and the underlying white matter as measured by fractional anisotropy (fa): a systematic review. *Neuroimage*, 153:369–381, 2017.
- [149] Qiuyi Lv, Guixing Xu, Yuxin Pan, Tongtong Liu, Xiaodong Liu, Linqing Miao, Xing Chen, Lan Jiang, Jie Chen, Yingjia He, et al. Effect of acupuncture on neuroplasticity of stroke patients with motor dysfunction: a meta-analysis of fmri studies. *Neural Plasticity*, 2021:1–10, 2021.
- [150] Mengxin Lu, Zhongming Du, Jiping Zhao, Lan Jiang, Ruoyi Liu, Muzhao Zhang, Tianjiao Xu, Jingpei Wei, Wei Wang, Lingling Xu, et al. Neuroimaging mechanisms of acupuncture on functional reorganization for post-stroke motor improvement: a machine learning-based functional magnetic resonance imaging study. *Frontiers in Neuroscience*, 17:1143239, 2023.
- [151] Pragya Srivastava, Panagiotis Fotiadis, Linden Parkes, and Dani S Bassett. The expanding horizons of network neuroscience: From description to prediction and control. *Neuroimage*, 258:119250, 2022.
- [152] Apollo M Nkwake and Apollo M Nkwake. Why are assumptions important? *Working with Assumptions in International Development Program Evaluation: With a Foreword by Michael Bamberger*, pages 93–111, 2013.
- [153] D Papo and JM Buldú. Does the brain behave like a (complex) network? i. dynamics. *Physics of Life Reviews*, 2023.
- [154] Farzad V Farahani, Waldemar Karwowski, and Nichole R Lighthall. Application of graph theory for identifying connectivity patterns in human brain networks: a systematic review. *frontiers in Neuroscience*, 13:585, 2019.
- [155] Qawi K Telesford, Sean L Simpson, Jonathan H Burdette, Satoru Hayasaka, and Paul J Laurienti. The brain as a complex system: using network science as a tool for understanding the brain. *Brain connectivity*, 1(4):295–308, 2011.
- [156] Bernadette CM Van Wijk, Cornelis J Stam, and Andreas Daffertshofer. Comparing brain networks of different size and connectivity density using graph theory. *PloS one*, 5(10):e13701, 2010.
- [157] Duncan J Watts and Steven H Strogatz. Collective dynamics of ‘small-world’ networks. *nature*, 393(6684):440–442, 1998.
- [158] Xuhong Liao, Athanasios V Vasilakos, and Yong He. Small-world human brain networks: perspectives and challenges. *Neuroscience & Biobehavioral Reviews*, 77:286–300, 2017.
- [159] Olaf Sporns and Christopher J Honey. Small worlds inside big brains. *Proceedings of the National Academy of Sciences*, 103(51):19219–19220, 2006.
- [160] Claus-C Hilgetag, Gully APC Burns, Marc A O’Neill, Jack W Scannell, and Malcolm P Young. Anatomical connectivity defines the organization of clusters of cortical areas in the macaque and the cat. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 355(1393):91–110, 2000.
- [161] Danielle Smith Bassett and ED Bullmore. Small-world brain networks. *The neuroscientist*, 12(6):512–523, 2006.
- [162] Xiaojian Xi, Shirin Panahi, Viet-Thanh Pham, Zhen Wang, Sajad Jafari, and Iqtadar Hussain. Optimum topology and coupling strength for synchronization. *Applied Mathematics and Computation*, 379:125226, 2020.
- [163] Anna Borne, C Lemaitre, Christine Bulteau, Monica Baciú, and Marcela Perrone-Bertolotti. Unveiling the cognitive network organization through cognitive performance. *Scientific Reports*, 14(1):11645, 2024.

- [164] Rong Wang, Mianxin Liu, Xinhong Cheng, Ying Wu, Andrea Hildebrandt, and Changsong Zhou. Segregation, integration, and balance of large-scale resting brain networks configure different cognitive abilities. *Proceedings of the National Academy of Sciences*, 118(23):e2022288118, 2021.
- [165] Jessica R Cohen and Mark D’Esposito. The segregation and integration of distinct brain networks and their relationship to cognition. *Journal of Neuroscience*, 36(48):12083–12094, 2016.
- [166] Yingying Wang, Caihong Wang, Peifang Miao, Jingchun Liu, Ying Wei, Luobing Wu, Kaiyu Wang, and Jingliang Cheng. An imbalance between functional segregation and integration in patients with pontine stroke: a dynamic functional network connectivity study. *NeuroImage: Clinical*, 28:102507, 2020.
- [167] Anna K Bonkhoff, Flor A Espinoza, Harshvardhan Gazula, Victor M Vergara, Lukas Hensel, Jochen Michely, Theresa Paul, Anne K Rehme, Lukas J Volz, Gereon R Fink, et al. Acute ischaemic stroke alters the brain’s preference for distinct dynamic connectivity states. *Brain*, 143(5):1525–1540, 2020.
- [168] Eckhard Schlemm, Robert Schulz, Marlene Bönstrup, Lutz Krawinkel, Jens Fiehler, Christian Gerloff, Götz Thomalla, and Bastian Cheng. Structural brain networks and functional motor outcome after stroke—a prospective cohort study. *Brain Communications*, 2(1):fcaa001, 2020.
- [169] Yongxin Li, Zeyun Yu, Ping Wu, and Jiaxu Chen. The disrupted topological properties of structural networks showed recovery in ischemic stroke patients: a longitudinal design study. *BMC neuroscience*, 22(1):1–15, 2021.
- [170] Rok Berlot, Claudia Metzler-Baddeley, M Arfan Ikram, Derek K Jones, and Michael J O’Sullivan. Global efficiency of structural networks mediates cognitive control in mild cognitive impairment. *Frontiers in aging neuroscience*, 8:292, 2016.
- [171] Matthew L Stanley, Sean L Simpson, Dale Dagenbach, Robert G Lyday, Jonathan H Burdette, and Paul J Laurienti. Changes in brain network efficiency and working memory performance in aging. *PLoS One*, 10(4):e0123950, 2015.
- [172] Milena Oehlers and Benjamin Fabian. Graph metrics for network robustness—a survey. *Mathematics*, 9(8):895, 2021.
- [173] Talia C Oughourlian, Shan Rizvi, Chencai Wang, Alex Kostiuk, Noriko Salamon, Langston T Holly, and Benjamin M Ellingson. Sex-specific alterations in functional connectivity and network topology in patients with degenerative cervical myelopathy. *Scientific Reports*, 14(1):16020, 2024.
- [174] Xiao Han, He Jin, Kuangshi Li, Yanzhe Ning, Lan Jiang, Pei Chen, Hongwei Liu, Yong Zhang, Hua Zhang, Zhongjian Tan, et al. Acupuncture modulates disrupted whole-brain network after ischemic stroke: evidence based on graph theory analysis. *Neural Plasticity*, 2020, 2020.
- [175] Olaf Sporns, Giulio Tononi, and Rolf Kötter. The human connectome: a structural description of the human brain. *PLoS computational biology*, 1(4):e42, 2005.
- [176] Nathalie Tzourio-Mazoyer, Brigitte Landeau, Dimitri Papathanassiou, Fabrice Crivello, Octave Etard, Nicolas Delcroix, Bernard Mazoyer, and Marc Joliot. Automated anatomical labeling of activations in spm using a macroscopic anatomical parcellation of the mni mri single-subject brain. *Neuroimage*, 15(1):273–289, 2002.
- [177] Chun-Hung Yeh, Derek K Jones, Xiaoyun Liang, Maxime Descoteaux, and Alan Connolly. Mapping structural connectivity using diffusion mri: challenges and opportunities. *Journal of Magnetic Resonance Imaging*, 53(6):1666–1682, 2021.

- [178] Jacob Tanner, Joshua Faskowitz, Andreia Sofia Teixeira, Caio Seguin, Ludovico Coletta, Alessandro Gozzi, Bratislav Mišić, and Richard F Betzel. A multi-modal, asymmetric, weighted, and signed description of anatomical connectivity. *Nature communications*, 15(1):5865, 2024.
- [179] Carlo Pierpaoli, Lindsay Walker, Mustafa Okan Irfanoglu, Alan Barnett, Peter Basser, Lin-Ching Chang, C Koay, Sinisia Pajevic, Gustavo Rohde, Joelle Sarlls, et al. Tortoise: an integrated software package for processing of diffusion mri data. In *ISMRM 18th annual meeting*, volume 1597. Stockholm, 2010.
- [180] Arun S Mahadevan, Ursula A Tooley, Maxwell A Bertolero, Allyson P Mackey, and Danielle S Bassett. Evaluating the sensitivity of functional connectivity measures to motion artifact in resting-state fmri data. *NeuroImage*, 241: 118408, 2021.
- [181] Xiaoying Liu, Shuting Qiu, Xiaoyang Wang, Hui Chen, Yuting Tang, and Yin Qin. Aberrant dynamic functional-structural connectivity coupling of large-scale brain networks in poststroke motor dysfunction. *NeuroImage: Clinical*, 37:103332, 2023.
- [182] Martin A Koch, David G Norris, and Margret Hund-Georgiadis. An investigation of functional and anatomical connectivity using magnetic resonance imaging. *Neuroimage*, 16(1):241–250, 2002.
- [183] Pawel Skudlarski, Kanchana Jagannathan, Vince D Calhoun, Michelle Hampson, Beata A Skudlarska, and Godfrey Pearlson. Measuring brain connectivity: diffusion tensor imaging validates resting state temporal correlations. *Neuroimage*, 43(3):554–561, 2008.
- [184] Hae-Jeong Park and Karl Friston. Structural and functional brain networks: from connections to cognition. *Science*, 342(6158):1238411, 2013.
- [185] J Michael Tyszka, Daniel P Kennedy, Ralph Adolphs, and Lynn K Paul. Intact bilateral resting-state networks in the absence of the corpus callosum. *Journal of Neuroscience*, 31(42):15154–15162, 2011.
- [186] Olaf Sporns, Dante R Chialvo, Marcus Kaiser, and Claus C Hilgetag. Organization, development and function of complex brain networks. *Trends in cognitive sciences*, 8(9):418–425, 2004.
- [187] David J Gladstone, Cynthia J Danells, and Sandra E Black. The fugl-meyer assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabilitation and neural repair*, 16(3):232–240, 2002.
- [188] Lisa Zeltzer. Fugl-meyer assessment of sensorimotor recovery after stroke (fma), 2010.
- [189] Susan Whitfield-Gabrieli and Alfonso Nieto-Castanon. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain connectivity*, 2(3):125–141, 2012.
- [190] Alfonso Nieto-Castanon. *Handbook of functional connectivity magnetic resonance imaging methods in CONN*. Hilbert Press, 2020.
- [191] Jesper LR Andersson, Chloe Hutton, John Ashburner, Robert Turner, and Karl Friston. Modeling geometric deformations in epi time series. *Neuroimage*, 13(5):903–919, 2001.
- [192] Karl J Friston, John Ashburner, Christopher D Frith, J-B Poline, John D Heather, and Richard SJ Frackowiak. Spatial registration and normalization of images. *Human brain mapping*, 3(3):165–189, 1995.
- [193] RNA Henson, C Buechel, O Josephs, and KJ Friston. The slice-timing problem in event-related fmri. *NeuroImage*, 9:125, 1999.
- [194] Ronald Sladky, Karl J Friston, Jasmin Tröstl, Ross Cunnington, Ewald Moser, and Christian Windischberger. Slice-timing effects and their correction in functional mri. *Neuroimage*, 58(2):588–594, 2011.

- [195] Alfonso Nieto-Castanon and Susan Whitfield-Gabrieli. Conn functional connectivity toolbox (rrid: Scr_009550), version 21. *Series CONN functional connectivity toolbox (RRID: SCR_009550), Version, 21, 2021.*
- [196] Jonathan D Power, Anish Mitra, Timothy O Laumann, Abraham Z Snyder, Bradley L Schlaggar, and Steven E Petersen. Methods to detect, characterize, and remove motion artifact in resting state fmri. *neuroimage*, 84: 320–341, 2014.
- [197] Alfonso Nieto-Castanon. Preparing fmri data for statistical analysis. *arXiv preprint arXiv:2210.13564*, 2022.
- [198] Jonathan D Power, Kelly A Barnes, Abraham Z Snyder, Bradley L Schlaggar, and Steven E Petersen. Spurious but systematic correlations in functional connectivity mri networks arise from subject motion. *Neuroimage*, 59(3): 2142–2154, 2012.
- [199] Vince D Calhoun, Tor D Wager, Anjali Krishnan, Keri S Rosch, Karen E Seymour, Mary Beth Nebel, Stewart H Mostofsky, Prashanth Nyalakanai, and Kent Kiehl. The impact of t1 versus epi spatial normalization templates for fmri data analyses. Technical report, Wiley Online Library, 2017.
- [200] John Ashburner and Karl J Friston. Unified segmentation. *neuroimage*, 26(3):839–851, 2005.
- [201] John Ashburner. A fast diffeomorphic image registration algorithm. *Neuroimage*, 38(1):95–113, 2007.
- [202] Zikuan Chen and Vince Calhoun. Effect of spatial smoothing on task fmri ica and functional connectivity. *Frontiers in neuroscience*, 12:15, 2018.
- [203] Karl J Friston, Steven Williams, Robert Howard, Richard SJ Frackowiak, and Robert Turner. Movement-related effects in fmri time-series. *Magnetic resonance in medicine*, 35(3):346–355, 1996.
- [204] Francesca Morfini, Susan Whitfield-Gabrieli, and Alfonso Nieto-Castañón. Functional connectivity mri quality control procedures in conn. *Frontiers in Neuroscience*, 17:1092125, 2023.
- [205] Michael N Hallquist, Kai Hwang, and Beatriz Luna. The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fmri preprocessing reintroduces noise and obscures functional connectivity. *Neuroimage*, 82:208–225, 2013.
- [206] Jonathan D Power, Mark Plitt, Prantik Kundu, Peter A Bandettini, and Alex Martin. Temporal interpolation alters motion in fmri scans: Magnitudes and consequences for artifact detection. *PloS one*, 12(9):e0182939, 2017.
- [207] Yashar Behzadi, Khaled Restom, Joy Liau, and Thomas T Liu. A component based noise correction method (compcor) for bold and perfusion based fmri. *Neuroimage*, 37(1):90–101, 2007.
- [208] Xiaoqian J Chai, Alfonso Nieto Castañón, Dost Öngür, and Susan Whitfield-Gabrieli. Anticorrelations in resting state networks without global signal regression. *Neuroimage*, 59(2):1420–1428, 2012.
- [209] Kevin Murphy, Rasmus M Birn, Daniel A Handwerker, Tyler B Jones, and Peter A Bandettini. The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *Neuroimage*, 44 (3):893–905, 2009.
- [210] Michael D Fox, Abraham Z Snyder, Justin L Vincent, Maurizio Corbetta, David C Van Essen, and Marcus E Raichle. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences*, 102(27):9673–9678, 2005.
- [211] Robert W Cox. Afni: what a long strange trip it's been. *Neuroimage*, 62(2):743–747, 2012.
- [212] Mark Jenkinson, Peter Bannister, Michael Brady, and Stephen Smith. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*, 17(2):825–841, 2002.
- [213] Peter J Basser, James Mattiello, and Denis LeBihan. Mr diffusion tensor spectroscopy and imaging. *Biophysical journal*, 66(1):259–267, 1994.

- [214] M Okan Irfanoglu, Pooja Modi, Amritha Nayak, Elizabeth B Hutchinson, Joelle Sarlls, and Carlo Pierpaoli. Dr-buddi (diffeomorphic registration for blip-up blip-down diffusion imaging) method for correcting echo planar imaging distortions. *Neuroimage*, 106:284–299, 2015.
- [215] Paul A Taylor and Ziad S Saad. Fatcat:(an efficient) functional and tractographic connectivity analysis toolbox. *Brain connectivity*, 3(5):523–535, 2013.
- [216] Robert W Cox. Afni: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical research*, 29(3):162–173, 1996.
- [217] Bruce Fischl, David H Salat, Evelina Busa, Marilyn Albert, Megan Dieterich, Christian Haselgrove, Andre Van Der Kouwe, Ron Killiany, David Kennedy, Shuna Klaveness, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron*, 33(3):341–355, 2002.
- [218] Randy L Buckner, Denise Head, Jamie Parker, Anthony F Fotenos, Daniel Marcus, John C Morris, and Abraham Z Snyder. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *Neuroimage*, 23(2):724–738, 2004.
- [219] Susumu Mori, Barbara J Crain, Vadappuram P Chacko, and Peter CM Van Zijl. Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 45(2):265–269, 1999.
- [220] Jinhui Wang, Xindi Wang, Mingrui Xia, Xuhong Liao, Alan Evans, and Yong He. Gretna: a graph theoretical network analysis toolbox for imaging connectomics. *Frontiers in human neuroscience*, 9:386, 2015.
- [221] Jin-Hui Wang, Xi-Nian Zuo, Suril Gohel, Michael P Milham, Bharat B Biswal, and Yong He. Graph theoretical analysis of functional brain networks: test-retest evaluation on short-and long-term resting-state functional mri data. *PloS one*, 6(7):e21976, 2011.
- [222] William Hedley Thompson and Peter Fransson. On stabilizing the variance of dynamic functional brain connectivity time series. *Brain connectivity*, 6(10):735–746, 2016.
- [223] Heath R Pardoe and Samantha P Martin. In-scanner head motion and structural covariance networks. *Human brain mapping*, 43(14):4335–4346, 2022.
- [224] Kristina P Vatcheva, MinJae Lee, Joseph B McCormick, and Mohammad H Rahbar. Multicollinearity in regression analyses conducted in epidemiologic studies. *Epidemiology (Sunnyvale, Calif.)*, 6(2):227, 2016.
- [225] Roser Sala-Llonch, David Bartrés-Faz, and Carme Junqué. Reorganization of brain networks in aging: a review of functional connectivity studies. *Frontiers in psychology*, 6:663, 2015.
- [226] Karl J Friston. Statistical parametric mapping. *Neuroscience databases: a practical guide*, pages 237–250, 2003.
- [227] Thomas Nichols and Satoru Hayasaka. Controlling the familywise error rate in functional neuroimaging: a comparative review. *Statistical methods in medical research*, 12(5):419–446, 2003.
- [228] Marie Delacre, Daniël Lakens, and Christophe Leys. Why psychologists should by default use welch’s t-test instead of student’s t-test. *International Review of Social Psychology*, 30(1):92–101, 2017.
- [229] Denes Szucs and John PA Ioannidis. Sample size evolution in neuroimaging research: An evaluation of highly-cited studies (1990–2012) and of latest practices (2017–2018) in high-impact journals. *NeuroImage*, 221:117164, 2020.
- [230] Codjo Emile Agbangba, Edmond Sacia Aide, Hermann Honfo, and Romain Glèlè Kakai. On the use of post-hoc tests in environmental and biological sciences: A critical review. *Heliyon*, 10(3), 2024.

- [231] Adrian G Guggisberg, Philipp J Koch, Friedhelm C Hummel, and Cathrin M Buetefisch. Brain networks and their relevance for stroke rehabilitation. *Clinical Neurophysiology*, 130(7):1098–1124, 2019.
- [232] Gabriel S Rocha, Marco AM Freire, André M Britto, Karina M Paiva, Rodrigo F Oliveira, Ivana AT Fonseca, Dayane P Araújo, Lucidio C Oliveira, Fausto P Guzen, Paulo LAG Morais, et al. Basal ganglia for beginners: the basic concepts you need to know and their role in movement control. *Frontiers in Systems Neuroscience*, 17: 1242929, 2023.
- [233] Jessica A Grahm, John A Parkinson, and Adrian M Owen. The cognitive functions of the caudate nucleus. *Progress in neurobiology*, 86(3):141–155, 2008.
- [234] Heinz Steiner and Kuei Y Tseng. *Handbook of basal ganglia structure and function*, volume 24. Academic Press, 2016.
- [235] Andreea C Bostan, Richard P Dum, and Peter L Strick. The basal ganglia communicate with the cerebellum. *Proceedings of the national academy of sciences*, 107(18):8452–8456, 2010.
- [236] Eiji Hoshi, Léon Tremblay, Jean Féger, Peter L Carras, and Peter L Strick. The cerebellum communicates with the basal ganglia. *Nature neuroscience*, 8(11):1491–1493, 2005.
- [237] M Jueptner, M Rijntjes, C Weiller, JH Faiss, D Timmann, SP Mueller, and HC Diener. Localization of a cerebellar timing process using pet. *Neurology*, 45(8):1540–1545, 1995.
- [238] Joseph J Paton and Dean V Buonomano. The neural basis of timing: distributed mechanisms for diverse functions. *Neuron*, 98(4):687–705, 2018.
- [239] Sundeep Teki, Manon Grube, Sukhbinder Kumar, and Timothy D Griffiths. Distinct neural substrates of duration-based and beat-based auditory timing. *Journal of Neuroscience*, 31(10):3805–3812, 2011.
- [240] Jean-Claude Dreher and Jordan Grafman. The roles of the cerebellum and basal ganglia in timing and error prediction. *European Journal of Neuroscience*, 16(8):1609–1619, 2002.
- [241] Ovidiu V Lungu, Martin Bares, Tao Liu, Christopher M Gomez, Ivica Cechova, and James Ashe. Trial-to-trial adaptation: parsing out the roles of cerebellum and bg in predictive motor timing. *Journal of cognitive neuroscience*, 28(7):920–934, 2016.
- [242] Martin Bares, Ovidiu V Lungu, Tao Liu, Tobias Waechter, Christopher M Gomez, and James Ashe. The neural substrate of predictive motor timing in spinocerebellar ataxia. *The cerebellum*, 10:233–244, 2011.
- [243] Warren H Meck. *Functional and neural mechanisms of interval timing*. CRC Press, 2003.
- [244] Jennifer T Coull, Ruey-Kuang Cheng, and Warren H Meck. Neuroanatomical and neurochemical substrates of timing. *Neuropsychopharmacology*, 36(1):3–25, 2011.
- [245] Howard Eichenbaum. Time cells in the hippocampus: a new dimension for mapping memories. *Nature Reviews Neuroscience*, 15(11):732–744, 2014.
- [246] Leila Reddy, Benedikt Zoefel, Jessy K Possel, Judith Peters, Doris E Dijksterhuis, Marlene Poncet, Elisabeth CW van Straaten, Johannes C Baayen, Sander Idema, and Matthew W Self. Human hippocampal neurons track moments in a sequence of events. *Journal of Neuroscience*, 41(31):6714–6725, 2021.
- [247] Russell Epstein and Nancy Kanwisher. A cortical representation of the local visual environment. *Nature*, 392 (6676):598–601, 1998.
- [248] Hugo Merchant, Deborah L Harrington, and Warren H Meck. Neural basis of the perception and estimation of time. *Annual review of neuroscience*, 36(1):313–336, 2013.

- [249] Jonathan W Mink. The basal ganglia: focused selection and inhibition of competing motor programs. *Progress in neurobiology*, 50(4):381–425, 1996.
- [250] DJ Sharp, V Bonnelle, X De Boissezon, CF Beckmann, SG James, MC Patel, and M Aa Mehta. Distinct frontal systems for response inhibition, attentional capture, and error processing. *Proceedings of the National Academy of Sciences*, 107(13):6106–6111, 2010.
- [251] Adam R Aron, Sarah Durston, Dawn M Eagle, Gordon D Logan, Cathy M Stinear, and Veit Stuphorn. Converging evidence for a fronto-basal-ganglia network for inhibitory control of action and cognition. *Journal of Neuroscience*, 27(44):11860–11864, 2007.
- [252] Hiroki Nakata, Kiwako Sakamoto, Antonio Ferretti, Mauro Gianni Perrucci, Cosimo Del Gratta, Ryusuke Kakigi, and Gian Luca Romani. Somato-motor inhibitory processing in humans: an event-related functional mri study. *Neuroimage*, 39(4):1858–1866, 2008.
- [253] Julià L Amengual, Thomas F Münte, Josep Marco-Pallarés, Nuria Rojo, Jennifer Grau-Sánchez, Francisco Rubio, Esther Duarte, Carles Grau, and Antoni Rodríguez-Fornells. Overactivation of the supplementary motor area in chronic stroke patients. *Journal of neurophysiology*, 112(9):2251–2263, 2014.
- [254] Huacong Liu, Lanpin Chen, Guifeng Zhang, Yijing Jiang, Shanshan Qu, Songyan Liu, Yong Huang, and Junqi Chen. Scalp acupuncture enhances the functional connectivity of visual and cognitive-motor function network of patients with acute ischemic stroke. *Evidence-Based Complementary and Alternative Medicine*, 2020(1): 8836794, 2020.
- [255] Yuxiang Liu, Jing Zhou, Jiawen Zheng, and Jing Chen. Efficacy of different acupuncture therapies on hand dysfunction in post-stroke patients: a systematic review and meta-analysis. *Frontiers in Neurology*, 16:1589874, 2025.
- [256] Ye Zhang, Hongliang Liu, Li Wang, Jun Yang, Rubing Yan, Jingna Zhang, Linqiong Sang, Pengyue Li, Jian Wang, and Mingguo Qiu. Relationship between functional connectivity and motor function assessment in stroke patients with hemiplegia: a resting-state functional mri study. *Neuroradiology*, 58:503–511, 2016.
- [257] Maria Laura Blefari, Roberto Martuzzi, Roy Salomon, Javier Bello-Ruiz, Bruno Herbelin, Andrea Serino, and Olaf Blanke. Bilateral rolandic operculum processing underlying heartbeat awareness reflects changes in bodily self-consciousness. *European Journal of Neuroscience*, 45(10):1300–1312, 2017.
- [258] Gerardo Salvato, Fabian Richter, Lucas Sedeño, Gabriella Bottini, and Eraldo Paulesu. Building the bodily self-awareness: Evidence for the convergence between interoceptive and exteroceptive information in a multilevel kernel density analysis study. *Human brain mapping*, 41(2):401–418, 2020.
- [259] Andrea Serino, Adrian Alsmith, Marcello Costantini, Alisa Mandrigin, Ana Tajadura-Jimenez, and Christophe Lopez. Bodily ownership and self-location: components of bodily self-consciousness. *Consciousness and cognition*, 22(4):1239–1252, 2013.
- [260] Giulio Mastria, Tommaso Bertoni, Henri Perrin, Nikita Akulenko, Gaia Risso, Michel Akselrod, Eleonora Guanziroli, Franco Molteni, Patric Hagmann, Michela Bassolino, et al. Body ownership alterations in stroke emerge from reduced proprioceptive precision and damage to the frontoparietal network. *Med*, 6(4), 2025.
- [261] Yong Huang, Jun-Qi Chen, Xin-Sheng Lai, Chun-Zhi Tang, Jun-Jun Yang, Hua Chen, Jun-Xian Wu, Hui-Ling Xiao, Shan-Shan Qu, Yi-Dan Zhang, et al. Lateralisation of cerebral response to active acupuncture in patients with unilateral ischaemic stroke: an fmri study. *Acupuncture in Medicine*, 31(3):290–296, 2013.

- [262] Geng Li, Clifford R Jack Jr, and Edward S Yang. An fmri study of somatosensory-implicated acupuncture points in stable somatosensory stroke patients. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine*, 24(5):1018–1024, 2006.
- [263] Hans-Otto Karnath. New insights into the functions of the superior temporal cortex. *Nature Reviews Neuroscience*, 2(8):568–576, 2001.
- [264] Bahar Khalighinejad, Prachi Patel, Jose L Herrero, Stephan Bickel, Ashesh D Mehta, and Nima Mesgarani. Functional characterization of human heschl's gyrus in response to natural speech. *Neuroimage*, 235:118003, 2021.
- [265] Leonardo Tozzi, Angela Carballedo, Friedrich Wetterling, Hazel McCarthy, Veronica O'keane, Michael Gill, Derrek Morris, Ciara Fahey, James Meaney, and Thomas Frodl. Single-nucleotide polymorphism of the fkbp5 gene and childhood maltreatment as predictors of structural changes in brain areas involved in emotional processing in depression. *Neuropsychopharmacology*, 41(2):487–497, 2016.
- [266] Stephanie Sutoko, Hirokazu Atsumori, Akiko Obata, Tsukasa Funane, Akihiko Kandori, Koji Shimonaga, Seiji Hama, Shigeto Yamawaki, and Toshio Tsuji. Lesions in the right rolandic operculum are associated with self-rating affective and apathetic depressive symptoms for post-stroke patients. *Scientific reports*, 10(1):20264, 2020.
- [267] Maree L Hackett, Chaturangi Yapa, Varsha Parag, and Craig S Anderson. Frequency of depression after stroke: a systematic review of observational studies. *Stroke*, 36(6):1330–1340, 2005.
- [268] Luis Ayerbe, Salma Ayis, Charles DA Wolfe, and Anthony G Rudd. Natural history, predictors and outcomes of depression after stroke: systematic review and meta-analysis. *The British journal of psychiatry*, 202(1):14–21, 2013.
- [269] Eran Chemerinski and Robert G Robinson. The neuropsychiatry of stroke. *Psychosomatics*, 41(1):5–14, 2000.
- [270] Rosemarie B King. Quality of life after stroke. *Stroke*, 27(9):1467–1472, 1996.
- [271] Patricia H Janak and Kay M Tye. From circuits to behaviour in the amygdala. *Nature*, 517(7534):284–292, 2015.
- [272] Volker Neugebauer. Amygdala physiology in pain. In *Handbook of behavioral neuroscience*, volume 26, pages 101–113. Elsevier, 2020.
- [273] Vinod Menon and Lucina Q Uddin. Saliency, switching, attention and control: a network model of insula function. *Brain structure and function*, 214:655–667, 2010.
- [274] James R Augustine. Circuitry and functional aspects of the insular lobe in primates including humans. *Brain research reviews*, 22(3):229–244, 1996.
- [275] Michael D Greicius, Ben Krasnow, Allan L Reiss, and Vinod Menon. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the national academy of sciences*, 100(1):253–258, 2003.
- [276] Devarajan Sridharan, Daniel J Levitin, and Vinod Menon. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proceedings of the National Academy of Sciences*, 105(34):12569–12574, 2008.
- [277] Peter H Rudebeck, Timothy E Behrens, Steven W Kennerley, Mark G Baxter, Mark J Buckley, Mark E Walton, and Matthew FS Rushworth. Frontal cortex subregions play distinct roles in choices between actions and stimuli. *Journal of Neuroscience*, 28(51):13775–13785, 2008.
- [278] Peter Fransson. Spontaneous low-frequency bold signal fluctuations: An fmri investigation of the resting-state default mode of brain function hypothesis. *Human brain mapping*, 26(1):15–29, 2005.

- [279] Marcus E Raichle, Ann Mary MacLeod, Abraham Z Snyder, William J Powers, Debra A Gusnard, and Gordon L Shulman. A default mode of brain function. *Proceedings of the national academy of sciences*, 98(2):676–682, 2001.
- [280] Daniel H Weissman, KC Roberts, KM Visscher, and MG Woldorff. The neural bases of momentary lapses in attention. *Nature neuroscience*, 9(7):971–978, 2006.
- [281] Ilan I Goldberg, Michal Harel, and Rafael Malach. When the brain loses its self: prefrontal inactivation during sensorimotor processing. *Neuron*, 50(2):329–339, 2006.
- [282] Diego Centonze, Alessandro Finazzi-Agro, Giorgio Bernardi, and Mauro Maccarrone. The endocannabinoid system in targeting inflammatory neurodegenerative diseases. *Trends in pharmacological sciences*, 28(4):180–187, 2007.
- [283] Shyang Chang. The meridian system and mechanism of acupuncture: a comparative review. part 3: mechanisms of acupuncture therapies. *Taiwanese Journal of Obstetrics and Gynecology*, 52(2):171–184, 2013.
- [284] Lin Chen, Jing Zhang, Fan Li, Yue Qiu, Lu Wang, Ying-hua Li, Jing Shi, Hui-Lin Pan, and Man Li. Endogenous anandamide and cannabinoid receptor-2 contribute to electroacupuncture analgesia in rats. *The journal of pain*, 10(7):732–739, 2009.
- [285] Stephanie C Tjen-A-Looi, Peng Li, and John C Longhurst. Processing cardiovascular information in the vlpag during electroacupuncture in rats: roles of endocannabinoids and gaba. *Journal of Applied Physiology*, 106(6):1793–1799, 2009.
- [286] Pál Pacher and George Kunos. Modulating the endocannabinoid system in human health and disease—successes and failures. *The FEBS journal*, 280(9):1918–1943, 2013.
- [287] Shu-Ming Wang, Zeev N Kain, and Paul F White. Acupuncture analgesia: li. clinical considerations. *Anesthesia & Analgesia*, 106(2):611–621, 2008.
- [288] Bashir Bietar, Sophie Tanner, and Christian Lehmann. Neuroprotection and beyond: the central role of cb1 and cb2 receptors in stroke recovery. *International journal of molecular sciences*, 24(23):16728, 2023.
- [289] Attila Köfalvi, Estefanía Moreno, Arnau Cordoní, Ning-Sheng Cai, Victor Fernández-Dueñas, Samira G Ferreira, Ramón Guixà-González, Marta Sánchez-Soto, Hideaki Yano, Verónica Casadó-Anguera, et al. Control of glutamate release by complexes of adenosine and cannabinoid receptors. *BMC biology*, 18:1–21, 2020.
- [290] Zeng-Hui Yue, Liang Li, Xiao-Rong Chang, Jing-Ming Jiang, Le-Le Chen, and Xiao-Shan Zhu. Comparative study on effects between electroacupuncture and acupuncture for spastic paralysis after stroke. *Zhongguo Zhen jiu= Chinese Acupuncture & Moxibustion*, 32(7):582–586, 2012.
- [291] J Shi. A study on the effect and mechanism of acupuncture suppression of neuronal apoptosis following cerebral ischemia. *Sheng li ke xue jin zhan [Progress in physiology]*, 30(4):326–329, 1999.
- [292] TS Zhang, Lu Yang, Rong Hu, XL Qiao, Xin Yang, and XG Liu. Effect of electroacupuncture on the contents of excitatory amino acids in cerebral tissue at different time courses in rats with cerebral ischemia and reperfusion injury. *Zhen ci yan jiu= Acupuncture Research*, 32(4):234–236, 2007.
- [293] XM Feng, SP Chen, JY Wang, YX Yan, SB Wang, YH Gao, JL Zhang, and JL Liu. Effect of electroacupuncture intervention on expression of pain sensory and affection processing related corticotropin-releasing factor receptor mrna, etc. in the amygdala in neuropathic pain and negative affection rats. *Zhen ci yan jiu= Acupuncture Research*, 39(6):448–455, 2014.

- [294] Bo Hu, Fuhai Bai, Lize Xiong, and Qiang Wang. The endocannabinoid system, a novel and key participant in acupuncture's multiple beneficial effects. *Neuroscience & Biobehavioral Reviews*, 77:340–357, 2017.
- [295] S Ruehle, A Aparisi Rey, F Remmers, and B Lutz. The endocannabinoid system in anxiety, fear memory and habituation. *Journal of psychopharmacology*, 26(1):23–39, 2012.
- [296] Kenneth E McCa Carson and SJ Enna. Gaba pharmacology: the search for analgesics. *Neurochemical research*, 39:1948–1963, 2014.
- [297] Stephen Birch. A review and analysis of placebo treatments, placebo effects, and placebo controls in trials of medical procedures when sham is not inert. *Journal of Alternative & Complementary Medicine*, 12(3):303–310, 2006.
- [298] Ian Appleyard, Thomas Lundeborg, and Nicola Robinson. Should systematic reviews assess the risk of bias from sham–placebo acupuncture control procedures? *European Journal of Integrative Medicine*, 6(2):234–243, 2014.
- [299] Hugh MacPherson, Emily Vertosick, George Lewith, Klaus Linde, Karen J Sherman, Claudia M Witt, Andrew J Vickers, and Acupuncture Trialists' Collaboration. Influence of control group on effect size in trials of acupuncture for chronic pain: a secondary analysis of an individual patient data meta-analysis. *PloS one*, 9(4):e93739, 2014.
- [300] George T Lewith and David Machin. On the evaluation of the clinical effects of acupuncture. *Pain*, 16(2):111–127, 1983.
- [301] Yiu Ming Wong. Think out of the box: revisiting sham acupuncture treatment. *Frontiers in Neurology*, 15:1479239, 2024.
- [302] Sujung Yeo, Bruce Rosen, Peggy Bosch, Maurits Van Den Noort, and Sabina Lim. Gender differences in the neural response to acupuncture: clinical implications. *Acupuncture in Medicine*, 34(5):364–372, 2016.
- [303] Klaus Linde, Claudia M Witt, Andrea Streng, Wolfgang Weidenhammer, Stefan Wagenpfeil, Benno Brinkhaus, Stefan N Willich, and Dieter Melchart. The impact of patient expectations on outcomes in four randomized controlled trials of acupuncture in patients with chronic pain. *Pain*, 128(3):264–271, 2007.
- [304] Lene Vase, Sara Baram, Nobuari Takakura, Hiroyoshi Yajima, Miho Takayama, Ted J Kaptchuk, Søren Schou, Troels Staehelin Jensen, Robert Zachariae, and Peter Svensson. Specifying the nonspecific components of acupuncture analgesia. *PAIN®*, 154(9):1659–1667, 2013.
- [305] Naomi P Friedman and Trevor W Robbins. The role of prefrontal cortex in cognitive control and executive function. *Neuropsychopharmacology*, 47(1):72–89, 2022.
- [306] Nancy Kanwisher, Josh McDermott, and Marvin M Chun. The fusiform face area: a module in human extrastriate cortex specialized for face perception. *Journal of neuroscience*, 17(11):4302–4311, 1997.
- [307] Marius V Peelen and Paul E Downing. Selectivity for the human body in the fusiform gyrus. *Journal of neurophysiology*, 93(1):603–608, 2005.
- [308] Kathleen M O'Craven and Nancy Kanwisher. Mental imagery of faces and places activates corresponding stimulus-specific brain regions. *Journal of cognitive neuroscience*, 12(6):1013–1023, 2000.
- [309] Karen P Liu, Chetwyn C Chan, Tatia M Lee, and Christina W Hui-Chan. Mental imagery for promoting relearning for people after stroke: a randomized controlled trial. *Archives of physical medicine and rehabilitation*, 85(9):1403–1408, 2004.
- [310] T Wolbers, C Weiller, and C Büchel. Contralateral coding of imagined body parts in the superior parietal lobe. *Cerebral cortex*, 13(4):392–399, 2003.

- [311] Ana Poveda-García, Carmen Moret-Tatay, and Miguel Gómez-Martínez. The association between mental motor imagery and real movement in stroke. In *Healthcare*, volume 9, page 1568. MDPI, 2021.
- [312] Xing Chen, Hua Zhang, and Yihuai Zou. A functional magnetic resonance imaging study on the effect of acupuncture at gb34 (yanglingquan) on motor-related network in hemiplegic patients. *brain research*, 1601:64–72, 2015.
- [313] Ziliang Zhu, Huichao Yang, Haojie Wen, Jinyi Hung, Yueqin Hu, Yanchao Bi, and Xi Yu. Innate network mechanisms of temporal pole for semantic cognition in neonatal and adult twin studies. *Nature Communications*, 16(1): 3835, 2025.
- [314] Regina M Sullivan, Donald A Wilson, Nadine Ravel, and Anne-Marie Mouly. Olfactory memory networks: from emotional learning to social behaviors, 2015.
- [315] Emma V Cooke, Kathryn Mares, Allan Clark, Raymond C Tallis, and Valerie M Pomeroy. The effects of increased dose of exercise-based therapies to enhance motor recovery after stroke: a systematic review and meta-analysis. *BMC medicine*, 8:1–13, 2010.
- [316] AW Dromerick, CE Lang, RL Birkenmeier, JM Wagner, JP Miller, TO Videen, WJ Powers, SL Wolf, and DF Edwards. Very early constraint-induced movement during stroke rehabilitation (vectors) a single-center rct. *Neurology*, 73(3):195–201, 2009.
- [317] Alain P Yelnik, Victorine Quintaine, Cedric Andriantsifanetra, Marie Wannepain, Peggy Reiner, Hélène Marnef, Mathilde Evrard, Elena Meseguer, Jean Pascal Devailly, Monica Lozano, et al. Amobes (active mobility very early after stroke). *Stroke*, 2017.
- [318] Marie-Claire Smith and Cathy M Stinear. Plasticity and motor recovery after stroke: Implications for physiotherapy. *New Zealand Journal of Physiotherapy*, 44(3):166–173, 2016.
- [319] Allen Newell and Paul S Rosenbloom. Mechanisms of skill acquisition and the law of practice. In *Cognitive skills and their acquisition*, pages 1–55. Psychology Press, 2013.
- [320] Janet H Carr and Roberta B Shepherd. A motor learning model for stroke rehabilitation. *Physiotherapy*, 75(7): 372–380, 1989.
- [321] Nagako Murase, Julie Duque, Riccardo Mazzocchio, and Leonardo G Cohen. Influence of interhemispheric interactions on motor function in chronic stroke. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 55(3):400–409, 2004.
- [322] Jo Nijs, Liesbeth Daenen, Patrick Cras, Filip Struyf, Nathalie Roussel, and Rob AB Oostendorp. Nociception affects motor output: a review on sensory-motor interaction with focus on clinical implications. *The Clinical journal of pain*, 28(2):175–181, 2012.
- [323] Bettina Pollok, Markus Butz, Joachim Gross, Martin Südmeyer, Lars Timmermann, and Alfons Schnitzler. Coupling between cerebellar hemispheres: behavioural, anatomic, and functional data. *The Cerebellum*, 5:212–219, 2006.
- [324] Shasha Zheng, Qixiang Lin, Miao Zhang, Hesheng Liu, Yong He, and Jie Lu. Secondary degeneration of white matter tract following basal ganglia infarction: A longitudinal diffusion tensor imaging study. *Current Medical Imaging*, 20(1):e15734056247080, 2024.
- [325] Meizhong Chen, Yufan Wu, Yuntao Wang, and Zhongming Li. Functional connectivity and white matter microstructural alterations in patients with left basal ganglia acute ischemic stroke. *Brain Imaging and Behavior*, pages 1–12, 2025.

- [326] Carlo Pierpaoli, Alan Barnett, Sinisa Pajevic, Robert Chen, LaRoy Penix, Anette Virta, and Peter Basser. Water diffusion changes in wallerian degeneration and their dependence on white matter architecture. *Neuroimage*, 13(6):1174–1185, 2001.
- [327] Michael D Greicius, Kaustubh Supekar, Vinod Menon, and Robert F Dougherty. Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cerebral cortex*, 19(1):72–78, 2009.
- [328] Jingna Zhang, Ye Zhang, Li Wang, Linqiong Sang, Jun Yang, Rubing Yan, Pengyue Li, Jian Wang, and Mingguo Qiu. Disrupted structural and functional connectivity networks in ischemic stroke patients. *Neuroscience*, 364: 212–225, 2017.
- [329] Yanan Wu, Chuanshuai Tian, Zhixuan Yu, Zaixing Liu, Han Wu, Jie Ming, Wenjun Hong, and Rong Xu. Structural and functional disconnections in non-acute post-stroke patients. *Frontiers in Neurology*, 16:1542292, 2025.
- [330] Changsheng Wang, Glenn T Stebbins, David L Nyenhuis, Leyla deToledo Morrell, Sally Freels, Eugenia Gencheva, Laura Pedelty, Kumar Sripathirathan, Michael E Moseley, David A Turner, et al. Longitudinal changes in white matter following ischemic stroke: a three-year follow-up study. *Neurobiology of aging*, 27(12):1827–1833, 2006.
- [331] Haiwen Chen, Jane Epstein, and Emily Stern. Neural plasticity after acquired brain injury: evidence from functional neuroimaging. *PM&R*, 2:S306–S312, 2010.
- [332] Albrecht Sigler, Majid H Mohajerani, and Timothy H Murphy. Imaging rapid redistribution of sensory-evoked depolarization through existing cortical pathways after targeted stroke in mice. *Proceedings of the National Academy of Sciences*, 106(28):11759–11764, 2009.
- [333] Stanley Milgram et al. The small world problem. *Psychology today*, 2(1):60–67, 1967.
- [334] Jinhui Wang, Xinian Zuo, and Yong He. Graph-based network analysis of resting-state functional mri. *Frontiers in systems neuroscience*, 4:1419, 2010.
- [335] Giulio Tononi, Olaf Sporns, and Gerald M Edelman. A measure for brain complexity: relating functional segregation and integration in the nervous system. *Proceedings of the National Academy of Sciences*, 91(11):5033–5037, 1994.
- [336] Boris C Bernhardt, Zhang Chen, Yong He, Alan C Evans, and Neda Bernasconi. Graph-theoretical analysis reveals disrupted small-world organization of cortical thickness correlation networks in temporal lobe epilepsy. *Cerebral cortex*, 21(9):2147–2157, 2011.
- [337] Anand S Pandit, Paul Expert, Renaud Lambiotte, Valerie Bonnelle, Robert Leech, Federico E Turkheimer, and David J Sharp. Traumatic brain injury impairs small-world topology. *Neurology*, 80(20):1826–1833, 2013.
- [338] Mengye Shi, Shenghua Liu, Huiyou Chen, Wen Geng, Xindao Yin, Yu-Chen Chen, and Liping Wang. Disrupted brain functional network topology in unilateral acute brainstem ischemic stroke. *Brain Imaging and Behavior*, 15(1):444–452, 2021.
- [339] Yifei Zhu, Lin Bai, Panpan Liang, Shan Kang, Hengbo Gao, and Haiqing Yang. Disrupted brain connectivity networks in acute ischemic stroke patients. *Brain imaging and behavior*, 11:444–453, 2017.
- [340] Shujun Zhang, Meiqin Li, Anqiang Chen, Liping Jiang, Hao Yu, and Yueqin Chen. Sex differences and age-related changes of large-scale brain networks. *BMC Medical Imaging*, 25(1):271, 2025.
- [341] Philipp Koch, Robert Schulz, and Friedhelm C Hummel. Structural connectivity analyses in motor recovery research after stroke. *Annals of clinical and translational neurology*, 3(3):233–244, 2016.

- [342] Mark D Humphries and Kevin Gurney. Network ‘small-world-ness’: a quantitative method for determining canonical network equivalence. *PloS one*, 3(4):e0002051, 2008.
- [343] Umberto Pensato, Andrew M Demchuk, Bijoy K Menon, Thanh N Nguyen, Gabriel Broocks, Bruce CV Campbell, Diego A Gutierrez Vasquez, Peter J Mitchell, Michael D Hill, Mayank Goyal, et al. Cerebral infarct growth: pathophysiology, pragmatic assessment, and clinical implications. *Stroke*, 56(1):219–229, 2025.
- [344] Leonardo Pantoni, Julio H Garcia, and Jorge A Gutierrez. Cerebral white matter is highly vulnerable to ischemia. *Stroke*, 27(9):1641–1647, 1996.
- [345] Michele Veldsman, Hsiao-Ju Cheng, Fang Ji, Emilio Werden, Mohamed Salah Khelif, Kwun Kei Ng, Joseph KW Lim, Xing Qian, Haoyong Yu, Juan Helen Zhou, et al. Degeneration of structural brain networks is associated with cognitive decline after ischaemic stroke. *Brain communications*, 2(2):fcaa155, 2020.
- [346] Stephanie Giezendanner, Melanie Sarah Fisler, Leila Maria Soravia, Jennifer Andreotti, Sebastian Walther, Roland Wiest, Thomas Dierks, and Andrea Federspiel. Microstructure and cerebral blood flow within white matter of the human brain: a tbss analysis. *PLoS One*, 11(3):e0150657, 2016.
- [347] Lijuan Wang, Zhiyong Zhang, John J McArdle, and Timothy A Salthouse. Investigating ceiling effects in longitudinal data analysis. *Multivariate behavioral research*, 43(3):476–496, 2008.
- [348] Claudia Alia, Cristina Spalletti, Stefano Lai, Alessandro Panarese, Giuseppe Lamola, Federica Bertolucci, Fabio Vallone, Angelo Di Garbo, Carmelo Chisari, Silvestro Micera, et al. Neuroplastic changes following brain ischemia and their contribution to stroke recovery: novel approaches in neurorehabilitation. *Frontiers in cellular neuroscience*, 11:76, 2017.
- [349] Y-X Tao, Y-H Wu, G-Q Zhu, and M Wang. Efficacy of acupuncture in the treatment of limb dyskinesia after stroke: a systematic review and meta-analysis. *European Review for Medical & Pharmacological Sciences*, 27(22), 2023.
- [350] Cheryl Bushnell, Janet Prvu Bettger, Kevin M Cockcroft, Steven C Cramer, Maria Orlando Edelen, Daniel Hanley, Irene L Katzan, Soeren Mattke, Dawn M Nilsen, Tepring Piquado, et al. Chronic stroke outcome measures for motor function intervention trials: expert panel recommendations. *Circulation: Cardiovascular Quality and Outcomes*, 8(6_suppl_3):S163–S169, 2015.
- [351] Pamela W Duncan, Larry B Goldstein, Ronnie D Horner, Pamela B Landsman, Gregory P Samsa, and David B Matchar. Similar motor recovery of upper and lower extremities after stroke. *Stroke*, 25(6):1181–1188, 1994.
- [352] Edgar D Hernandez, Claudia P Galeano, Nubia E Barbosa, Sandra M Forero, Åsa Nordin, Katharina S Sunnerhagen, and Margit Alt Murphy. Intra-and inter-rater reliability of fugl-meyer assessment of upper extremity in stroke. *Journal of rehabilitation medicine*, 51(9):652–659, 2019.
- [353] Nanako Hijikata, Michiyuki Kawakami, Ryota Ishii, Keita Tsuzuki, Takuya Nakamura, Kohei Okuyama, and Meigen Liu. Item difficulty of fugl-meyer assessment for upper extremity in persons with chronic stroke with moderate-to-severe upper limb impairment. *Frontiers in neurology*, 11:577855, 2020.
- [354] Ru-Lan Hsieh, Liang-Yi Wang, and Wen-Chung Lee. Additional therapeutic effects of electroacupuncture in conjunction with conventional rehabilitation for patients with first-ever ischaemic stroke. *Deutsche Zeitschrift für Akupunktur*, 51(1):56–57, 2008.
- [355] Guoliang Hu, Hongqiu Gu, Yingyu Jiang, Xin Yang, Yong Jiang, Chunjuan Wang, Zixiao Li, Yongjun Wang, and Yilong Wang. Revisiting the smoking paradox in acute ischemic stroke patients: findings from the chinese stroke center alliance study. *Journal of the American Heart Association*, 12(16):e029963, 2023.

- [356] Janet Prvu Bettger, Xin Zhao, Cheryl Bushnell, Louise Zimmer, Wenqin Pan, Linda S Williams, and Eric D Peterson. The association between socioeconomic status and disability after stroke: findings from the adherence evaluation after ischemic stroke longitudinal (avail) registry. *BMC public health*, 14(1):281, 2014.
- [357] Floor Buma, Gert Kwakkel, and Nick Ramsey. Understanding upper limb recovery after stroke. *Restorative neurology and neuroscience*, 31(6):707–722, 2013.
- [358] Gert Kwakkel, Janne M Veerbeek, Erwin EH van Wegen, and Steven L Wolf. Constraint-induced movement therapy after stroke. *The Lancet Neurology*, 14(2):224–234, 2015.
- [359] Bingkai Wang, Elizabeth Ogburn, and Michael Rosenblum. Analysis of covariance (ancova) in randomized trials: more precision, less conditional bias, and valid confidence intervals, without model assumptions. 2018.
- [360] Lisa M Sullivan and Ralph B D’Agostino. Robustness and power of analysis of covariance applied to data distorted from normality by floor effects: non-homogeneous regression slopes. *Journal of Statistical Computation and Simulation*, 72(2):141–165, 2002.
- [361] Bruce E Blaine. Winsorizing. *The SAGE encyclopedia of educational research, measurement, and evaluation*, page 1817, 2018.
- [362] Hongbo Chen, Lin Fan, Wenyu Jiang, and Yueqiang Hu. Increased functional connectivity after acupuncture treatment for patients with post-stroke depression. In *Proceedings of the 2nd International Conference on Biomedical Engineering and Bioinformatics*, pages 78–84, 2018.
- [363] CM Sackley, BI Baguley, S Gent, and P Hodgson. The use of a balance performance monitor in the treatment of weight-bearing and weight-transference problems after stroke. *Physiotherapy*, 78(12):907–913, 1992.
- [364] Lynsey M Keator, Grigori Yourganov, Alexandra Basilakos, Argye E Hillis, Gregory Hickok, Leonardo Bonilha, Christopher Rorden, and Julius Fridriksson. Independent contributions of structural and functional connectivity: Evidence from a stroke model. *Network Neuroscience*, 5(4):911–928, 2021.
- [365] Chase R Figley, Md Nasir Uddin, Kaihim Wong, Jennifer Kornelsen, Josep Puig, and Teresa D Figley. Potential pitfalls of using fractional anisotropy, axial diffusivity, and radial diffusivity as biomarkers of cerebral white matter microstructure. *Frontiers in Neuroscience*, 15:799576, 2022.
- [366] Jens H Jensen and Joseph A Helpert. Mri quantification of non-gaussian water diffusion by kurtosis analysis. *NMR in Biomedicine*, 23(7):698–710, 2010.
- [367] Hui Zhang, Torben Schneider, Claudia A Wheeler-Kingshott, and Daniel C Alexander. Noddi: practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage*, 61(4):1000–1016, 2012.
- [368] M Okan Irfanoglu, Lindsay Walker, Steffen Sammet, Carlo Pierpaoli, and Raghu Machiraju. Susceptibility distortion correction for echo planar images with non-uniform b-spline grid sampling: a diffusion tensor image study. In *Medical Image Computing and Computer-Assisted Intervention–MICCAI 2011: 14th International Conference, Toronto, Canada, September 18–22, 2011, Proceedings, Part II 14*, pages 174–181. Springer, 2011.
- [369] Ran Tao, P Thomas Fletcher, Samuel Gerber, and Ross T Whitaker. A variational image-based approach to the correction of susceptibility artifacts in the alignment of diffusion weighted and structural mri. In *Information Processing in Medical Imaging: 21st International Conference, IPMI 2009, Williamsburg, VA, USA, July 5–10, 2009. Proceedings 21*, pages 664–675. Springer, 2009.
- [370] Kurt G Schilling, Justin Blaber, Colin Hansen, Leon Cai, Baxter Rogers, Adam W Anderson, Seth Smith, Praitayini Kanakaraj, Tonia Rex, Susan M Resnick, et al. Distortion correction of diffusion weighted mri without reverse phase-encoding scans or field-maps. *PloS one*, 15(7):e0236418, 2020.

- [371] Hiroki Togo, Jaroslav Rokicki, Kenji Yoshinaga, Tatsuhiko Hisatsune, Hiroshi Matsuda, Nobuhiko Haga, and Takashi Hanakawa. Effects of field-map distortion correction on resting state functional connectivity mri. *Frontiers in neuroscience*, 11:656, 2017.
- [372] Wei Qiao Qiu, Joshua Claunch, Jian Kong, Erika E Nixon, Jiliang Fang, Ming Li, Mark Vangel, and Kathleen Kin-Sang Hui. The effects of acupuncture on the brain networks for emotion and cognition: an observation of gender differences. *Brain Research*, 1362:56–67, 2010.
- [373] Onella Athnaiel, Santiago Cantillo, Stephania Paredes, and Nebojsa Nick Knezevic. The role of sex hormones in pain-related conditions. *International journal of molecular sciences*, 24(3):1866, 2023.
- [374] Yan Yang and Gerhard Litscher. Gender differences in manual, electro, and laser acupuncture, 2020.
- [375] Sandy McCombe Waller and Jill Whittall. Hand dominance and side of stroke affect rehabilitation in chronic stroke. *Clinical rehabilitation*, 19(5):544–551, 2005.
- [376] Christer PO Carlsson and Bengt H Sjölund. Acupuncture for chronic low back pain: a randomized placebo-controlled study with long-term follow-up. *The Clinical journal of pain*, 17(4):296–305, 2001.
- [377] Zahra Yazdanpanahi, Mehrnosh Ghaemmaghami, Marzieh Akbarzadeh, Najaf Zare, and Amir Azisi. Comparison of the effects of dry cupping and acupressure at acupuncture point (bl23) on the women with postpartum low back pain (plbp) based on short form mcgill pain questionnaires in iran: a randomized controlled trial. *Journal of family & reproductive health*, 11(2):82, 2017.
- [378] Robin ST Ho, Fai Fai Ho, Jon Adams, Holger Cramer, Brenda Leung, Lesley Ward, Yan Zhang, and Vincent CH Chung. Patients' perceptions on non-specific effects of acupuncture: Qualitative comparison between responders and non-responders. *Integrative medicine research*, 11(1):100771, 2022.
- [379] Cindy L Grines, Eric J Topol, William W O'Neill, Barry S George, Dean Kereiakes, Harry R Phillips, Jeffrey D Leimberger, Lynn H Woodlief, and Robert M Califf. Effect of cigarette smoking on outcome after thrombolytic therapy for myocardial infarction. *Circulation*, 91(2):298–303, 1995.
- [380] Fumi Irie, Ryu Matsuo, Satomi Mezuki, Yoshinobu Wakisaka, Masahiro Kamouchi, Takanari Kitazono, and Tetsuro Ago. Effect of smoking status on clinical outcomes after reperfusion therapy for acute ischemic stroke. *Scientific reports*, 14(1):9290, 2024.
- [381] Tian Song, Yuesong Pan, Ruoling Chen, Hao Li, Xingquan Zhao, Liping Liu, Chunxue Wang, Yilong Wang, and Yongjun Wang. Is there a correlation between socioeconomic disparity and functional outcome after acute ischemic stroke? *PloS one*, 12(7):e0181196, 2017.

Chapter 12

Appendices

12.1 Appendix A: FC Processing Steps and QC

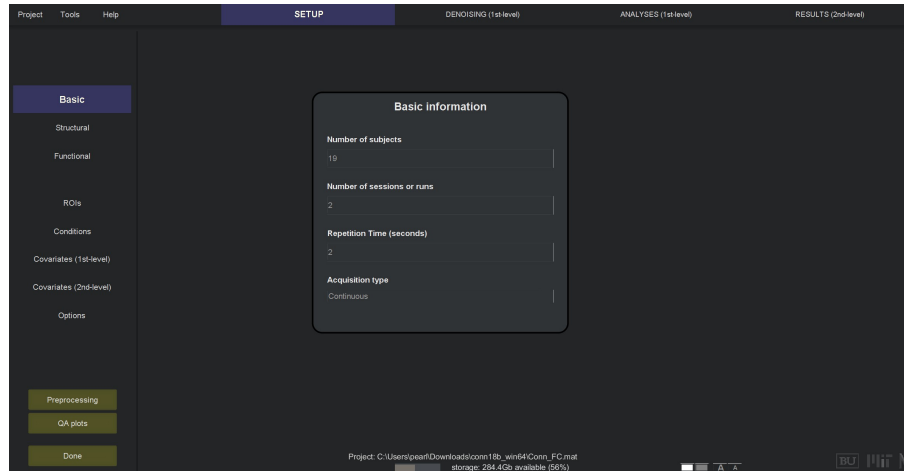


Figure 33: Basic information for set-up in the CONN Toolbox.

12.1.1 AAL116 Atlas

Table 25: **AAL116 brain regions and their coordinates.** This table lists the 116 brain regions defined by the Automated Anatomical Labeling (AAL116) atlas, used for structural and functional connectivity analysis. Columns include: (1) **Region ID**, the abbreviated identifier for the brain region; (2) **Region Name**, the full anatomical name; (3) **X**, (4) **Y**, and (5) **Z**, the MNI coordinates of the region's center of mass. Data are ordered as per the AAL116 atlas specification and are used for mapping structural and functional connectivity measures, such as Fractional Anisotropy (FA), in neuroimaging studies.

Region ID	Region Name	X	Y	Z
Precentral_L	Precentral gyrus (Left)	-40	-6	51
Precentral_R	Precentral gyrus (Right)	40	-8	52
Frontal_Sup_L	Superior frontal gyrus, dorsolateral (Left)	-19	35	42
Frontal_Sup_R	Superior frontal gyrus, dorsolateral (Right)	20	31	44
Frontal_Sup_Orb_L	Superior frontal gyrus, orbital part (Left)	-18	47	-13
Frontal_Sup_Orb_R	Superior frontal gyrus, orbital part (Right)	17	48	-14
Frontal_Mid_L	Middle frontal gyrus (Left)	-34	33	35
Frontal_Mid_R	Middle frontal gyrus (Right)	37	33	34
Frontal_Mid_Orb_L	Middle frontal gyrus orbital part (Left)	-32	50	-10
Frontal_Mid_Orb_R	Middle frontal gyrus orbital part (Right)	32	53	-11
Frontal_Inf_Oper_L	Inferior frontal gyrus, opercular part (Left)	-49	13	19
Frontal_Inf_Oper_R	Inferior frontal gyrus, opercular part (Right)	49	15	21
Frontal_Inf_Tri_L	Inferior frontal gyrus, triangular part (Left)	-47	30	14
Frontal_Inf_Tri_R	Inferior frontal gyrus, triangular part (Right)	49	30	14
Frontal_Inf_Orb_L	Inferior frontal gyrus, orbital part (Left)	-37	31	-12
Frontal_Inf_Orb_R	Inferior frontal gyrus, orbital part (Right)	40	32	-12
Rolandic_Oper_L	Rolandic operculum (Left)	-48	-8	14
Rolandic_Oper_R	Rolandic operculum (Right)	52	-6	15

Table 25: (Continued)

Region ID	Region Name	X	Y	Z
Supp_Motor_Area_L	Supplementary motor area (Left)	-6	5	61
Supp_Motor_Area_R	Supplementary motor area (Right)	8	0	62
Olfactory_L	Olfactory cortex (Left)	-9	15	-12
Olfactory_R	Olfactory cortex (Right)	8	16	-11
Frontal_Sup_Medial_L	Superior frontal gyrus, medial (Left)	-6	49	31
Frontal_Sup_Medial_R	Superior frontal gyrus, medial (Right)	8	51	30
Frontal_Med_Orb_L	Superior frontal gyrus, medial orbital (Left)	-6	54	-7
Frontal_Med_Orb_R	Superior frontal gyrus, medial orbital (Right)	7	52	-7
Rectus_L	Gyrus rectus (Left)	-6	37	-18
Rectus_R	Gyrus rectus (Right)	7	36	-18
Insula_L	Insula (Left)	-36	7	3
Insula_R	Insula (Right)	38	6	2
Cingulum_Ant_L	Anterior cingulate and paracingulate gyri (Left)	-5	35	14
Cingulum_Ant_R	Anterior cingulate and paracingulate gyri (Right)	7	37	16
Cingulum_Mid_L	Median cingulate and paracingulate gyri (Left)	-6	-15	42
Cingulum_Mid_R	Median cingulate and paracingulate gyri (Right)	7	-9	40
Cingulum_Post_L	Posterior cingulate gyrus (Left)	-6	-43	25
Cingulum_Post_R	Posterior cingulate gyrus (Right)	6	-42	22
Hippocampus_L	Hippocampus (Left)	-26	-21	-10
Hippocampus_R	Hippocampus (Right)	28	-20	-10
ParaHippocampal_L	Parahippocampal gyrus (Left)	-22	-16	-21
ParaHippocampal_R	Parahippocampal gyrus (Right)	24	-15	-20
Amygdala_L	Amygdala (Left)	-24	-1	-17
Amygdala_R	Amygdala (Right)	26	1	-18
Calcarine_L	Calcarine fissure and surrounding cortex (Left)	-8	-79	6
Calcarine_R	Calcarine fissure and surrounding cortex (Right)	15	-73	9
Cuneus_L	Cuneus (Left)	-7	-80	27
Cuneus_R	Cuneus (Right)	13	-79	28
Lingual_L	Lingual gyrus (Left)	-16	-68	-5
Lingual_R	Lingual gyrus (Right)	15	-67	-4
Occipital_Sup_L	Superior occipital gyrus (Left)	-18	-84	28
Occipital_Sup_R	Superior occipital gyrus (Right)	23	-81	31
Occipital_Mid_L	Middle occipital gyrus (Left)	-33	-81	16
Occipital_Mid_R	Middle occipital gyrus (Right)	36	-80	19
Occipital_Inf_L	Inferior occipital gyrus (Left)	-37	-78	-8
Occipital_Inf_R	Inferior occipital gyrus (Right)	37	-82	-8
Fusiform_L	Fusiform gyrus (Left)	-32	-40	-20
Fusiform_R	Fusiform gyrus (Right)	33	-39	-20
Postcentral_L	Postcentral gyrus (Left)	-43	-23	49
Postcentral_R	Postcentral gyrus (Right)	40	-25	53
Parietal_Sup_L	Superior parietal gyrus (Left)	-24	-60	59
Parietal_Sup_R	Superior parietal gyrus (Right)	25	-59	62
Parietal_Inf_L	Inferior parietal, but supramarginal and angular gyri (Left)	-44	-46	47
Parietal_Inf_R	Inferior parietal, but supramarginal and angular gyri (Right)	45	-46	50
SupraMarginal_L	Supramarginal gyrus (Left)	-57	-34	30
SupraMarginal_R	Supramarginal gyrus (Right)	57	-32	34
Angular_L	Angular gyrus (Left)	-45	-61	36
Angular_R	Angular gyrus (Right)	45	-60	39
Precuneus_L	Precuneus (Left)	-8	-56	48
Precuneus_R	Precuneus (Right)	9	-56	44
Paracentral_Lobule_L	Paracentral lobule (Left)	-9	-25	70
Paracentral_Lobule_R	Paracentral lobule (Right)	6	-32	68

Table 25: (Continued)

Region ID	Region Name	X	Y	Z
Caudate_L	Caudate nucleus (Left)	-12	11	9
Caudate_R	Caudate nucleus (Right)	14	12	9
Putamen_L	Lenticular nucleus, putamen (Left)	-25	4	2
Putamen_R	Lenticular nucleus, putamen (Right)	27	5	2
Pallidum_L	Lenticular nucleus, pallidum (Left)	-19	0	0
Pallidum_R	Lenticular nucleus, pallidum (Right)	20	0	0
Thalamus_L	Thalamus (Left)	-12	-18	8
Thalamus_R	Thalamus (Right)	12	-18	8
Heschl_L	Heschl gyrus (Left)	-43	-19	10
Heschl_R	Heschl gyrus (Right)	45	-17	10
Temporal_Sup_L	Superior temporal gyrus (Left)	-54	-21	7
Temporal_Sup_R	Superior temporal gyrus (Right)	57	-22	7
Temporal_Pole_Sup_L	Temporal pole: superior temporal gyrus (Left)	-41	15	-20
Temporal_Pole_Sup_R	Temporal pole: superior temporal gyrus (Right)	47	15	-17
Temporal_Mid_L	Middle temporal gyrus (Left)	-57	-34	-2
Temporal_Mid_R	Middle temporal gyrus (Right)	56	-37	-1
Temporal_Pole_Mid_L	Temporal pole: middle temporal gyrus (Left)	-37	15	-34
Temporal_Pole_Mid_R	Temporal pole: middle temporal gyrus (Right)	43	15	-32
Temporal_Inf_L	Inferior temporal gyrus (Left)	-51	-28	-23
Temporal_Inf_R	Inferior temporal gyrus (Right)	53	-31	-22
Cerebelum_Crus1_L	Cerebelum_Crus1_L	-36	-67	-29
Cerebelum_Crus1_R	Cerebelum_Crus1_R	37	-67	-30
Cerebelum_Crus2_L	Cerebelum_Crus2_L	-29	-73	-38
Cerebelum_Crus2_R	Cerebelum_Crus2_R	32	-69	-40
Cerebelum_3_L	Cerebelum_3_L	-9	-37	-19
Cerebelum_3_R	Cerebelum_3_R	12	-34	-19
Cerebelum_4_5_L	Cerebelum_4_5_L	-15	-43	-17
Cerebelum_4_5_R	Cerebelum_4_5_R	17	-43	-18
Cerebelum_6_L	Cerebelum_6_L	-23	-59	-22
Cerebelum_6_R	Cerebelum_6_R	25	-58	-24
Cerebelum_7b_L	Cerebelum_7b_L	-33	-60	-43
Cerebelum_7b_R	Cerebelum_7b_R	35	-64	-47
Cerebelum_8_L	Cerebellum_8_L	-26	-55	-48
Cerebelum_8_R	Cerebellum_8_R	25	-56	-49
Cerebelum_9_L	Cerebellum_9_L	-11	-49	-46
Cerebelum_9_R	Cerebellum_9_R	9	-49	-46
Cerebelum_10_L	Cerebellum_10_L	-23	-34	-42
Cerebelum_10_R	Cerebellum_10_R	26	-34	-41
Vermis_1_2	Vermis_1_2	1	-39	-20
Vermis_3	Vermis_3	1	-40	-11
Vermis_4_5	Vermis_4_5	1	-52	-6
Vermis_6	Vermis_6	1	-67	-15
Vermis_7	Vermis_7	1	-72	-25
Vermis_8	Vermis_8	1	-64	-34
Vermis_9	Vermis_9	1	-55	-35
Vermis_10	Vermis_10	0	-46	-32

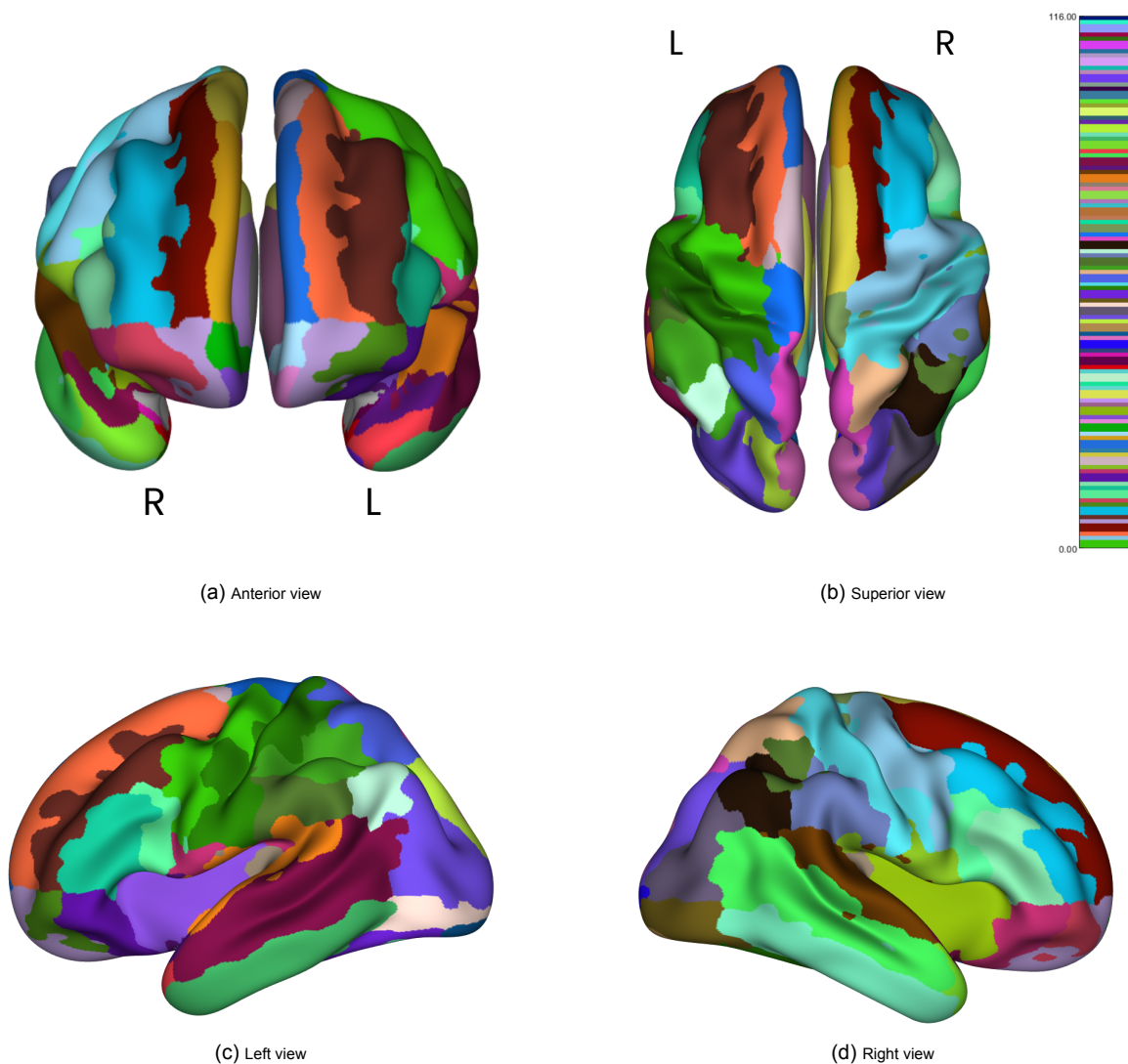


Figure 34: **3D surface projections of the AAL116 atlas in CONN toolbox using the random activation colormap.** (A) Anterior, (B) Superior, (C) Left, and (D) Right views showing the spatial distribution of 116 brain regions. The color bar indicates region-specific colors, with names listed in Appendix A (Table 22) in the same order as the table and color scheme (starting from region 1). "L" indicates the left hemisphere, and "R" indicates the right hemisphere.

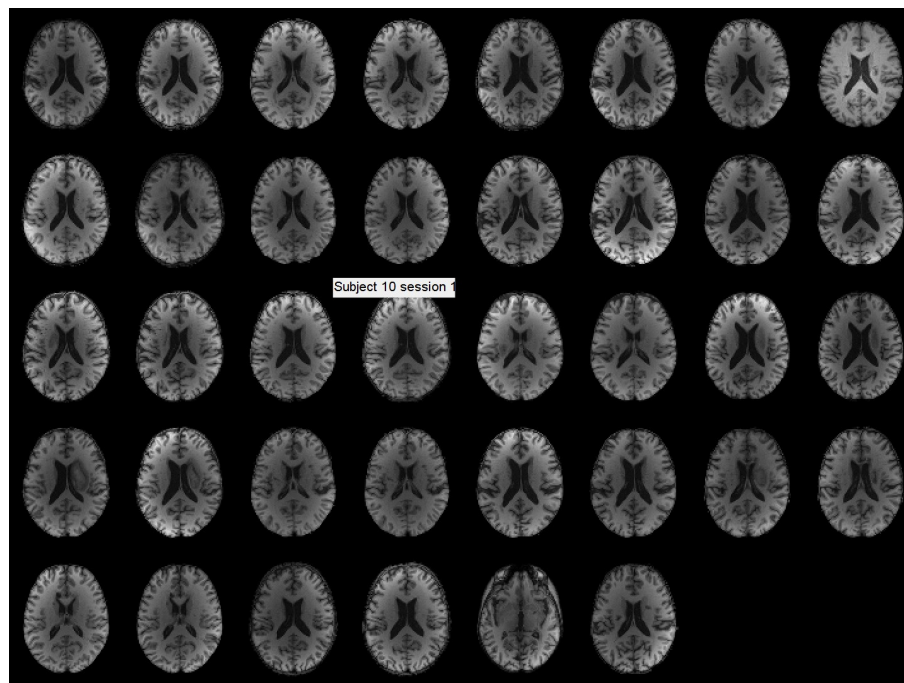


Figure 35: **Montage of structural scans (T1) for all 19 subjects before and after treatment**

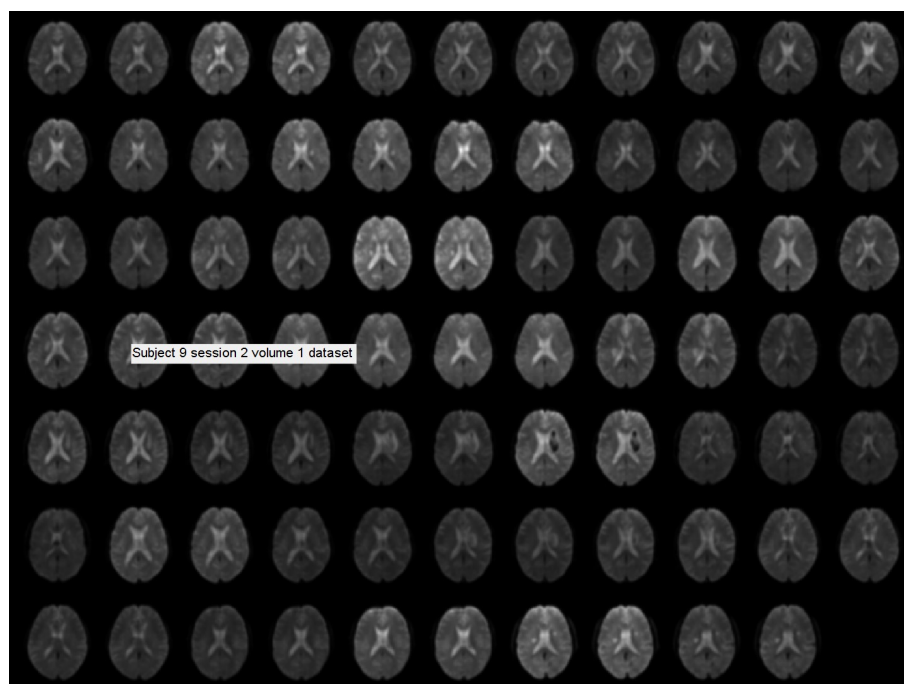


Figure 36: **Montage of rs-fMRI scans for all 19 subjects before and after treatment**

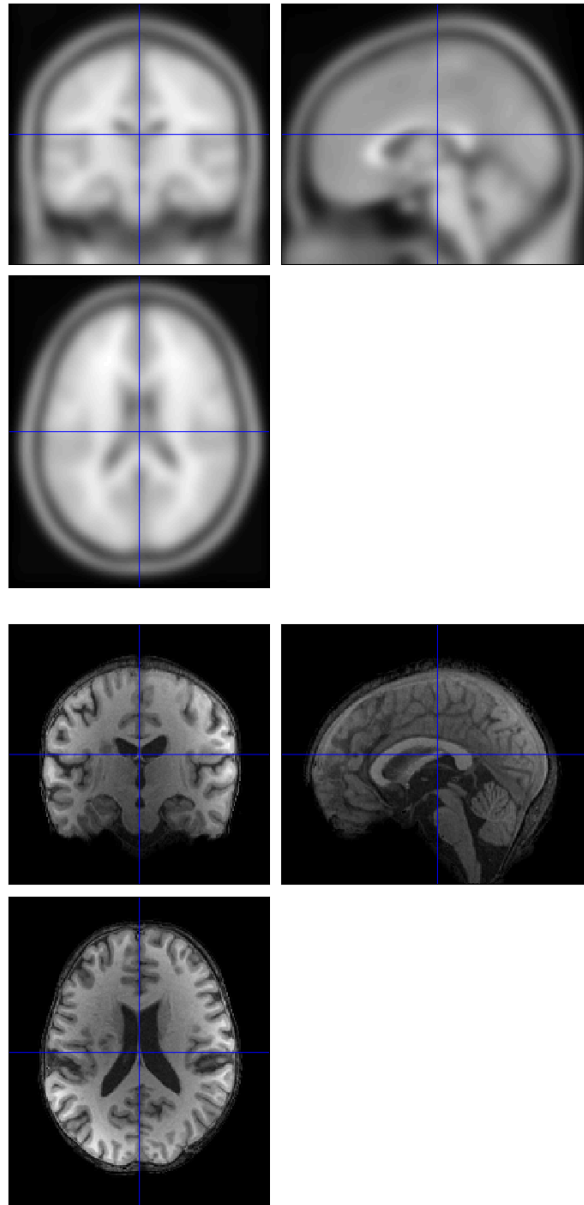


Figure 37: Inspection of T1 registration to MNI space.

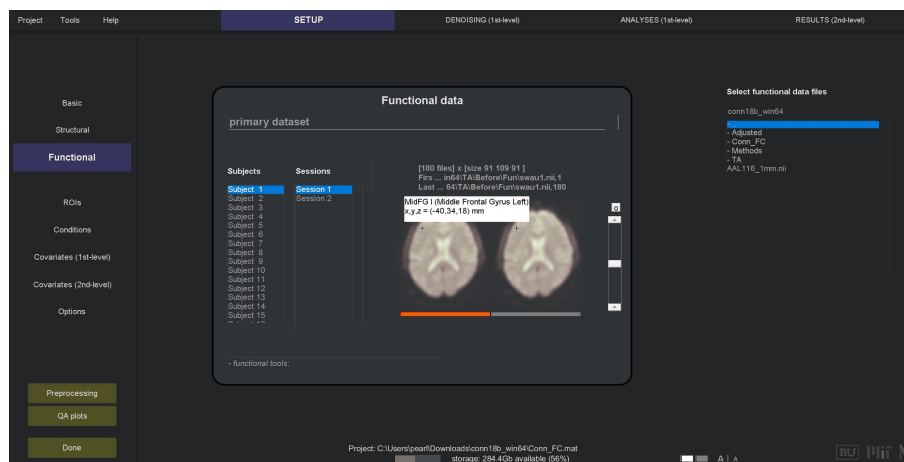


Figure 38: Making sure the rs-fMRI data loaded correctly.

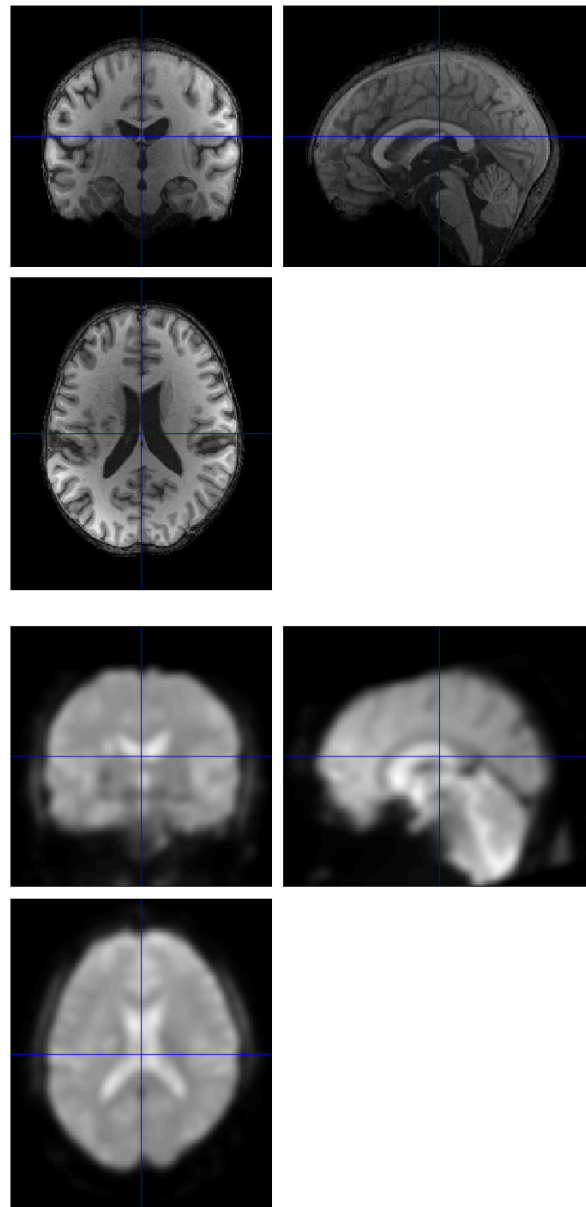


Figure 39: Inspection of rs-fMRI to T1 space

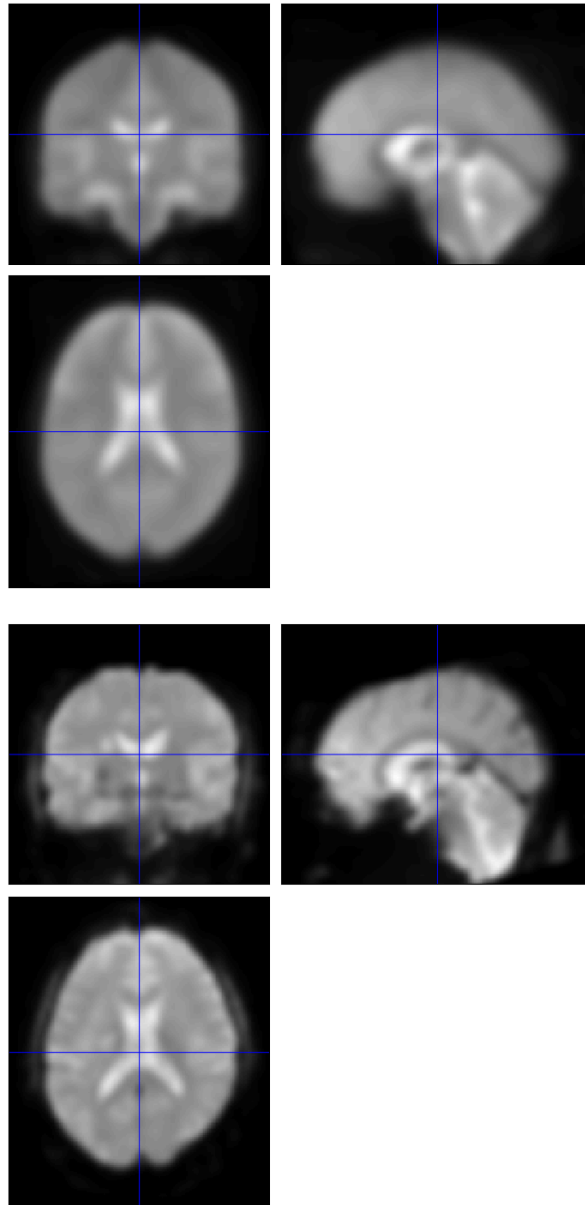


Figure 40: Inspection of rs-fMRI to MNI space

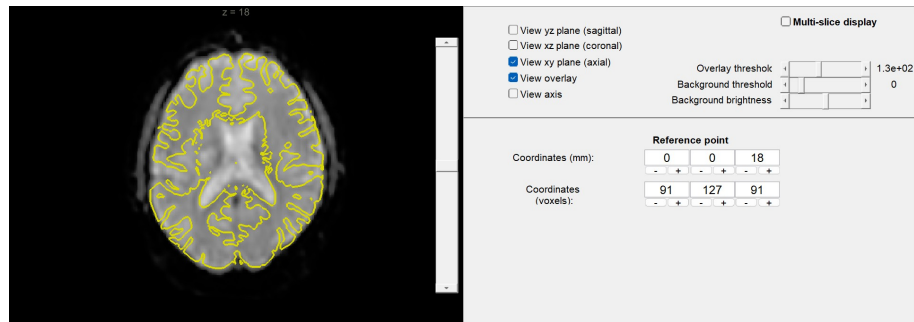


Figure 41: Inspection of anatomical overlay

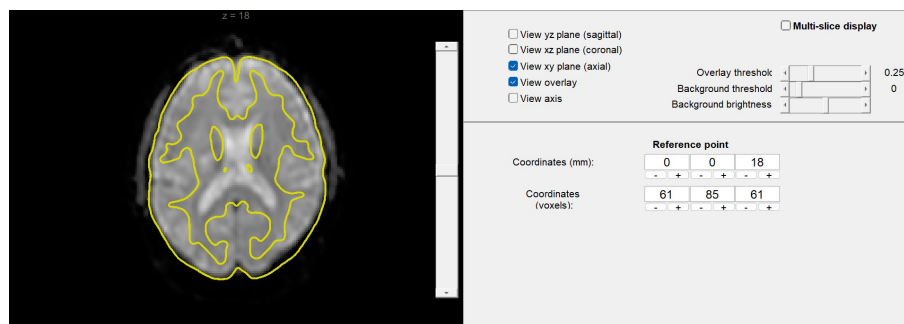


Figure 42: Inspection of MNI overlay

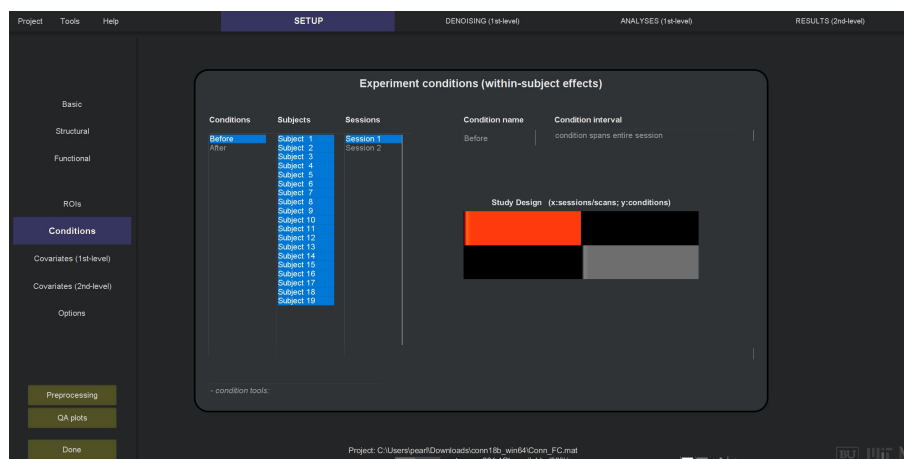


Figure 43: CONN toolbox set-up for condition 1 (MRI data before treatment intervention).

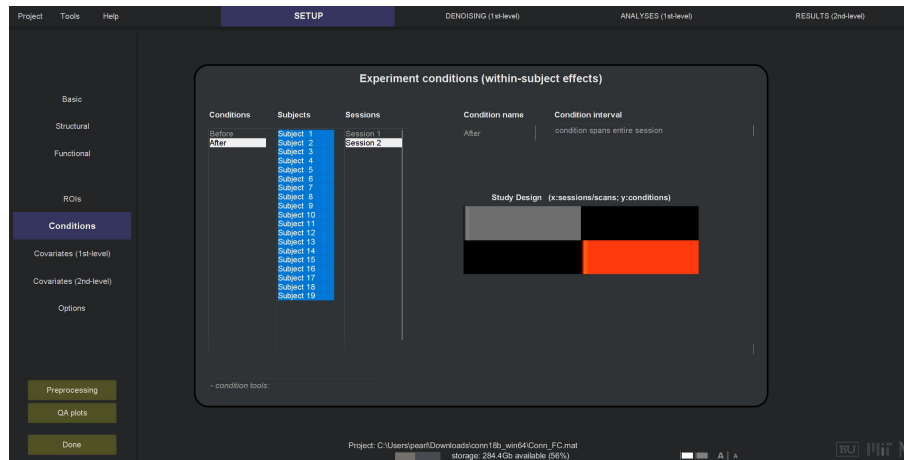


Figure 44: CONN toolbox set-up for condition 2 (MRI data after treatment intervention).

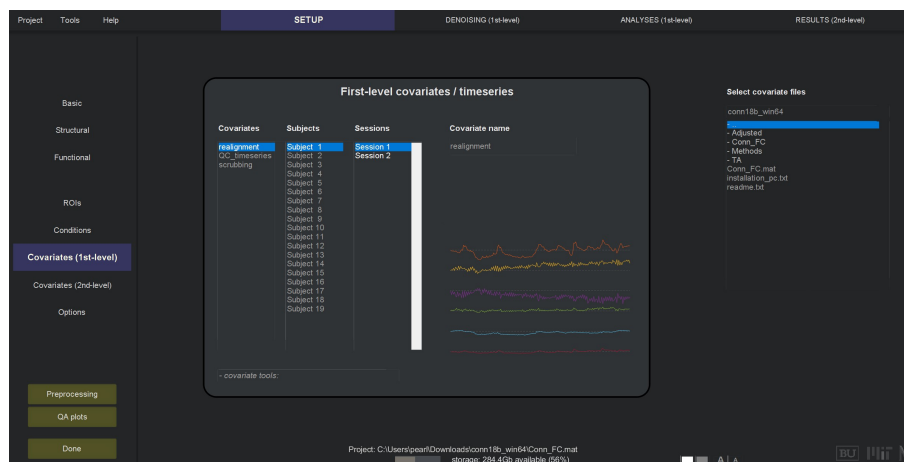


Figure 45: First-level covariates

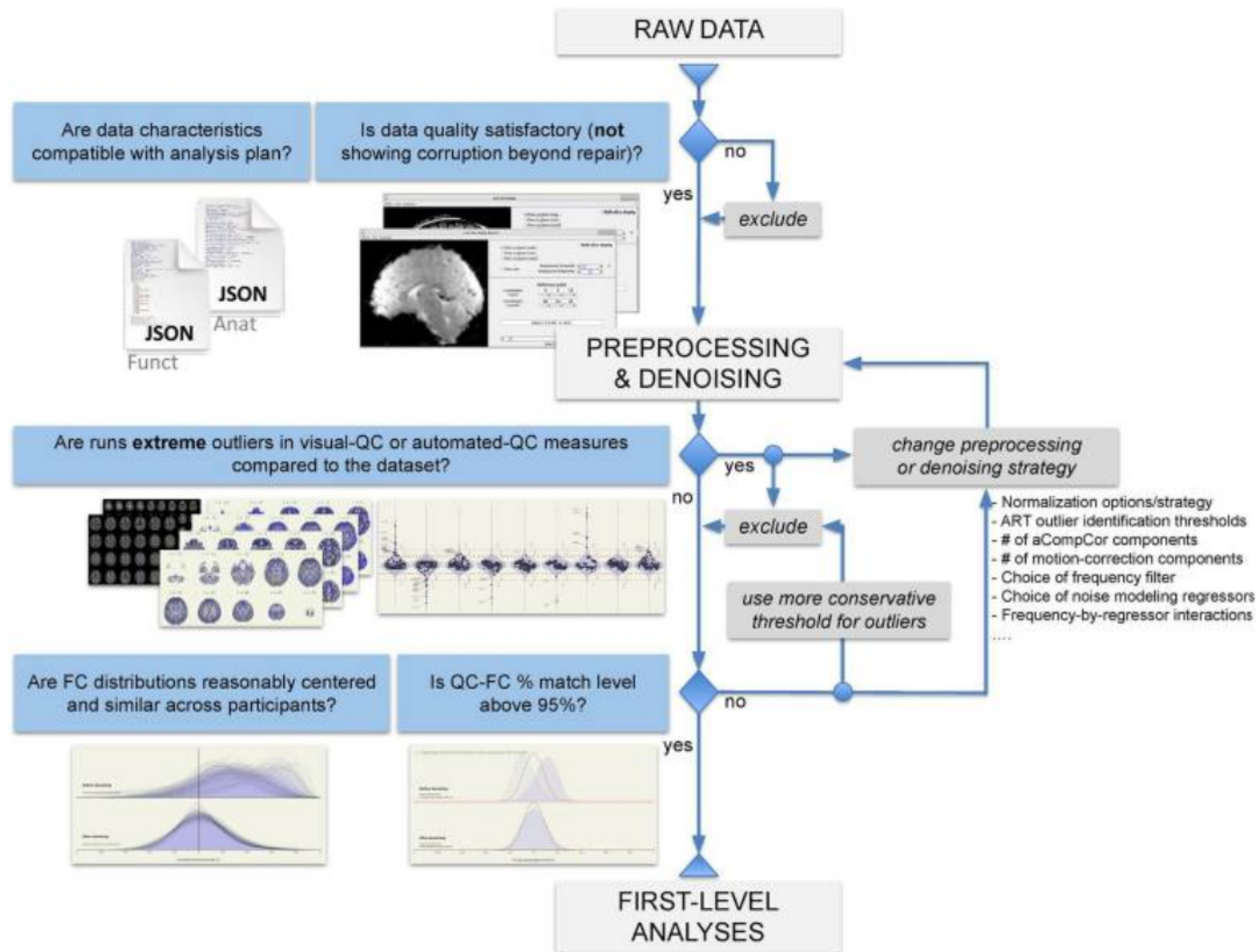


Figure 46: Quality Control (QC) pipeline used in the CONN toolbox. [218]

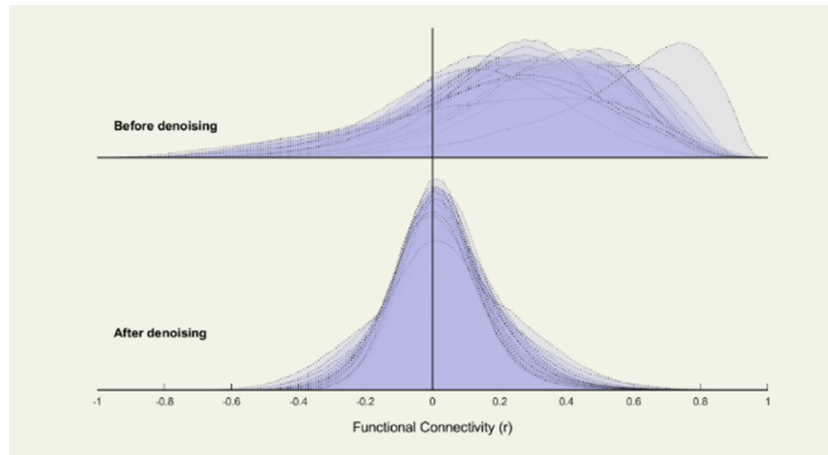


Figure 47: **Quality assurance plot of functional connectivity distribution across sessions.** This figure displays the distribution of functional connectivity values (r) for all subjects in Session 1 (before) and Session 2 (after), across all treatment groups. The plot compares the distribution before and after denoising. Post-denoising, the functional connectivity values exhibit a closer alignment to a Gaussian distribution, indicating improved data quality.

Table 26: **Hypotheses and statistical tests for between-subject effects in functional connectivity analysis using the CONN toolbox.** The analysis evaluates differences across conditions (before vs. after) for each treatment group: TA (n=5), TA+PT (n=6), SA+PT (n=8). Each hypothesis is represented by contrast vectors, where 1s indicate the variables of interest being tested, 0s denote covariates adjusted for (e.g., age, motion), and negative signs (e.g., -1) specify the direction of the comparison.

Analysis Type	Subject Effects [Between-Subjects Contrast]	Conditions [Between-Conditions Contrasts]
<i>Hypothesis: Differences in the TA group after compared to before treatment</i>		
ANCOVA covariate control	TA, Age, Motion [1 0 0]	Pre, Post [-1 1]
<i>Hypothesis: Differences in the TA+PT group after compared to before treatment</i>		
ANCOVA covariate control	TA+PT, Age, Motion [1 0 0]	Pre, Post [-1 1]
<i>Hypothesis: Differences in the SA+PT group after compared to before treatment</i>		
ANCOVA covariate control	SA+PT, Age, Motion [1 0 0]	Pre, Post [-1 1]

12.2 Appendix B: Scripts for Processing DWI Data.

12.2.1 dcm2nii Command: Converting DICOM Images to NifTI Images.

```
#!/bin/bash

# --- Get current username ---
user=`whoami`

# --- Define base directory containing subject data ---
base=/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/SA+PT/BEFORE

# --- Navigate to base directory ---
cd $base

# List directories ending with '-1' (assumed subject/session IDs)
input_dir=`ls -1 -d *-1`

# --- Loop through each subject/session directory ---
for dir_index in $input_dir
do
    # Define the output directory for converted NIfTI files
    output_dir=$base/*_nifti/dcm2niix

    # Convert DICOM to NIfTI using dcm2niix
    # -o : output directory
    # Last argument : input DICOM directory
    dcm2niix -o $output_dir $base/$input_dir
done
```

12.2.2 Motion Parameter Extraction from DWI Data.

```
# Run motion correction on the input data and generate transformation matrices, plots,
and RMS values
mcflirt -in input_data.nii.gz -out output_data -mats -plots -rmsrel -rmsabs -report

awk 'BEGIN {
    max = 0    # Initialize maximum displacement to zero
}
{
    # Compute Euclidean norm (displacement) of first six fields
    displacement = sqrt($1^2 + $2^2 + $3^2 + $4^2 + $5^2 + $6^2)
    # Update max if current displacement is larger
    if(displacement > max) max = displacement
} END {
    # Print the maximum displacement found
    print max
}' dwi_1.par
```

12.2.3 Motion Estimates in DWI Data

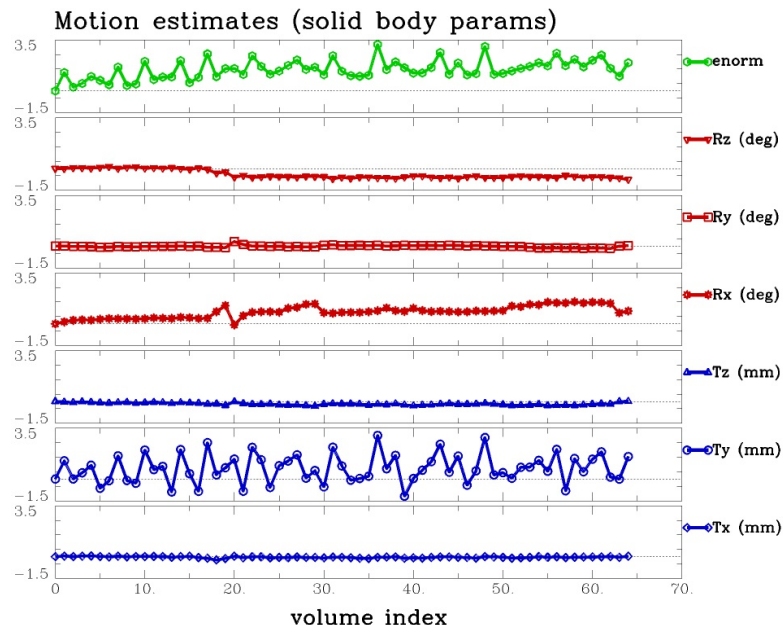


Figure 48: Motion estimates in subject 5 of the TA group post-treatment.

12.2.4 Linux Code to Import Data to TORTOISE.

```
#!/bin/bash

# --- Print status message ---
echo "VARIABLES...."

# Get current username
user=`whoami`

# --- Define paths ---
# Path to TORTOISE ImportNIFTI executable
base_import=/usr/local/TORTOISE_V3.1.4/DIFFPREPV314/bin/bin
# Base directory containing the subject data
base=/home/pearly/researchdata_mri/UserFolders/Pearly/TRUE_ACU/BEFORE

# --- Navigate to base directory ---
cd $base

# List directories ending with '27-1' (assumed to be subject/session IDs)
input_dir=`ls -1 -d *27-1`

# --- Loop through each subject/session directory ---
for dir_index in $input_dir
do
    # Path to the anatomical (ANATOM) folder inside the subject directory
    base_diff=$base/$dir_index/dcm2niix/ANATOM

    # Print status update
    echo "Importing TA BEFORE group data to TORTOISE"

    # Run TORTOISE ImportNIFTI to import NifTI + bval/bvec files
    # -i : input NifTI file
    # -b : b-values
    # -v : b-vectors
```

```
# -p : phase encoding direction (here, 'vertical')
$base_import/./ImportNIFTI -i $base_diff/*_8.nii -b $base_diff/*.bval -v
    $base_diff/*.bvec -p vertical
done
```

12.2.5 Script to Run Auto ACPC Detect.

```
#!/bin/bash

# --- Print status message ---
echo "VARIABLES...."

# Get current username
user=`whoami`

# --- Set environment variable for ARTHOME ---
export ARTHOME=/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/art

# --- Define base directories ---
# Path to subject data
base=/home/pearly/researchdata_mri/UserFolders/Pearly/TRUE_ACU/AFTER
# Path to TORTOISE ACPC detect binary
acpc=/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/bin

# --- Navigate to base directory ---
cd $base

# List directories ending with '2' (assumed subject/session IDs)
input_dir=`ls -1 -d *2`

# --- Loop through each subject/session directory ---
for dir_index in $input_dir
do
    # Path to anatomical (ANATOM) folder inside the subject directory
    base_detect=$base/$dir_index/dcm2niix/ANATOM

    # Run ACPC detect on the T1-weighted NIfTI image
    $acpc/./acpcdetect -i $base_detect/T1.nii
done
```

12.2.6 DIFF_PREP Command in TORTOISE Software.

```
#!/bin/bash

# Get current username
user=`whoami`

# --- Define base paths ---
base_import=/usr/local/TORTOISE_V3.1.4/DIFFPREPV314/bin/bin
base_diffprep=/usr/local/TORTOISE_V3.1.4/DIFFPREPV314/bin/bin
base=/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/SA+PT/BEFORE
base_drBUDDI=/usr/local/TORTOISE_V3.1.4/DRBUDDIV314/bin
base_diff_calc=/usr/local/TORTOISE_V3.1.4/DIFFCALC/DIFFCALCV314

# --- Navigate to base directory ---
cd $base

# List directories ending with 'nifti2'
input_dir=`ls -1 -d *nifti2`
```

```
# --- Loop through each subject/session directory ---
for dir_index in $(ls $input_dir)
do
    # Path to anatomical folder
    base_diff=$base/$dir_index/dcm2niix/ANATOM

    # Print processing step
    echo " ##### DIFFPREP for PA direction #####"

    # Run DIFFPREP
    # -i : input list file
    # -s : secondary image (T2-weighted)
    # -r : reference image (T1 in RAS orientation)
    $base_diffprep/./DIFFPREP -i $base_diff/*_proc/*.list -s $base_diff/T2.nii -r
    $base_diff/T1_RAS.nii
done
```

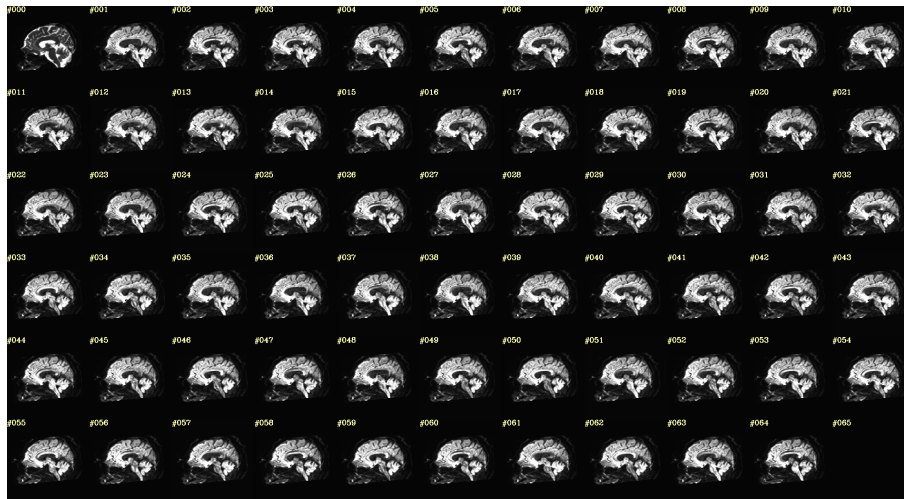


Figure49: Visualization of the diffusion data after basic preprocessing to spot intensity outliers, signal dropouts, motion artifacts, or slices with unusually high/low intensity.

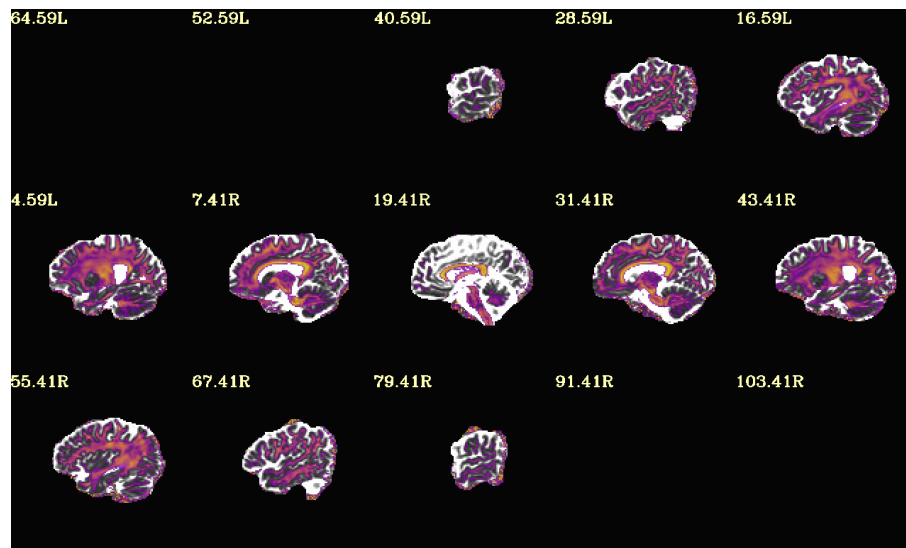


Figure 50: QC overlay of FA (fractional anisotropy) and MD (mean diffusivity) maps after running `fat_proc_dwi_to_dt` command to check that tensor-derived maps look anatomically plausible.

12.2.7 GradFlipTest and fat_proc_dwi_to_dt Command to Calculate the Diffusion Tensor Parameters.

```
#!/bin/bash

# Define input file paths for DWI data and TORTOISE matrix
IN="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/MRKY128-1
_nifti2/dcm2niix/ANATOM/MRKY128-1_ep2d_diff_mddw64_2mm_20230306130851_6_proc/
LPI_DMC.nii"
IN_TORT="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/
MRKY128-1_nifti2/dcm2niix/ANATOM/MRKY128-1
_ep2d_diff_mddw64_2mm_20230306130851_6_proc/LPI_DMC.bmtxt"
# Define output file path for gradient flip test results
OUT="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/MRKY128-1
_nifti2/dcm2niix/ANATOM/MRKY128-1_ep2d_diff_mddw64_2mm_20230306130851_6_proc/
Gradflip.txt"

# Run gradient flip test to check for gradient orientation issues
@GradFlipTest \
-in_dwi $IN \
-in_col_matT $IN_TORT \
-prefix $OUT

# Print message to indicate start of fat processing command
echo "-----starting with fat proc command-----"
# Read gradient flip results into variable
set my_flip = `cat $OUT`

# Define input file paths for fat processing
IN_fat="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/MRKY128
-1_nifti2/dcm2niix/ANATOM/MRKY128-1_ep2d_diff_mddw64_2mm_20230306130851_6_proc/
LPI_DMC.nii"
IN_TORT_fat="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/
MRKY128-1_nifti2/dcm2niix/ANATOM/MRKY128-1
_ep2d_diff_mddw64_2mm_20230306130851_6_proc/LPI_DMC.bmtxt"

# Define structural and reference image paths
T1_struc="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/
MRKY128-1_nifti2/dcm2niix/ANATOM/MRKY128-1
_ep2d_diff_mddw64_2mm_20230306130851_6_proc/T1_aligned.nii"
T2_in="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/MRKY128
-1_nifti2/dcm2niix/ANATOM/MRKY128-1_ep2d_diff_mddw64_2mm_20230306130851_6_proc/
T2_bet.nii"

# Define output prefix for fat processing results
PREFIX="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE/MRKY128
-1_nifti2/dcm2niix/ANATOM/MRKY128-1_ep2d_diff_mddw64_2mm_20230306130851_6_proc/
fat_proc_"

# Commented out mask file path (not currently used)
#MASK="/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/SA+PT/AFTER/MRKY115-2
_nifti4/dcm2niix/ANATOM/MRKY115-2_ep2d_diff_mddw64_2mm_20220112123930_6_proc/
T1_bet_mask.nii"

# Run fat processing to compute diffusion tensor from DWI data
fat_proc_dwi_to_dt \
-in_dwi $IN_fat \
-in_col_matT $IN_TORT_fat \
-in_struc_res $T1_struc \
```

```
-in_ref_orig $T2_in \
-prefix $PREFIX \
-mask_from_struct \
$my_flip
```

12.2.8 Inverse Warping of AAL116 Atlas from MNI to eac Subject's Native Space.

```
# Step 1: Register AAL116 atlas (MNI space) to subject T1 space using FLIRT
flirt -in AAL116.nii -ref T1.nii -out AAL116_in_T1.nii \
      -cost mutualinfo -omat AAL116_to_T1.mat

# Step 2: Non-linear registration of T1 to MNI (or vice versa) using FNIRT
fnirt --in=T1.nii --ref=MNI152_T1_2mm.nii --refmask=MNI_mask.nii \
      --iout=T1_warped_to_MNI.nii --aff=AAL116_to_T1.mat --cout=T1_to_MNI_warpcoef.nii

# Step 3: Invert the warp field to map MNI space back to T1 space
invwarp -w T1_to_MNI_warpcoef.nii -o invwarp_MNI_to_T1.nii -r T1.nii

# Step 4: Register T1 to DTI space (e.g. FA image) with FLIRT
flirt -in T1.nii -ref DT_FA.nii -out T1_in_DTI.nii -omat T1_to_DTI.mat -cost
      mutualinfo

# Step 5: Invert affine transform from DTI to T1 space
convert_xfm -omat inv_T1_to_DTI.mat -inverse T1_to_DTI.mat

# Step 6: Apply inverse warp + inverse affine to warp AAL116 atlas from T1 space to
          DTI space
applywarp -i AAL116_in_T1.nii -o AAL116_in_DTI.nii -r DT_FA.nii \
          -w invwarp_MNI_to_T1.nii --premat=inv_T1_to_DTI.mat
```

12.2.9 3dTrackID to Determine the FA Values Between the 116 Brain Regions Specified using the AAL116 Atlas.

```
#!/bin/bash

# Print current user (commented out)
#echo "VARIABLES...."
user=`whoami`

# Define base directory for research data
base=/home/pearly/researchdata_mri/UserFolders/Pearly/TORTOISE/TA+PT/BEFORE

# Change to base directory
cd $base

# List directories ending in nifti2
input_dir=`ls -1 -d *nifti2`

# Loop through each nifti2 directory
for dir_index in $input_dir
do
    # Define paths for diffusion and atlas data
    base_diff=$base/$dir_index/dcm2niix/ANATOM
    base_atlas=$base/ATLAS

    # Run 3dTrackID for probabilistic tractography
    3dTrackID -mode PROB -dti_in $base_diff/dt_ -netrois $base_atlas/Atlas_warped_dt.
              nii -uncert $base_diff/UNC/dt UNC.nii -dump_rois AFNI -nifti -prefix track_out
done
```

12.2.10 Extracting FA Matrices from .grid Files (Output After Running 3dTrackID.)

```
# 1. Copy the entire content of play_around.grid to gridout.txt
awk '{print $0}' play_around.grid > gridout.txt

# 2. Extract lines between markers starting with "# FA" and "# sFA" (inclusive)
awk '/^# FA/,/^# sFA/' gridout.txt > extractedDataFA.txt

# 3. Format each field in extractedDataFA.txt to 15 decimal places and tab-separate
    fields
awk -v OFS='\t' '{
    for(i=1; i<=NF; i++)
        $i = sprintf("%.15f", $i) # format each field with 15 decimal places
}1' extractedDataFA.txt > extractedDataFAFormat.txt

# 4. Check that total number of entries equals 116 x 116 = 13456

# Count total number of fields in entire file
total_entries=$(awk '{sum += NF} END {print sum}' extractedDataFAFormat.txt)

echo "Total entries in matrix: $total_entries"

# Validate total entries
if [ "$total_entries" -eq 13456 ]; then
    echo "FA matrix has 116 x 116 = 13456 entries as expected."
else
    echo "FA matrix does NOT have 13456 entries! Check data."
fi
```

12.3 Appendix C: Scripts for Statistical Analysis.

12.3.1 Covariate Comparison for Structural Connectivity Data.

```

1 import pandas as pd
2 import numpy as np
3 from scipy import stats
4 import statsmodels.api as sm
5 from statsmodels.formula.api import ols
6
7 # Function to calculate SEM (Standard Error of the Mean)
8 def calculate_sem(data):
9     return stats.sem(data)
10
11 # Read the Excel file
12 df = pd.read_excel('SC_covariates.xlsx')
13
14 # Map group numbers to names for clarity
15 group_map = {1: 'TA', 2: 'TA+PT', 3: 'SA+PT'}
16 df['Group'] = df['Group'].map(group_map)
17
18 # Define variables to analyze
19 variables = ['Side', 'Age', 'Motion', 'BrainVolume']
20
21 # Initialize lists to store results
22 anova_results = []
23
24 # Perform ANOVA for each variable
25 for var in variables:
26     # Separate data by group
27     ta_data = df[df['Group'] == 'TA'][var].dropna()
28     tapt_data = df[df['Group'] == 'TA+PT'][var].dropna()
29     sapt_data = df[df['Group'] == 'SA+PT'][var].dropna()
30
31     # Perform ANOVA
32     f_statistic, p_value = stats.f_oneway(ta_data, tapt_data, sapt_data)
33
34     # Calculate Eta-squared
35     ss_total = np.sum((df[var].dropna() - df[var].dropna().mean())**2)
36     ss_between = ((ta_data.mean() - df[var].dropna().mean())**2 * len(ta_data) +
37                  (tapt_data.mean() - df[var].dropna().mean())**2 * len(tapt_data) +
38                  (sapt_data.mean() - df[var].dropna().mean())**2 * len(sapt_data))
39     eta_squared = ss_between / ss_total if ss_total > 0 else 0 # Avoid division by
40         zero
41
42     # Calculate descriptive statistics
43     ta_mean_sd = f"{ta_data.mean():.2f} ± {ta_data.std():.2f}"
44     tapt_mean_sd = f"{tapt_data.mean():.2f} ± {tapt_data.std():.2f}"
45     sapt_mean_sd = f"{sapt_data.mean():.2f} ± {sapt_data.std():.2f}"
46
47     ta_sem = calculate_sem(ta_data)
48     tapt_sem = calculate_sem(tapt_data)
49     sapt_sem = calculate_sem(sapt_data)
50
51     # Append results for this variable
52     anova_results.extend([
53         {'Variable': var, 'Statistic': 'F-statistic', 'Value': f_statistic},
54         {'Variable': var, 'Statistic': 'p-value', 'Value': p_value},
55         {'Variable': var, 'Statistic': 'Eta-squared', 'Value': eta_squared},
56         {'Variable': var, 'Statistic': 'TA Mean ± SD', 'Value': ta_mean_sd},

```

```

56         {'Variable': var, 'Statistic': 'TA+PT Mean ± SD', 'Value': tapt_mean_sd},
57         {'Variable': var, 'Statistic': 'SA+PT Mean ± SD', 'Value': sapt_mean_sd},
58         {'Variable': var, 'Statistic': 'TA SEM', 'Value': ta_sem},
59         {'Variable': var, 'Statistic': 'TA+PT SEM', 'Value': tapt_sem},
60         {'Variable': var, 'Statistic': 'SA+PT SEM', 'Value': sapt_sem}
61     ])
62
63     # Create results DataFrame
64     results_df = pd.DataFrame(anova_results)
65
66     # Pivot the table for better readability (optional)
67     results_pivot = results_df.pivot(index='Statistic', columns='Variable', values='Value'
68     )
69
70     # Save to Excel
71     results_df.to_excel('ANOVA_SC.xlsx', index=False)
72     # Optionally save the pivoted version as well
73     results_pivot.to_excel('ANOVA_SC_pivot.xlsx')
74
75     # Display results
76     print("ANOVA Results:")
77     print(results_df)
78     print("\nPivoted ANOVA Results:")
79     print(results_pivot)

```

12.3.2 Covariate Comparison for Functional Connectivity Data.

```

1  import pandas as pd
2  import numpy as np
3  from scipy import stats
4
5  # Function to calculate SEM (Standard Error of the Mean)
6  def calculate_sem(data):
7      return stats.sem(data)
8
9  # Read the Excel file
10 df = pd.read_excel('/content/FC_variables.xlsx')
11
12 # Map group numbers to names for clarity
13 group_map = {1: 'TA', 2: 'TA+PT', 3: 'SA+PT'}
14 df['Group'] = df['Group'].map(group_map)
15
16 # Separate Motion data by group
17 ta_motion = df[df['Group'] == 'TA']['Motion'].dropna()
18 tapt_motion = df[df['Group'] == 'TA+PT']['Motion'].dropna()
19 sapt_motion = df[df['Group'] == 'SA+PT']['Motion'].dropna()
20
21 # Perform ANOVA
22 f_statistic, p_value = stats.f_oneway(ta_motion, tapt_motion, sapt_motion)
23
24 # Calculate Eta-squared
25 ss_total = np.sum((df['Motion'].dropna() - df['Motion'].dropna().mean())**2)
26 ss_between = ((ta_motion.mean() - df['Motion'].dropna().mean())**2 * len(ta_motion) +
27               (tapt_motion.mean() - df['Motion'].dropna().mean())**2 * len(tapt_motion)
28               +
29               (sapt_motion.mean() - df['Motion'].dropna().mean())**2 * len(sapt_motion)
30               )
31 eta_squared = ss_between / ss_total if ss_total > 0 else 0 # Avoid division by zero

```

```

31 # Calculate descriptive statistics
32 ta_mean_sd = f"{ta_motion.mean():.2f} ± {ta_motion.std():.2f}"
33 tapt_mean_sd = f"{tapt_motion.mean():.2f} ± {tapt_motion.std():.2f}"
34 sapt_mean_sd = f"{sapt_motion.mean():.2f} ± {sapt_motion.std():.2f}"
35
36 ta_sem = calculate_sem(ta_motion)
37 tapt_sem = calculate_sem(tapt_motion)
38 sapt_sem = calculate_sem(sapt_motion)
39
40 # Create results DataFrame
41 anova_results = pd.DataFrame({
42     'Statistic': ['F-statistic', 'p-value', 'Eta-squared',
43                 'TA Mean ± SD', 'TA+PT Mean ± SD', 'SA+PT Mean ± SD',
44                 'TA SEM', 'TA+PT SEM', 'SA+PT SEM'],
45     'Value': [f_statistic, p_value, eta_squared,
46              ta_mean_sd, tapt_mean_sd, sapt_mean_sd,
47              ta_sem, tapt_sem, sapt_sem]
48 })
49
50 # Save to Excel
51 anova_results.to_excel('ANOVA_FC.xlsx', index=False)
52
53 # Display results
54 print("ANOVA Results for Motion:")
55 print(anova_results)

```

12.3.3 Infarct Side

Table 27: **Side of ischemic infarct.** This table presents data for subjects across three groups: TA (n=5), TA+PT (n=6), SA+PT (n=8), including group assignment, subject ID, hemispheric infarct side, and a variable value. The groups represent different treatment or assessment conditions, with the number of subjects indicated (e.g., TA, n=5). The hemispheric infarct side indicates the affected brain hemisphere, and the variable column represents a coded value (0 = Unknown, 1 = Right, 2 = Left).

Group	Subjects	Infarct side	Variable
TA (n=5)	1	Left	2
	2	Left	2
	3	Unknown	0
	4	Right	1
	5	Right	1
TA+PT (n=6)	6	Left	2
	7	Left	2
	8	Left	2
	9	Left	2
	10	Right	1
	11	Left	2
SA+PT (n=8)	12	Right	1
	13	Right	1
	14	Left	2
	15	Left	2
	16	Right	1
	17	Right	1
	18	Right	1
	19	Unknown	0

12.3.4 Fisher's Exact Test to Compare the Distribution of a Binary Variable ("Side") Across the Three Treatment Groups.

```

1 import pandas as pd
2 import scipy.stats as stats
3 import numpy as np
4 from openpyxl import Workbook
5 from openpyxl.utils.dataframe import dataframe_to_rows
6
7 # Load the Excel file
8 df = pd.read_excel('SC_covariates.xlsx')
9
10 # Filter out rows where Side == 0
11 df = df[df['Side'] != 0]
12
13 # Map Side values: 1=Right, 2=Left
14 df['Side'] = df['Side'].map({1: 'Right', 2: 'Left'})
15
16 # Function to create contingency table and perform Fisher's Exact Test
17 def fisher_exact_test(group1, group2, group1_name, group2_name):
18     # Filter data for the two groups
19     df_filtered = df[df['Group'].isin([group1, group2])]
20
21     # Create contingency table
22     contingency_table = pd.crosstab(
23         df_filtered['Group'],
24         df_filtered['Side'],
25         values=[group1_name, group2_name],
26         index=[group1, group2],
27         columns=[group1_name, group2_name])
28
29     # Ensure table has both Left and Right columns

```



```

28     if 'Left' not in contingency_table.columns:
29         contingency_table['Left'] = 0
30     if 'Right' not in contingency_table.columns:
31         contingency_table['Right'] = 0
32
33     # Reorder columns
34     contingency_table = contingency_table[['Left', 'Right']]
35
36     # Perform Fisher's Exact Test
37     odds_ratio, p_value = stats.fisher_exact(contingency_table)
38
39     # Calculate 95% confidence interval for odds ratio
40     # Using the formula:  $\exp(\log(OR) \pm 1.96 * \sqrt{1/a + 1/b + 1/c + 1/d})$ 
41     a = contingency_table.iloc[0, 0] # Group1, Left
42     b = contingency_table.iloc[0, 1] # Group1, Right
43     c = contingency_table.iloc[1, 0] # Group2, Left
44     d = contingency_table.iloc[1, 1] # Group2, Right
45
46     # Avoid division by zero
47     if any(x == 0 for x in [a, b, c, d]):
48         ci_lower, ci_upper = np.nan, np.nan
49     else:
50         se_log_or = np.sqrt(1/a + 1/b + 1/c + 1/d)
51         log_or = np.log(odds_ratio)
52         ci_lower = np.exp(log_or - 1.96 * se_log_or)
53         ci_upper = np.exp(log_or + 1.96 * se_log_or)
54
55     return contingency_table, odds_ratio, p_value, ci_lower, ci_upper
56
57 # Define group mappings
58 group_map = {1: 'TA', 2: 'TA+PT', 3: 'SA+PT'}
59
60 # Perform tests for the three comparisons
61 comparisons = [
62     (1, 2, 'TA', 'TA+PT'), # Table 1: TA vs TA+PT
63     (1, 3, 'TA', 'SA+PT'), # Table 2: TA vs SA+PT
64     (2, 3, 'TA+PT', 'SA+PT') # Table 3: TA+PT vs SA+PT
65 ]
66
67 results = []
68 for group1, group2, name1, name2 in comparisons:
69     table, odds_ratio, p_value, ci_lower, ci_upper = fisher_exact_test(group1, group2,
70     name1, name2)
71     results.append({
72         'Comparison': f'{name1} vs {name2}',
73         'Contingency Table': table,
74         'Odds Ratio': odds_ratio,
75         'P-value': p_value,
76         'CI Lower': ci_lower,
77         'CI Upper': ci_upper
78     })
79
80 # Create descriptive statistics and save to Excel
81 wb = Workbook()
82 ws = wb.active
83 ws.title = 'Fisher Exact Test Results'
84
85 # Write results
86 row_idx = 1

```

```

86 for result in results:
87     # Write comparison title
88     ws.cell(row=row_idx, column=1, value=result['Comparison'])
89     row_idx += 1
90
91     # Write contingency table
92     table_df = result['Contingency Table']
93     for r in dataframe_to_rows(table_df, index=True, header=True):
94         ws.append(r)
95     row_idx += len(table_df) + 2
96
97     # Write statistics
98     ws.cell(row=row_idx, column=1, value='Odds Ratio')
99     ws.cell(row=row_idx, column=2, value=result['Odds Ratio'])
100    row_idx += 1
101    ws.cell(row=row_idx, column=1, value='P-value')
102    ws.cell(row=row_idx, column=2, value=result['P-value'])
103    row_idx += 1
104    ws.cell(row=row_idx, column=1, value='95% CI Lower')
105    ws.cell(row=row_idx, column=2, value=result['CI Lower'])
106    row_idx += 1
107    ws.cell(row=row_idx, column=1, value='95% CI Upper')
108    ws.cell(row=row_idx, column=2, value=result['CI Upper'])
109    row_idx += 2
110
111 # Save the workbook
112 wb.save('FisherExact.xlsx')

```

12.3.5 Matlab Code to Convert Fisher Z-values to r-values in the 116 by 116 Functional Connectivity Matrices

```

1 % Specify the folder containing .mat files
2 inputFolder = 'path_to_your_folder'; % Replace with your folder path
3 outputFolder = 'path_to_output_folder'; % Replace with your desired output folder
4 path
5
6 % Get a list of all .mat files in the input folder
7 matFiles = dir(fullfile(inputFolder, '*.mat'));
8
9 % Loop through each .mat file
10 for k = 1:length(matFiles)
11     % Construct full file name
12     inputFileName = fullfile(inputFolder, matFiles(k).name);
13
14     % Load the .mat file
15     data = load(inputFileName);
16
17     % Check available variables
18     whos('-file', inputFileName);
19
20     % Access and process the Z matrix
21     if isfield(data, 'Z') % Check if Z exists in the loaded data
22         Z = data.Z; % Adjust based on actual variable name in your file
23
24         % Delete the last 3 columns of Z
25         Z(:, end-2:end) = []; % Remove last three columns
26
27         % Replace NaN values with 1 in the updated Z matrix

```

```

27     Z(isnan(Z)) = 1;
28
29     % Convert updated Z values to r values using tanh
30     r = tanh(Z); % Use hyperbolic tangent to convert Z to r
31
32     % Replace diagonal values of r with 1s
33     n = size(r, 1); % Get the number of rows (and columns for a square matrix)
34     r(1:n+1:end) = 1; % Set diagonal elements to 1
35
36     % Create output file name
37     [~, name, ~] = fileparts(matFiles(k).name); % Get the base name without
           extension
38     outputFileName = fullfile(outputFolder, [name '_processed.mat']); % Add
           suffix to output file
39
40     % Save the results to a new .mat file
41     save(outputFileName, 'r'); % Save processed r values
42 else
43     fprintf('Variable Z not found in file: %s\n', matFiles(k).name);
44 end
45 end
46
47 disp('Processing complete.');
```

12.3.6 Converting r-value Back to Z-values

```

1 % Specify the folder containing .mat files
2 inputFolder = 'path_to_your_folder'; % <- replace
3 outputFolder = 'path_to_output_folder'; % <- replace
4 if ~exist(outputFolder, 'dir'); mkdir(outputFolder); end
5
6 % Get a list of all .mat files in the input folder
7 matFiles = dir(fullfile(inputFolder, '*.mat'));
8
9 % Loop through each .mat file
10 for k = 1:length(matFiles)
11     % Construct full file name
12     inputFileName = fullfile(inputFolder, matFiles(k).name);
13
14     % Load the .mat file
15     data = load(inputFileName);
16
17     % Check available variables
18     whos('-file', inputFileName);
19
20     % Access and process the Z matrix
21     if isfield(data, 'Z') % Check if Z exists in the loaded data
22         Z = data.Z; % Adjust based on actual variable name in your file
23
24         % --- Safety check for column deletion ---
25         if size(Z, 2) >= 3
26             % Delete the last 3 columns of Z
27             Z(:, end-2:end) = [];
28         else
29             warning('File %s: Z has < 3 cols; skipping column deletion.', matFiles(k).
30                 name);
31         end
32
33         % Replace NaN values with 1 in the updated Z matrix
```

```

33     Z(isnan(Z)) = 1;
34
35     % Convert updated Z values to r values using tanh
36     r = tanh(Z); % Fisher Z -> Pearson r
37
38     % Replace diagonal values of r with 1s (self-correlation)
39     n = size(r, 1);
40     r(1:n+1:end) = 1;
41
42     % ===== NEW: Convert r back to Fisher Z safely =====
43     % Clamp r to (-1, 1) to avoid Inf from atanh(±1)
44     tol = 1 - 1e-12;
45     r_clamped = max(min(r, tol), -tol);
46
47     % Convert back
48     Z_back = atanh(r_clamped);
49
50     % Set diagonal to 0 (common convention for Fisher Z diagonals)
51     % (atanh(1) would be Inf; we choose 0 for clarity; change if you prefer NaN)
52     Z_back(1:n+1:end) = 0;
53     % =====
54
55     % Create output file name
56     [~, name, ~] = fileparts(matFiles(k).name);
57     outputFileName = fullfile(outputFolder, [name '_processed.mat']);
58
59     % Save the results to a new .mat file
60     save(outputFileName, 'r', 'Z_back'); % Save processed r and back-converted Z
61 else
62     fprintf('Variable Z not found in file: %s\n', matFiles(k).name);
63 end
64 end
65
66 disp('Processing complete.');
```

12.3.7 SC Adjacency Matrices of 13 Brain Connections Identified using the CONN Toolbox.

```

1  #!/usr/bin/env python3
2  # -*- coding: utf-8 -*-
3
4  """
5  Create thesis-ready adjacency matrices (Before/After) for each group (TA, TA+PT, SA+PT
6  )
7  from SC_FA_Winsor.xlsx with columns:
8  - Group: "TA", "TA+PT", "SA+PT" (optional; falls back to Subject mapping)
9  - Subject: integers 1..19 (1-5=TA, 6-11=TA+PT, 12-19=SA+PT)
10 - Connection: e.g. "Supp_Motor_Area_L to Thalamus_R"
11 - Before: FA value before treatment
12 - After: FA value after treatment
13 """
14
15 import os
16 import math
17 import textwrap
18 import numpy as np
19 import pandas as pd
20 import matplotlib.pyplot as plt
21
22 # =====
```

```

22 # Config
23 # =====
24 INPUT_XLSX = "/content/SC_FA_Winsor.xlsx"
25 OUTPUT_DIR = "adjacency_matrices"
26 CMAP = "viridis"
27 DPI = 300 # high-res for thesis figures
28 TITLE_WRAP = 75 # wrap long titles nicely
29
30 # Subject → Group fallback map
31 SUBJECT_GROUP_RANGES = {
32     "TA": range(1, 6),          # -15
33     "TA+PT": range(6, 12),      # -611
34     "SA+PT": range(12, 20),     # -1219
35 }
36
37 GROUPS = ["TA", "TA+PT", "SA+PT"]
38
39
40 # =====
41 # Helpers
42 # =====
43 def subject_to_group(subject_value: int) -> str | None:
44     for g, rng in SUBJECT_GROUP_RANGES.items():
45         if subject_value in rng:
46             return g
47     return None
48
49
50 def ensure_group_column(df: pd.DataFrame) -> pd.DataFrame:
51     if "Group" not in df.columns:
52         df["Group"] = None
53     mask_missing = df["Group"].isna() | (df["Group"].astype(str).strip() == "")
54     if mask_missing.any():
55         inferred = df.loc[mask_missing, "Subject"].apply(
56             lambda s: subject_to_group(int(s)) if pd.notna(s) else None
57         )
58         df.loc[mask_missing, "Group"] = inferred
59     df["Group"] = df["Group"].astype(str)
60     return df
61
62
63 def parse_connection(conn: str) -> tuple[str, str]:
64     if not isinstance(conn, str):
65         return "", ""
66     parts = conn.split(" to ")
67     if len(parts) != 2:
68         parts = [p.strip() for p in conn.replace(">", " to ").replace("-", " to ").
69                 split(" to ") if p.strip()]
70         if len(parts) != 2:
71             return (conn.strip(), conn.strip())
72     return parts[0].strip(), parts[1].strip()
73
74
75 def make_node_list(df: pd.DataFrame) -> list[str]:
76     nodes = set()
77     for conn in df["Connection"].dropna().astype(str):
78         a, b = parse_connection(conn)
79         if a: nodes.add(a)
80         if b: nodes.add(b)

```

```

80     return sorted(nodes)
81
82
83 def build_group_matrices(
84     df: pd.DataFrame, group: str, nodes: list[str]
85 ) -> tuple[np.ndarray, np.ndarray]:
86     gdf = df.loc[df["Group"] == group].copy()
87     if gdf.empty:
88         n = len(nodes)
89         return np.full((n, n), np.nan), np.full((n, n), np.nan)
90
91     agg = (
92         gdf.groupby("Connection", as_index=False)[["Before", "After"]]
93         .mean(numeric_only=True)
94     )
95
96     n = len(nodes)
97     before_mat = np.full((n, n), np.nan, dtype=float)
98     after_mat = np.full((n, n), np.nan, dtype=float)
99
100    node_index = {name: i for i, name in enumerate(nodes)}
101    for _, row in agg.iterrows():
102        a, b = parse_connection(str(row["Connection"]))
103        if a in node_index and b in node_index and a != b:
104            i, j = node_index[a], node_index[b]
105            before_val = row.get("Before", np.nan)
106            after_val = row.get("After", np.nan)
107            before_mat[i, j] = before_val
108            before_mat[j, i] = before_val
109            after_mat[i, j] = after_val
110            after_mat[j, i] = after_val
111
112    return before_mat, after_mat
113
114
115 def finite_minmax(*arrays: np.ndarray) -> tuple[float, float]:
116     vals = []
117     for arr in arrays:
118         a = np.array(arr).ravel()
119         a = a[np.isfinite(a)]
120         if a.size:
121             vals.append(a.min())
122             vals.append(a.max())
123     if not vals:
124         return (0.0, 1.0)
125     return (float(np.min(vals)), float(np.max(vals)))
126
127
128 def dynamic_figsize(n_nodes: int) -> tuple[float, float]:
129     base = 6.0
130     scale = min(1.0 + n_nodes/16.0, 2.2)
131     size = base * scale
132     return (size, size)
133
134
135 def prettify_title(s: str) -> str:
136     return "\n".join(textwrap.wrap(s, TITLE_WRAP))
137
138

```

```

139 def plot_matrix(
140     mat: np.ndarray,
141     nodes: list[str],
142     title: str,
143     vmin: float,
144     vmax: float,
145     outfile: str,
146     cmap: str = CMAP,
147     annotate_minmax: tuple[float, float] | None = None,
148 ):
149     n = len(nodes)
150     fig_size = dynamic_figsize(n)
151     fig, ax = plt.subplots(figsize=fig_size, dpi=DPI)
152
153     # Show matrix
154     im = ax.imshow(mat, interpolation="nearest", aspect="equal", cmap=cmap, vmin=vmin,
155                    vmax=vmax)
156
157     # Axis labels (BOLD + larger font)
158     ax.set_xticks(range(n))
159     ax.set_yticks(range(n))
160     ax.set_xticklabels(nodes, rotation=90, fontsize=14, fontweight="bold") # larger +
161     bold
162     ax.set_yticklabels(nodes, fontsize=14, fontweight="bold") # larger +
163     bold
164
165     # Axis tick params (make tick marks bold + bigger)
166     ax.tick_params(axis="x", labelsize=14, width=1.2)
167     ax.tick_params(axis="y", labelsize=14, width=1.2)
168
169     # Gridlines
170     ax.set_xticks(np.arange(-0.5, n, 1), minor=True)
171     ax.set_yticks(np.arange(-0.5, n, 1), minor=True)
172     ax.grid(which="minor", linestyle="-", linewidth=0.3, alpha=0.3)
173     ax.tick_params(which="both", length=0)
174
175     # Title
176     ax.set_title(prettify_title(title), fontsize=14, pad=12, fontweight="bold")
177
178     # Colorbar
179     cbar = fig.colorbar(im, ax=ax, fraction=0.046, pad=0.04)
180     cbar.ax.set_ylabel("Mean FA", rotation=270, labelpad=12, fontsize=12, fontweight="
181     bold")
182     cbar.ax.tick_params(labelsize=12, width=1.2) # bigger colorbar ticks
183
184     if annotate_minmax is not None:
185         gmin, gmax = annotate_minmax
186         txt = f"Group scale (FA): min={gmin:.3f}, max={gmax:.3f}"
187         fig.text(0.995, 0.02, txt, ha="right", va="bottom", fontsize=9)
188
189     fig.tight_layout()
190     os.makedirs(os.path.dirname(outfile) or ".", exist_ok=True)
191     fig.savefig(outfile, dpi=DPI, bbox_inches="tight")
192     plt.close(fig)
193
194 # =====
195 # Main
196 # =====

```

```

194 def main():
195     if not os.path.exists(INPUT_XLSX):
196         raise FileNotFoundError(f"Input file not found: {INPUT_XLSX}")
197
198     df = pd.read_excel(INPUT_XLSX)
199
200     expected_cols = {"Subject", "Connection", "Before", "After"}
201     missing = expected_cols - set(df.columns)
202     if missing:
203         raise ValueError(f"Missing required column(s): {'', '.join(sorted(missing))}")
204
205     df["Subject"] = pd.to_numeric(df["Subject"], errors="coerce").astype("Int64")
206     df["Before"] = pd.to_numeric(df["Before"], errors="coerce")
207     df["After"] = pd.to_numeric(df["After"], errors="coerce")
208
209     df = ensure_group_column(df)
210
211     nodes = make_node_list(df)
212     if len(nodes) == 0:
213         raise ValueError("No brain regions parsed from 'Connection' column.")
214
215     print(f"Detected {len(nodes)} unique regions across all connections.")
216     print(f"Regions: {nodes}")
217
218     for group in GROUPS:
219         before_mat, after_mat = build_group_matrices(df, group, nodes)
220         gmin, gmax = finite_minmax(before_mat, after_mat)
221         if math.isclose(gmin, gmax, rel_tol=1e-12, abs_tol=1e-12):
222             gmin, gmax = (gmin - 1e-6, gmax + 1e-6)
223
224         title_before = f"{group}: Structural Connectivity (FA) - Before"
225         outfile_before = os.path.join(OUTPUT_DIR, f"Adjacency_{group}_Before.png")
226         plot_matrix(before_mat, nodes, title_before, gmin, gmax, outfile_before, cmap=
227             CMAP, annotate_minmax=(gmin, gmax))
228         print(f"Saved: {outfile_before}")
229
230         title_after = f"{group}: Structural Connectivity (FA) - After"
231         outfile_after = os.path.join(OUTPUT_DIR, f"Adjacency_{group}_After.png")
232         plot_matrix(after_mat, nodes, title_after, gmin, gmax, outfile_after, cmap=
233             CMAP, annotate_minmax=(gmin, gmax))
234         print(f"Saved: {outfile_after}")
235
236     print("Done.")
237
238 if __name__ == "__main__":
239     main()

```

12.3.8 Calculating SC-FC Coupling: Pearson Correlation Between the Mean FA and Correlated Mean Time Series.

```

1 import pandas as pd
2 import numpy as np
3 import matplotlib.pyplot as plt
4 from scipy.stats import pearsonr
5
6 # Read the Excel files
7 sc_data = pd.read_excel("/content/SC_FA_Winsor.xlsx")

```



```

8 fc_data = pd.read_excel("/content/FC_Zvalues_Winsor.xlsx")
9
10 # Define group assignments based on Subject
11 groups = {
12     'TA': range(1, 6),
13     'TA+PT': range(6, 12),
14     'SA+PT': range(12, 20)
15 }
16
17 # Define colors for each group
18 colors = {
19     'TA': {'before': '#D8BFD8', 'after': '#800080'}, # Light purple, Dark purple
20     'TA+PT': {'before': '#FFDAB9', 'after': '#FF8C00'}, # Light orange, Dark orange
21     'SA+PT': {'before': '#98FB98', 'after': '#006400'} # Light green, Dark green
22 }
23
24 # Function to create scatter plot for a group
25 def create_scatter_plot(group_name, group_data, ax):
26     # Extract data for before and after
27     sc_before = group_data['SC_Before_FA']
28     fc_before = group_data['FC_Before_Z']
29     sc_after = group_data['SC_After_FA']
30     fc_after = group_data['FC_After_Z']
31
32     # Plot before data
33     ax.scatter(sc_before, fc_before, c=colors[group_name]['before'], label='Before',
34               alpha=0.6)
35     # Plot after data
36     ax.scatter(sc_after, fc_after, c=colors[group_name]['after'], label='After', alpha
37               =0.6)
38
39     # Fit line for before data (solid blue)
40     z_before = np.polyfit(sc_before, fc_before, 1)
41     p_before = np.poly1d(z_before)
42     ax.plot(sc_before, p_before(sc_before), "--", color="blue", label='Before Fit')
43
44     # Fit line for after data (solid red)
45     z_after = np.polyfit(sc_after, fc_after, 1)
46     p_after = np.poly1d(z_after)
47     ax.plot(sc_after, p_after(sc_after), "--", color="red", label='After Fit')
48
49     # Calculate Pearson correlation for before data
50     r_before, p_before = pearsonr(sc_before, fc_before)
51     # Calculate Pearson correlation for after data
52     r_after, p_after = pearsonr(sc_after, fc_after)
53
54     # Add correlation info for before (blue)
55     ax.text(0.05, 0.95, f'Before: r = {r_before:.3f}, p = {p_before:.3f}',
56            transform=ax.transAxes, verticalalignment='top',
57            color='blue', bbox=dict(boxstyle='round', facecolor='white', alpha=0.8))
58
59     # Add correlation info for after (red, slightly below before)
60     ax.text(0.05, 0.85, f'After: r = {r_after:.3f}, p = {p_after:.3f}',
61            transform=ax.transAxes, verticalalignment='top',
62            color='red', bbox=dict(boxstyle='round', facecolor='white', alpha=0.8))
63
64     ax.set_xlabel('SC (FA)')
65     ax.set_ylabel('FC (Z-value)')
66     ax.set_title(f'Group: {group_name}')

```

```

65     ax.legend()
66     ax.grid(True, linestyle='--', alpha=0.7)
67
68     # Merge SC and FC data on common columns
69     merged_data = pd.merge(sc_data, fc_data, on=['Group', 'Subject', 'Connection'])
70
71     # Process each group and save separate plots
72     for group_name, subject_range in groups.items():
73         # Filter data for the group
74         group_data = merged_data[merged_data['Subject'].isin(subject_range)]
75
76         # Create a new figure for each group
77         fig, ax = plt.subplots(figsize=(5, 5))
78
79         # Create scatter plot
80         create_scatter_plot(group_name, group_data, ax)
81
82         # Adjust layout and save the plot
83         plt.tight_layout()
84         plt.savefig(f'{group_name}_plot.png')
85         plt.close(fig) # Close the figure to free memory

```

Figure 51: A snippet of the SC_FA_Winsor input file used above for the SC-FC Coupling analysis.

Group	Subject	Connection	SC_Before_FA	SC_After_FA
TA	1	Supp_Motor_Area_L to Thalamus_R	0,4946895	0,4696195
TA	1	Supp_Motor_Area_L to Rolandic_Oper_R	0,4714279	0,4445678
TA	1	Supp_Motor_Area_L to Rolandic_Oper_L	0,4678508	0,4429753
TA	1	Vermis_7 to Caudate_R	0,3944551	0,4140446
TA	1	Cerebelum_7b_R to Cerebelum_8_L	0,3679784	0,494465
TA	1	Vermis_7 to Thalamus_R	0,412672	0,4788551
TA	1	Insula_R to Vermis_1_2	0,5224399	0,4828921
TA	1	Temporal_Mid_L to Vermis_7	0,2866099	0,5649805
TA	1	Amygdala_L to Frontal_Sup_Orb_L	0,4771516	0,4667701
TA	1	Amygdala_L to Frontal_Sup_Orb_R	0,474317	0,4656437
TA	1	Amygdala_R to Frontal_Sup_Orb_L	0,4777154	0,4665208
TA	1	Amygdala_R to Frontal_Sup_Orb_R	0,4777488	0,4655313
TA	1	Amygdala_L to Frontal_Mid_Orb_R	0,4773707	0,4700491
TA	2	Supp_Motor_Area_L to Thalamus_R	0,4306847	0,4333843
TA	2	Supp_Motor_Area_L to Rolandic_Oper_R	0,4102202	0,4240009
TA	2	Supp_Motor_Area_L to Rolandic_Oper_L	0,415592525	0,4220753
TA	2	Vermis_7 to Caudate_R	0,377324	0,4400654
TA	2	Cerebelum_7b_R to Cerebelum_8_L	0,4112278	0,4272984
TA	2	Vermis_7 to Thalamus_R	0,3484481	0,4031877
TA	2	Insula_R to Vermis_1_2	0,4345573	0,4440463
TA	2	Temporal_Mid_L to Vermis_7	0,3530609	0,2942494
TA	2	Amygdala_L to Frontal_Sup_Orb_L	0,423706	0,4358836
TA	2	Amygdala_L to Frontal_Sup_Orb_R	0,424727925	0,4362964
TA	2	Amygdala_R to Frontal_Sup_Orb_L	0,4258812	0,4325234
TA	2	Amygdala_R to Frontal_Sup_Orb_R	0,4220928	0,4338563
TA	2	Amygdala_L to Frontal_Mid_Orb_R	0,435538	0,4341973
TA	3	Supp_Motor_Area_L to Thalamus_R	0,4746561	0,4679972
TA	3	Supp_Motor_Area_L to Rolandic_Oper_R	0,4592425	0,4512618
TA	3	Supp_Motor_Area_L to Rolandic_Oper_L	0,4567359	0,4501615

Figure 52: A snippet of the FC_Zvalues_Winsor input file used above for the SC-FC Coupling analysis.

Group	Subject	Connection	FC_Before_Z	FC_After_Z
TA	1	Supp_Motor_Area_L to Thalamus	0,050022851	0,084559427
TA	1	Supp_Motor_Area_L to Rolandic_	0,366589552	0,296647479
TA	1	Supp_Motor_Area_L to Rolandic_	0,475646701	0,380815026
TA	1	Vermis_7 to Caudate_R	0,306806582	0,241382022
TA	1	Cerebelum_7b_R to Cerebelum_8	0,916031433	0,560767978
TA	1	Vermis_7 to Thalamus_R	0,165474299	0,497288567
TA	1	Insula_R to Vermis_1_2	0,066268544	0,007592878
TA	1	Temporal_Mid_L to Vermis_7	0,279163276	0,213613601
TA	1	Amygdala_L to Frontal_Sup_Orb_	0,51571338	0,066689075
TA	1	Amygdala_L to Frontal_Sup_Orb_	0,271586273	0,075086039
TA	1	Amygdala_R to Frontal_Sup_Orb_	0,431826801	0,151196687
TA	1	Amygdala_R to Frontal_Sup_Orb_	0,23098624	0,086311879
TA	1	Amygdala_L to Frontal_Mid_Orb_	0,265560734	0,135751344
TA	2	Supp_Motor_Area_L to Thalamus	0,065929389	0,396830472
TA	2	Supp_Motor_Area_L to Rolandic_	0,201915386	0,045646846
TA	2	Supp_Motor_Area_L to Rolandic_	0,107166772	0,009029307
TA	2	Vermis_7 to Caudate_R	0,446396884	0,150930526
TA	2	Cerebelum_7b_R to Cerebelum_8	0,946950031	0,627933565
TA	2	Vermis_7 to Thalamus_R	0,16578644	0,194327485
TA	2	Insula_R to Vermis_1_2	0,256210822	0,277584978
TA	2	Temporal_Mid_L to Vermis_7	0,06635827	0,353165437
TA	2	Amygdala_L to Frontal_Sup_Orb_	0,129231035	0,443694909
TA	2	Amygdala_L to Frontal_Sup_Orb_	0,287243271	0,113387097
TA	2	Amygdala_R to Frontal_Sup_Orb_	0,267698565	0,153069396
TA	2	Amygdala_R to Frontal_Sup_Orb_	0,383571171	0,341632862
TA	2	Amygdala_L to Frontal_Mid_Orb_	0,080725536	0,105532889
TA	3	Supp_Motor_Area_L to Thalamus	0,327105216	0,131123579
TA	3	Supp_Motor_Area_L to Rolandic_	0,573659978	0,249210394
TA	3	Supp_Motor_Area_L to Rolandic_	0,647000416	0,355974137
TA	3	Vermis_7 to Caudate_R	0,552552756	0,309224891
TA	3	Cerebelum_7b_R to Cerebelum_8	0,896058695	0,544203924
TA	3	Vermis_7 to Thalamus_R	0,129257282	0,00220667
TA	3	Insula_R to Vermis_1_2	0,202031834	0,358429413
TA	3	Temporal_Mid_L to Vermis_7	0,024795393	0,145375413

12.3.9 Graph measures: Adjusting for the Motion Covariate when Comparing FC Graph Measures using a Repeated Measures ANCOVA (within-group differences) and one-way ANCOVA (between-group differences).

```

1 import pandas as pd
2 import numpy as np
3 import pingouin as pg
4 from statsmodels.formula.api import ols
5 from statsmodels.stats.anova import anova_lm
6 from statsmodels.stats.multicomp import MultiComparison
7 from scipy import stats
8
9 # =====
10 # Load Data
11 # =====
12 motion_df = pd.read_excel("SC_Motion.xlsx")
13 winsor_df = pd.read_excel("winsor.xlsx")
14
15 # Make sure the subjects are aligned across files
16 assert len(motion_df) == len(winsor_df), "Files must have same number of rows (
    subjects)."

```

```

17
18 # Add motion covariate to winsor_df for easier handling
19 winsor_df["Motion"] = motion_df["Motion"]
20 winsor_df["Group"] = motion_df["Group"]
21
22 # Graph measures
23 measures = ["Cp", "Lp", "Sigma", "Eg", "Eloc"]
24
25 # =====
26 # 1. Repeated Measures ANCOVA
27 # =====
28 rep_results = []
29
30 for m in measures:
31     # Build long-format dataframe for repeated measures
32     long_df = pd.DataFrame({
33         "Subject": np.repeat(winsor_df.index, 2),
34         "Time": np.tile(["Before", "After"], len(winsor_df)),
35         "Value": pd.concat([winsor_df[f"{m}_before"], winsor_df[f"{m}_after"]], axis
36                             =0).values,
37         "Group": np.repeat(winsor_df["Group"].values, 2),
38         "Motion": np.repeat(winsor_df["Motion"].values, 2)
39     })
40
41     # Run repeated measures ANCOVA (pingouin supports mixed models via rm_anova with
42     # covariates)
43     # Note: pingouin does not natively support covariates in rm_anova, so we use
44     # ancova for each time point.
45     # Instead, approximate by running mixed ANCOVA via statsmodels.
46     model = ols("Value ~ C(Time) * C(Group) + Motion", data=long_df).fit()
47     aov_table = anova_lm(model, typ=2)
48
49     # Partial eta squared
50     aov_table["eta_sq_partial"] = aov_table["sum_sq"] / (aov_table["sum_sq"] +
51                                                         aov_table.loc["Residual", "sum_sq"])
52
53     # Collect results
54     for effect in aov_table.index:
55         if effect != "Residual":
56             rep_results.append({
57                 "Measure": m,
58                 "Effect": effect,
59                 "F": aov_table.loc[effect, "F"],
60                 "p-uncorrected": aov_table.loc[effect, "PR(>F)"],
61                 "eta_sq_partial": aov_table.loc[effect, "eta_sq_partial"]
62             })
63
64     # Descriptives
65     desc = long_df.groupby(["Time"])["Value"].agg(["mean", "std", "count"])
66     desc["sem"] = desc["std"] / np.sqrt(desc["count"])
67     desc = desc.reset_index()
68     desc["Measure"] = m
69     rep_results.append({"Measure": m, "Descriptives": desc.to_dict()})
70
71 rep_df = pd.DataFrame(rep_results)
72 rep_df.to_excel("ANCOVA_repeated.xlsx", index=False)
73
74 # =====
75 # 2. One-Way ANCOVA (Post-treatment only)

```

```

72 # =====
73 one_results = []
74
75 for m in measures:
76     df_after = winsor_df[["Group", "Motion", f"{m}_after"]].rename(columns={f"{m}_after": "DV"})
77
78     # One-way ANCOVA model
79     model = ols("DV ~ C(Group) + Motion", data=df_after).fit()
80     aov_table = anova_lm(model, typ=2)
81
82     # Partial eta squared
83     aov_table["eta_sq_partial"] = aov_table["sum_sq"] / (aov_table["sum_sq"] +
84         aov_table.loc["Residual", "sum_sq"])
85
86     # Save ANCOVA results
87     for effect in aov_table.index:
88         if effect != "Residual":
89             one_results.append({
90                 "Measure": m,
91                 "Effect": effect,
92                 "F": aov_table.loc[effect, "F"],
93                 "p-uncorrected": aov_table.loc[effect, "PR(>F)"],
94                 "eta_sq_partial": aov_table.loc[effect, "eta_sq_partial"]
95             })
96
97     # Multiple comparisons (Sidak correction)
98     mc = MultiComparison(df_after["DV"], df_after["Group"])
99     comp = mc.allpairtest(stats.ttest_ind, method="sidak")[0]
100
101     # Descriptives
102     desc = df_after.groupby("Group")["DV"].agg(["mean", "std", "count"])
103     desc["sem"] = desc["std"] / np.sqrt(desc["count"])
104     desc = desc.reset_index()
105     desc["Measure"] = m
106     one_results.append({"Measure": m, "Descriptives": desc.to_dict()})
107
108 one_df = pd.DataFrame(one_results)
109 one_df.to_excel("ANCOVA_one.xlsx", index=False)
110
111 print("Analysis complete. Results saved to ANCOVA_repeated.xlsx and ANCOVA_one.xlsx")

```

12.3.10 Comparing Motion Covariate Before and After Treatment in the DWI Data.

```

1
2 !pip install XlsxWriter
3
4
5 #!/usr/bin/env python3
6 # -*- coding: utf-8 -*-
7
8 """
9 Paired t-tests: Before vs After within each group (TA=1, TA+PT=2, SA+PT=3)
10
11 Input:
12 - Motion_Before_After_SC.xlsx
13   Columns:
14     Group (1=TA, 2=TA+PT, 3=SA+PT)
15     Before (numeric)

```

```

16         After (numeric)
17
18 Output:
19 - Motion_within.xlsx [intentional filename per user request]
20     Sheets:
21     - Paired_t_within: descriptive stats + t, df, p, 95% CI for mean diff
22     - By_Subject_Diffs: per-subject difference (After-Before) for sanity checks
23     """
24
25 import pandas as pd
26 import numpy as np
27 from scipy import stats
28
29 # -----
30 # Config
31 # -----
32 INPUT_XLSX = "/content/Motion_Before_After_SC.xlsx"
33 OUTPUT_XLSX = "Motion_within.xlsx" # keep the exact filename requested
34
35 GROUP_MAP = {1: "TA", 2: "TA+PT", 3: "SA+PT"}
36
37 # -----
38 # Load data
39 # -----
40 df = pd.read_excel(INPUT_XLSX)
41
42 # Ensure expected columns exist
43 required_cols = {"Group", "Before", "After"}
44 missing = required_cols - set(df.columns)
45 if missing:
46     raise ValueError(f"Missing required columns in input file: {missing}")
47
48 # Coerce numeric (silently turn bad strings into NaN)
49 for c in ["Before", "After"]:
50     df[c] = pd.to_numeric(df[c], errors="coerce")
51
52 # -----
53 # Helper: summary + paired t per group
54 # -----
55 rows = []
56 diff_rows = [] # for optional per-subject export
57
58 for g_code, sub in df.groupby("Group", dropna=True):
59     g_name = GROUP_MAP.get(g_code, str(g_code))
60
61     # Drop rows where either timepoint is missing (pairwise complete)
62     sub = sub.dropna(subset=["Before", "After"]).copy()
63     n = len(sub)
64     if n < 2:
65         # Not enough data for a paired t-test
66         rows.append({
67             "Group": g_name,
68             "N": n,
69             "Before_mean": np.nan, "Before_SD": np.nan, "Before_SEM": np.nan,
70             "After_mean": np.nan, "After_SD": np.nan, "After_SEM": np.nan,
71             "Diff_mean": np.nan, "Diff_SD": np.nan, "Diff_SEM": np.nan,
72             "t": np.nan, "df": np.nan, "p_value": np.nan,
73             "CI95_low": np.nan, "CI95_high": np.nan
74         })

```

```

75         continue
76
77     # Descriptives
78     b = sub["Before"].values
79     a = sub["After"].values
80     d = a - b
81
82     before_mean, before_sd = float(np.nanmean(b)), float(np.nanstd(b, ddof=1))
83     after_mean, after_sd = float(np.nanmean(a)), float(np.nanstd(a, ddof=1))
84     diff_mean, diff_sd = float(np.nanmean(d)), float(np.nanstd(d, ddof=1))
85
86     before_sem = before_sd / np.sqrt(n)
87     after_sem = after_sd / np.sqrt(n)
88     diff_sem = diff_sd / np.sqrt(n)
89
90     # Paired t-test
91     t_stat, p_val = stats.ttest_rel(a, b, nan_policy="omit")
92     dfree = n - 1
93
94     # 95% CI for mean difference (paired): mean(d) ± t_crit * SE(d)
95     if n > 1 and np.isfinite(diff_sem):
96         t_crit = stats.t.ppf(0.975, dfree)
97         ci_low = diff_mean - t_crit * diff_sem
98         ci_high = diff_mean + t_crit * diff_sem
99     else:
100         ci_low = ci_high = np.nan
101
102     rows.append({
103         "Group": g_name,
104         "N": n,
105         "Before_mean": before_mean, "Before_SD": before_sd, "Before_SEM": before_sem,
106         "After_mean": after_mean, "After_SD": after_sd, "After_SEM": after_sem,
107         "Diff_mean": diff_mean, "Diff_SD": diff_sd, "Diff_SEM": diff_sem,
108         "t": t_stat, "df": dfree, "p_value": p_val,
109         "CI95_low": ci_low, "CI95_high": ci_high
110     })
111
112     # Keep per-subject differences for reference
113     tmp = sub[["Group", "Before", "After"]].copy()
114     tmp["Group"] = g_name
115     tmp["Diff"] = tmp["After"] - tmp["Before"]
116     diff_rows.append(tmp)
117
118 # -----
119 # Build output DataFrames
120 # -----
121 out_df = pd.DataFrame(rows)
122
123 # Order columns nicely
124 col_order = [
125     "Group", "N",
126     "Before_mean", "Before_SD", "Before_SEM",
127     "After_mean", "After_SD", "After_SEM",
128     "Diff_mean", "Diff_SD", "Diff_SEM",
129     "t", "df", "p_value", "CI95_low", "CI95_high"
130 ]
131 out_df = out_df[col_order]
132
133 # Optional: round for readability

```

```

134 round_cols = [c for c in out_df.columns if c not in ["Group", "N", "df"]]
135 out_df[round_cols] = out_df[round_cols].round(4)
136
137 diff_df = pd.concat(diff_rows, axis=0, ignore_index=True) if diff_rows else pd.
    DataFrame(columns=["Group", "Before", "After", "Diff"])
138 diff_df = diff_df.sort_values(["Group"]).reset_index(drop=True)
139
140 # -----
141 # Export to Excel
142 # -----
143 with pd.ExcelWriter(OUTPUT_XLSX, engine="xlsxwriter") as writer:
144     out_df.to_excel(writer, sheet_name="Paired_t_within", index=False)
145     diff_df.to_excel(writer, sheet_name="By_Subject_Diffs", index=False)
146
147 print(f"Done. Results saved to: {OUTPUT_XLSX}")

```

12.3.11 Adjusting for Motion in the SC Graph Measures using Repeated Measures ANCOVA for Within-group Comparisons, with the Mean Motion as a Covariate.

```

1
2 #!/usr/bin/env python3
3 # -*- coding: utf-8 -*-
4
5 import pandas as pd
6 import numpy as np
7 from statsmodels.formula.api import ols
8 from statsmodels.stats.anova import anova_lm
9
10 # =====
11 # Load Data
12 # =====
13 motion_df = pd.read_excel("SC_Motion.xlsx") # must include columns: Group, Motion
14 winsor_df = pd.read_excel("winsor.xlsx")    # must include columns like Cp_before,
    Cp_after, etc.
15
16 # Make sure the subjects are aligned across files
17 assert len(motion_df) == len(winsor_df), "Files must have same number of rows (
    subjects)."
18
19 # Add motion covariate and group to winsor_df for easier handling
20 winsor_df["Motion"] = motion_df["Motion"]
21 winsor_df["Group"] = motion_df["Group"]
22
23 # Graph measures to analyze
24 measures = ["Cp", "Lp", "Sigma", "Eg", "Eloc"]
25
26 # =====
27 # Repeated Measures ANCOVA
28 # Value ~ C(Time) * C(Group) + Motion
29 # (fixed-effects OLS; no subject random effect)
30 # =====
31 aov_rows = []
32 desc_rows = []
33
34 for m in measures:
35     # Long format: Before/After stacked
36     long_df = pd.DataFrame({
37         "Subject": np.repeat(winsor_df.index, 2),

```



```

38     "Time":      np.tile(["Before", "After"], len(winsor_df)),
39     "Value":     pd.concat([winsor_df[f"{m}_before"], winsor_df[f"{m}_after"]], axis
40                       =0).values,
41     "Group":     np.repeat(winsor_df["Group"].values, 2),
42     "Motion":    np.repeat(winsor_df["Motion"].values, 2)
43 })
44
45 # OLS with Time, Group, Interaction, and Motion covariate
46 model = ols("Value ~ C(Time) * C(Group) + Motion", data=long_df).fit()
47
48 # Type-II ANOVA
49 aov = anova_lm(model, typ=2)
50
51 # Partial eta squared
52 aov["eta_sq_partial"] = aov["sum_sq"] / (aov["sum_sq"] + aov.loc["Residual", "
53                               sum_sq"])
54
55 # Collect ANOVA rows (exclude residual)
56 for effect in aov.index:
57     if effect == "Residual":
58         continue
59     aov_rows.append({
60         "Measure":      m,
61         "Effect":        effect,
62         "df":            aov.loc[effect, "df"],
63         "sum_sq":        aov.loc[effect, "sum_sq"],
64         "mean_sq":       aov.loc[effect, "mean_sq"],
65         "F":             aov.loc[effect, "F"],
66         "p_value":       aov.loc[effect, "PR(>F)"],
67         "eta_sq_partial": aov.loc[effect, "eta_sq_partial"]
68     })
69
70 # Descriptives by Group × Time (mean, SD, n, SEM)
71 d = (long_df
72     .groupby(["Group", "Time"])["Value"]
73     .agg(mean="mean", sd="std", n="count")
74     .reset_index())
75 d["sem"] = d["sd"] / np.sqrt(d["n"])
76 d.insert(0, "Measure", m)
77 desc_rows.append(d)
78
79 # Combine and export
80 aov_df = pd.DataFrame(aov_rows)
81 desc_df = pd.concat(desc_rows, ignore_index=True)
82
83 with pd.ExcelWriter("ANCOVA_repeated.xlsx", engine="xlsxwriter") as writer:
84     aov_df.to_excel(writer, sheet_name="ANOVA", index=False)
85     desc_df.to_excel(writer, sheet_name="Descriptives_GxT", index=False)
86
87 print("Repeated-measures ANCOVA complete. Saved to ANCOVA_repeated.xlsx")

```

12.3.12 Adjusting for Motion in the SC Graph Measures Using One-way ANCOVA for Comparing Pre- and Post-treatment, Between Groups.

```

1
2 #!/usr/bin/env python3
3 # -*- coding: utf-8 -*-

```

```

4
5 """
6 One-way ANCOVA (between-group) adjusting for Motion
7 + pairwise (motion-adjusted) comparisons for Lp ONLY.
8
9 Input:
10 - /content/SC_Before_Graph.xlsx
11   Columns:
12     Group (1=TA, 2=TA+PT, 3=SA+PT)
13     Motion
14     Cp, Lp, Sigma, Eg, Eloc
15
16 Outputs:
17 - ANCOVA_before.xlsx (overall group effect, descriptives)
18 - Pairwise_before.xlsx (pairwise, motion-adjusted comparisons for Lp only)
19 """
20
21 import pandas as pd
22 import numpy as np
23 import statsmodels.formula.api as smf
24 from statsmodels.stats.anova import anova_lm
25
26 # -----
27 # Config
28 # -----
29 INPUT_XLSX = "/content/SC_After_Graph.xlsx"
30 ANCOVA_OUT = "ANCOVA_after.xlsx"
31 PAIRWISE_OUT = "Pairwise_after.xlsx"
32
33 MEASURES_ALL = ["Cp", "Lp", "Sigma", "Eg", "Eloc"] # ANCOVA across all
34 MEASURES_PAIRWISE = ["Lp"] # pairwise for Lp only
35 GROUP_MAP = {1: "TA", 2: "TA+PT", 3: "SA+PT"}
36
37 # -----
38 # Load & check
39 # -----
40 df = pd.read_excel(INPUT_XLSX)
41
42 required = {"Group", "Motion"} | set(MEASURES_ALL)
43 missing = required - set(df.columns)
44 if missing:
45     raise ValueError(f"Missing required columns in input file: {missing}")
46
47 df["Group"] = pd.to_numeric(df["Group"], errors="coerce")
48 df["Motion"] = pd.to_numeric(df["Motion"], errors="coerce")
49 for m in MEASURES_ALL:
50     df[m] = pd.to_numeric(df[m], errors="coerce")
51
52 df = df[df["Group"].isin([1, 2, 3])].copy()
53 df["GroupLabel"] = df["Group"].map(GROUP_MAP)
54
55 # -----
56 # Helpers
57 # -----
58 def group_descriptives(data, measure):
59     out = {}
60     grp = data.groupby("Group")[measure]
61     for g in [1, 2, 3]:

```

```

62     vals = grp.get_group(g).dropna() if g in grp.groups else pd.Series(dtype=float
63     )
64     n = int(vals.shape[0])
65     mean = float(vals.mean()) if n > 0 else np.nan
66     sd = float(vals.std(ddof=1)) if n > 1 else (0.0 if n == 1 else np.nan)
67     sem = (sd / np.sqrt(n)) if n > 0 and np.isfinite(sd) else np.nan
68     label = GROUP_MAP[g]
69     out[f"N_{label}"] = n
70     out[f"{label}_mean"] = mean
71     out[f"{label}_SD"] = sd
72     out[f"{label}_SEM"] = sem
73     out[f"{label}_mean±SD(SEM)"] = "" if (np.isnan(mean) or np.isnan(sd) or np.
74        .isnan(sem)) else f"{mean:.3f} ± {sd:.3f} ({sem:.3f})"
75     out["N_total"] = int(np.sum([out.get("N_TA", 0), out.get("N_TA+PT", 0), out.get("
76         N_SA+PT", 0)]))
77     return out
78
79 def sidak(p, m):
80     if pd.isna(p):
81         return np.nan
82     p = min(max(p, 0.0), 1.0)
83     return 1.0 - (1.0 - p) ** m
84
85 # -----
86 # ANCOVA per measure (overall effect + descriptives)
87 # -----
88 rows = []
89 p_adj_list = []
90
91 for measure in MEASURES_ALL:
92     sub = df[["Group", "Motion", measure]].dropna().copy()
93     sub.rename(columns={measure: "Y"}, inplace=True)
94
95     desc = group_descriptives(df[["Group", measure]].dropna(), measure)
96
97     # Unadjusted one-way ANOVA
98     p_unadj = np.nan
99     try:
100         m0 = smf.ols("Y ~ C(Group)", data=sub).fit()
101         a0 = anova_lm(m0, typ=2)
102         if "C(Group)" in a0.index:
103             p_unadj = float(a0.loc["C(Group)", "PR(>F)"])
104     except Exception:
105         pass
106
107     # Adjusted ANCOVA
108     p_adj = np.nan
109     F_adj = np.nan
110     df_effect = np.nan
111     df_error = np.nan
112     partial_eta_sq = np.nan
113     try:
114         m1 = smf.ols("Y ~ C(Group) + Motion", data=sub).fit()
115         a1 = anova_lm(m1, typ=2)
116         if {"C(Group)", "Residual"}.issubset(set(a1.index)):
117             ss_g = float(a1.loc["C(Group)", "sum_sq"])
118             df_g = float(a1.loc["C(Group)", "df"])
119             F_adj = float(a1.loc["C(Group)", "F"])
120             p_adj = float(a1.loc["C(Group)", "PR(>F)"])

```

```

118         ss_r = float(a1.loc["Residual", "sum_sq"])
119         df_r = float(a1.loc["Residual", "df"])
120         df_effect = df_g
121         df_error = df_r
122         partial_eta_sq = ss_g / (ss_g + ss_r) if (ss_g + ss_r) > 0 else np.nan
123     except Exception:
124         pass
125
126     rows.append({
127         "Measure": measure,
128         **desc,
129         "p-unadj": p_unadj,
130         "p-adj": p_adj,
131         "p-Sidak": np.nan, # filled below
132         "partial_eta_sq": partial_eta_sq,
133         "F_adj": F_adj,
134         "df_effect": df_effect,
135         "df_error": df_error
136     })
137     p_adj_list.append(p_adj)
138
139 # Šidák across measures (family = valid adjusted 'ps)
140 valid_p = [p for p in p_adj_list if pd.notna(p)]
141 m_family = len(valid_p) if valid_p else 1
142 for r in rows:
143     r["p-Sidak"] = sidak(r["p-adj"], m_family)
144
145 ancova_df = pd.DataFrame(rows)
146 # Reorder and round
147 ancova_cols = [
148     "Measure",
149     "N_total", "N_TA", "N_TA+PT", "N_SA+PT",
150     "TA_mean", "TA_SD", "TA_SEM", "TA_mean±SD(SEM)",
151     "TA+PT_mean", "TA+PT_SD", "TA+PT_SEM", "TA+PT_mean±SD(SEM)",
152     "SA+PT_mean", "SA+PT_SD", "SA+PT_SEM", "SA+PT_mean±SD(SEM)",
153     "p-unadj", "p-adj", "p-Sidak",
154     "partial_eta_sq", "F_adj", "df_effect", "df_error",
155 ]
156 ancova_cols = [c for c in ancova_cols if c in ancova_df.columns]
157 ancova_df = ancova_df[ancova_cols]
158 fmt_cols = {"TA_mean±SD(SEM)", "TA+PT_mean±SD(SEM)", "SA+PT_mean±SD(SEM)"}
159 num_cols = [c for c in ancova_df.columns if c not in fmt_cols and c not in ["Measure",
160 ]]
161 ancova_df[num_cols] = ancova_df[num_cols].apply(pd.to_numeric, errors="coerce")
162 round_cols = [c for c in num_cols if c not in ["N_total", "N_TA", "N_TA+PT", "N_SA+PT",
163 , "df_effect", "df_error"]]
164 ancova_df[round_cols] = ancova_df[round_cols].round(4)
165 for c in ["N_total", "N_TA", "N_TA+PT", "N_SA+PT"]:
166     if c in ancova_df.columns:
167         ancova_df[c] = ancova_df[c].astype("Int64")
168
169 # -----
170 # Pairwise (motion-adjusted) - Lp ONLY
171 # -----
172 pairwise_rows = []
173
174 def adjusted_means(model, motion_mean):
175     emms = {}
176     for g in [1, 2, 3]:

```

```

175     X = pd.DataFrame({"Group": [g], "Motion": [motion_mean]})
176     emms[g] = float(model.predict(X))
177     return emms
178
179 def contrast_t(model, L):
180     res = model.t_test(L)
181     eff = float(res.effect)
182     tval = float(res.tvalue)
183     pval = float(res.pvalue)
184     df = float(model.df_resid)
185     return eff, tval, pval, df
186
187 # Only for Lp
188 measure = "Lp"
189 sub = df[["Group", "Motion", measure]].dropna().copy()
190 sub.rename(columns={measure: "Y"}, inplace=True)
191
192 if not sub.empty:
193     model = smf.ols("Y ~ C(Group) + Motion", data=sub).fit()
194     params = list(model.params.index)
195     pnames = {name: i for i, name in enumerate(params)}
196
197     rmse = float(np.sqrt(model.mse_resid))
198     df_resid = float(model.df_resid)
199     J = (1.0 - 3.0 / (4.0 * df_resid - 1.0)) if df_resid > 1 else np.nan
200
201     m_motion = float(sub["Motion"].mean())
202     emms = adjusted_means(model, m_motion)
203
204     def L_vec(**coef_weights):
205         v = np.zeros(len(params))
206         for name, w in coef_weights.items():
207             if name in pnames:
208                 v[pnames[name]] = w
209         return v
210
211     g2 = "C(Group)[T.2]"
212     g3 = "C(Group)[T.3]"
213     if g2 not in pnames or g3 not in pnames:
214         cand2 = [k for k in pnames if "C(Group)" in k and "T.2" in k]
215         cand3 = [k for k in pnames if "C(Group)" in k and "T.3" in k]
216         g2 = cand2[0] if cand2 else g2
217         g3 = cand3[0] if cand3 else g3
218
219     comparisons = [
220         ("TA vs TA+PT", 1, 2, L_vec(**{g2: -1.0})),
221         ("TA vs SA+PT", 1, 3, L_vec(**{g3: -1.0})),
222         ("TA+PT vs SA+PT", 2, 3, L_vec(**{g2: 1.0, g3: -1.0})),
223     ]
224
225     pvals = []
226     tmp_rows = []
227     for label, gA, gB, L in comparisons:
228         eff, tval, pval, df_t = contrast_t(model, L)
229         pvals.append(pval)
230
231         diff = emms[gA] - emms[gB]
232         d = diff / rmse if rmse > 0 else np.nan
233         g = (J * d) if (not np.isnan(d) and not np.isnan(J)) else np.nan

```

```

234     t_df_str = f"{tval:.2f} ({int(round(df_t))})"
235
236
237     tmp_rows.append({
238         "Measure": measure,
239         "Comparison": label,
240         "Adj mean 1": emms[gA],
241         "Adj mean 2": emms[gB],
242         "t(df)": t_df_str,
243         "p-unc": pval,
244         # p-Sidak added after loop
245         "cohen's d": d,
246         "Hedge's g": g
247     })
248
249     # Šidák within-measure (m=3, since 3 pairwise for Lp)
250     m_within = len([p for p in pvals if pd.notna(p)]) or 1
251     for row in tmp_rows:
252         row["p-Sidak"] = sidak(row["p-unc"], m_within)
253         pairwise_rows.append(row)
254
255     # -----
256     # Save outputs
257     # -----
258     with pd.ExcelWriter(ANCOVA_OUT, engine="openpyxl") as writer:
259         ancova_df.to_excel(writer, sheet_name="ANCOVA", index=False)
260
261     pairwise_df = pd.DataFrame(pairwise_rows)
262     if not pairwise_df.empty:
263         pw_cols = ["Measure", "Comparison", "Adj mean 1", "Adj mean 2", "t(df)", "p-unc",
264             "p-Sidak", "cohen's d", "Hedge's g"]
265         pairwise_df = pairwise_df[pw_cols]
266         for c in ["Adj mean 1", "Adj mean 2", "p-unc", "p-Sidak", "cohen's d", "Hedge's g"
267             ]:
268             pairwise_df[c] = pd.to_numeric(pairwise_df[c], errors="coerce").round(4)
269         with pd.ExcelWriter(PAIRWISE_OUT, engine="openpyxl") as writer:
270             pairwise_df.to_excel(writer, sheet_name="Pairwise", index=False)
271     else:
272         pairwise_df = pd.DataFrame(columns=["Measure", "Comparison", "Adj mean 1", "Adj mean
273             2", "t(df)", "p-unc", "p-Sidak", "cohen's d", "Hedge's g"])
274         with pd.ExcelWriter(PAIRWISE_OUT, engine="openpyxl") as writer:
275             pairwise_df.to_excel(writer, sheet_name="Pairwise", index=False)
276
277     print(f"Done. Results saved to: {ANCOVA_OUT} and {PAIRWISE_OUT}")

```

12.3.13 Statistical Analysis of SC Graph Measures that Violated Assumptions of ANCOVA

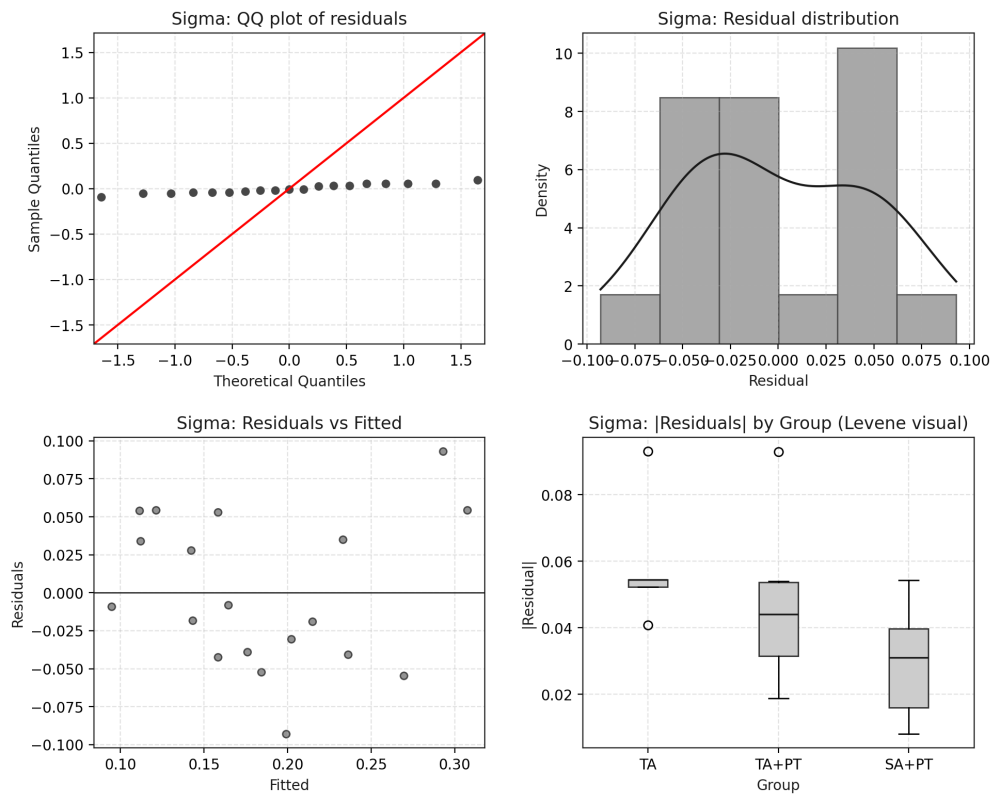


Figure 53: Pre-treatment sigma graph measure that violated the assumption of ANCOVA: Homogeneity of slopes

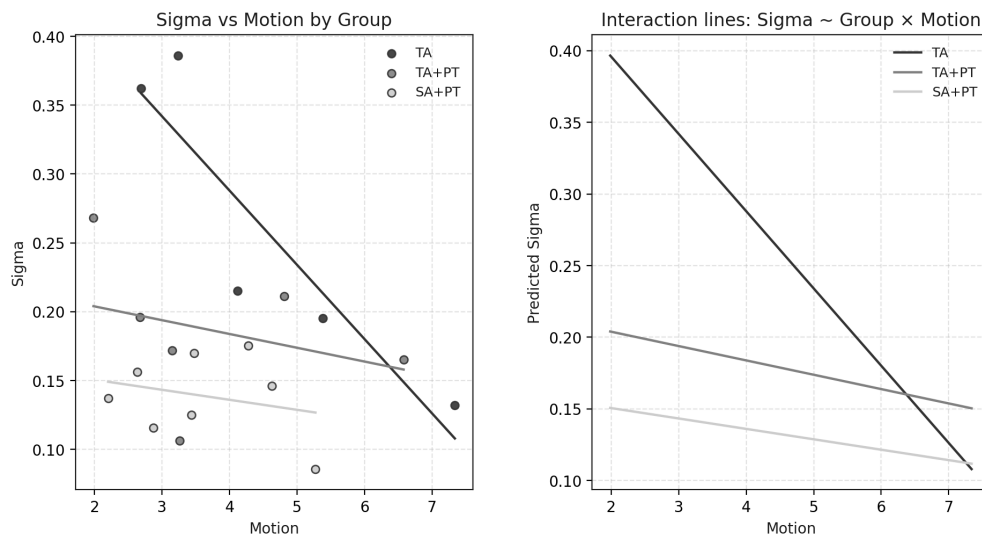


Figure 54: Pre-treatment linearity check for Sigma.

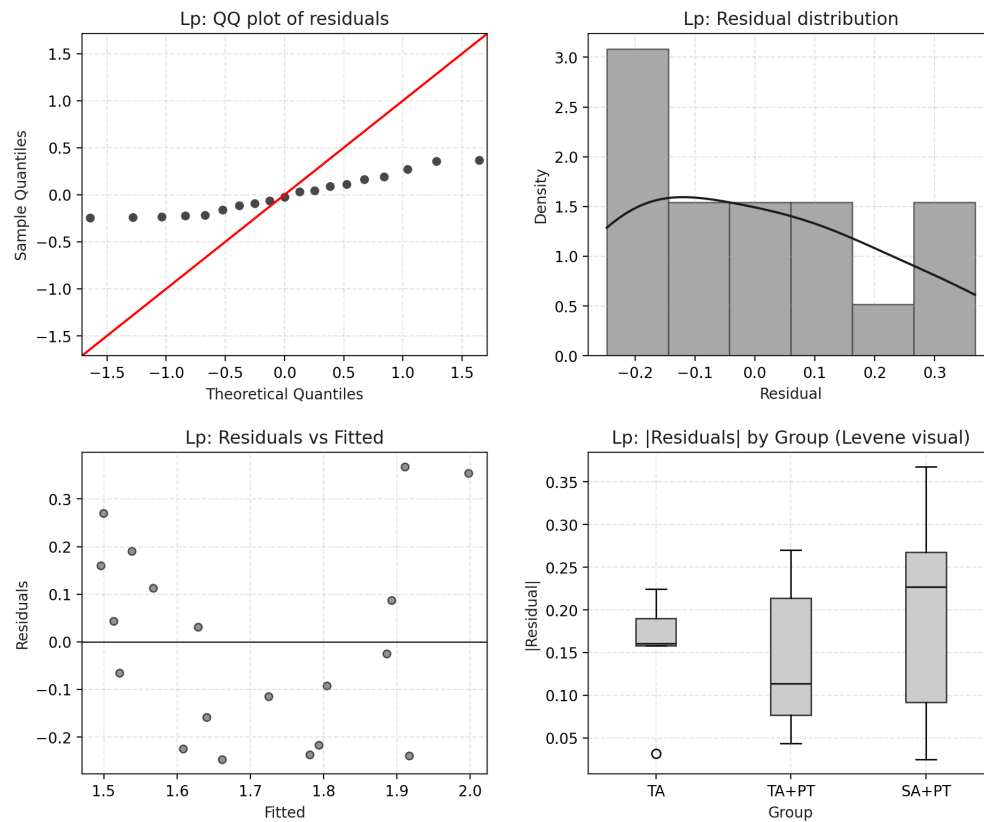


Figure 55: Post-treatment Lp that violated the Homogeneity of Slopes assumption of ANCOVA.

12.3.14 Statistical Analysis of FC Graph Measures that Violated Assumptions of ANCOVA

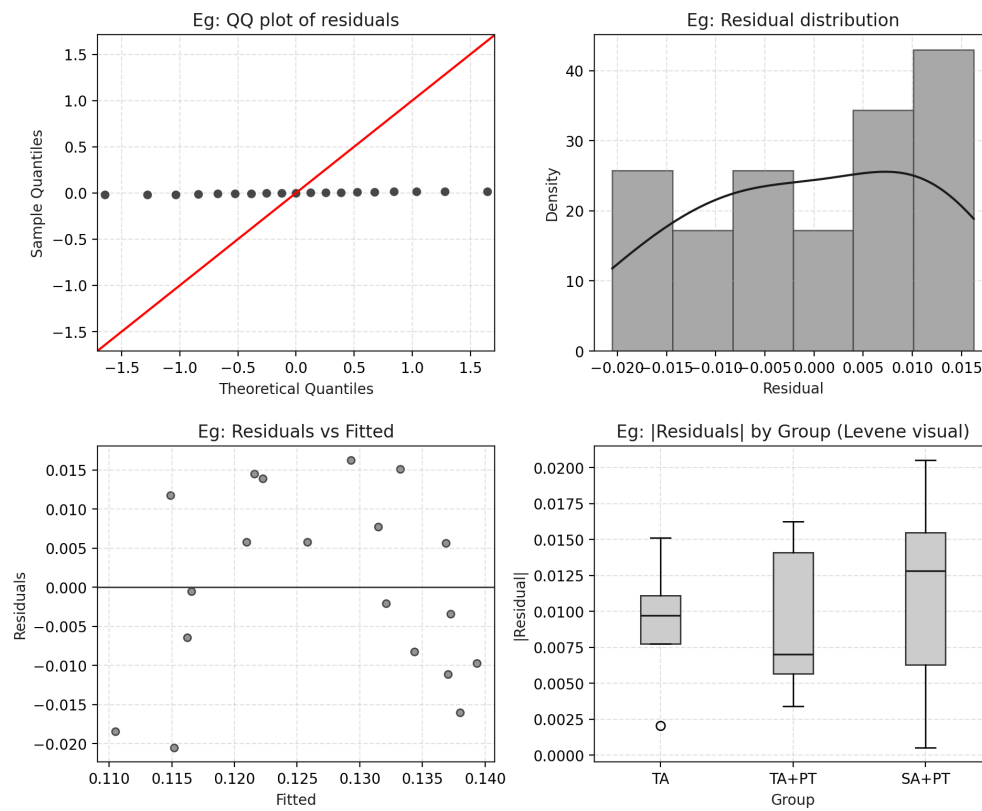


Figure 56: Post-treatment Eg that violated the Homogeneity of Slopes assumption of ANCOVA.

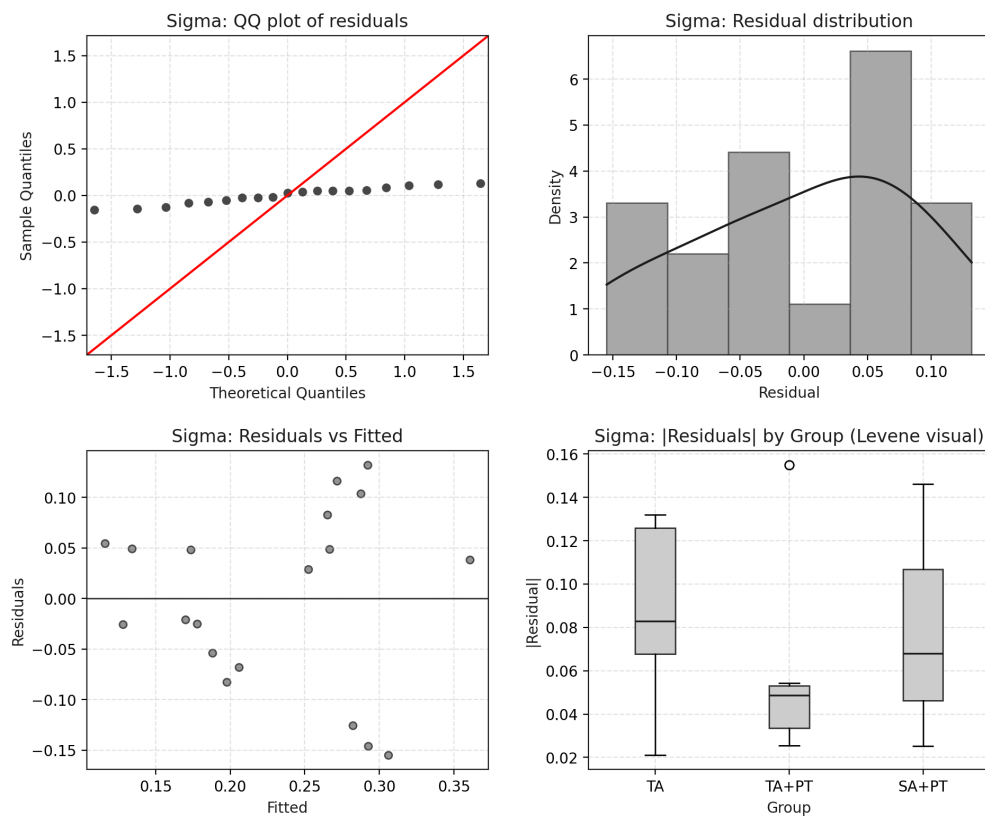


Figure 57: Post-treatment Sigma that violated the normality assumption of ANCOVA.

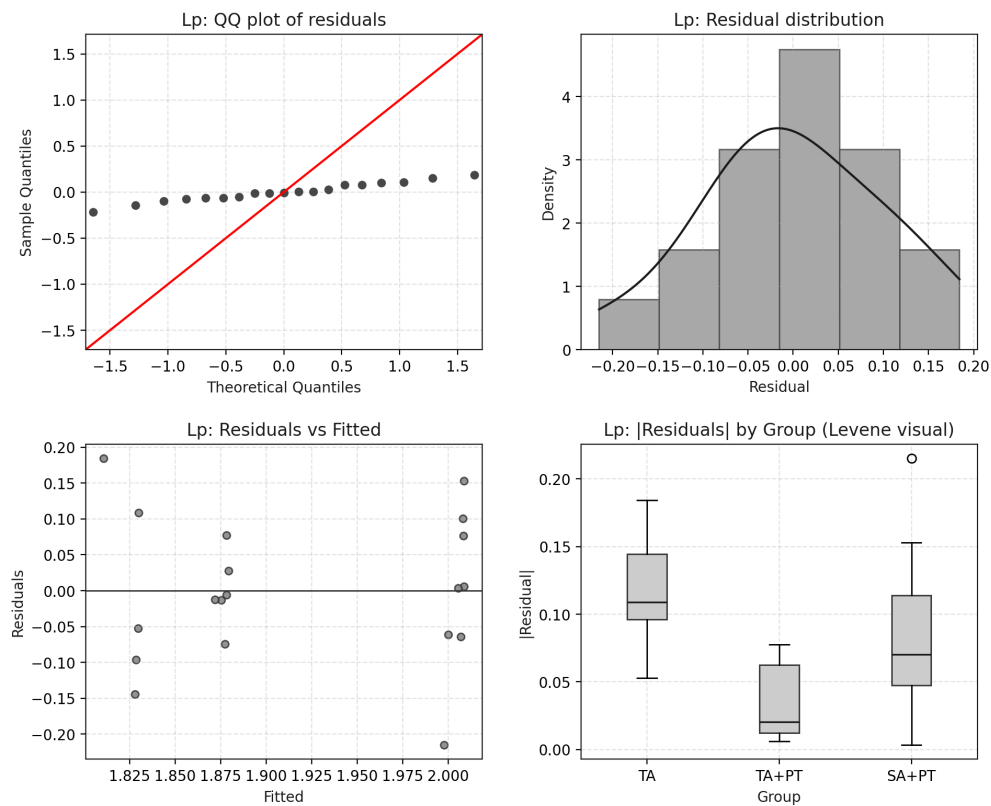


Figure 58: Pre-treatment Lp that violated the homogeneity of slopes assumption of ANCOVA.

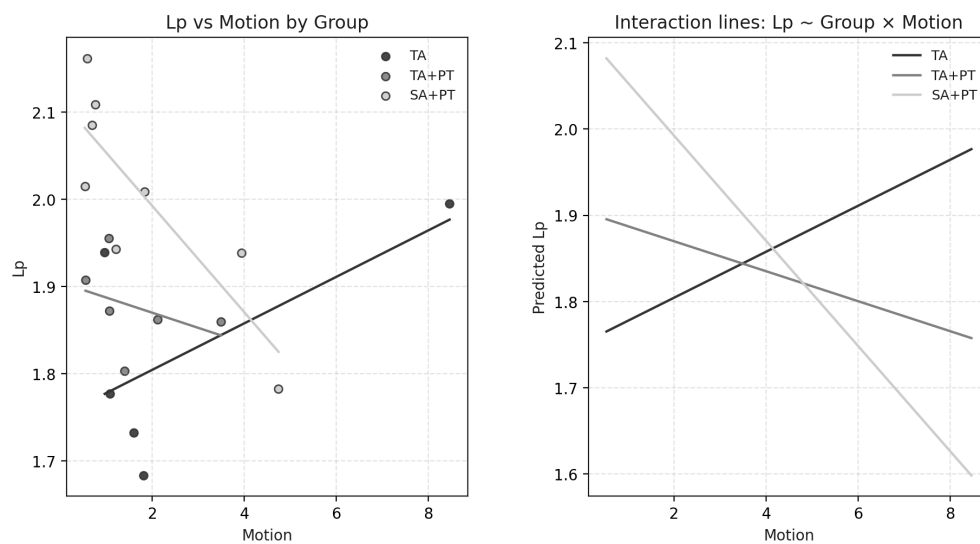


Figure 59: Pre-treatment Lp that violated the linearity assumption of ANCOVA.

12.3.15 Outlier Detection to use for Winsorizing Graph Theoretical Data

```

1 # Import required libraries
2 import numpy as np
3
4 # Step 1: Load data from .txt file line-by-line as a list
5 file_path = "/content/testing.txt" # Adjust this to your actual .txt file path/name
6 with open(file_path, 'r') as file:
7     values = [float(line.strip()) for line in file if line.strip()] # Read each line
8     as a float
9
10 # Step 2: Detect outliers using IQR method
11 Q1 = np.quantile(values, 0.25) # Calculate Q1 directly from list
12 Q3 = np.quantile(values, 0.75) # Calculate Q3 directly from list
13 IQR = Q3 - Q1
14 lower_bound = Q1 - 1.5 * IQR
15 upper_bound = Q3 + 1.5 * IQR
16
17 # Identify outliers
18 outliers = [x for x in values if x < lower_bound or x > upper_bound]
19
20 # Step 3: Display Q1, Q3, IQR, bounds, and outliers
21 print("Q1 (25th percentile):", Q1)
22 print("Q3 (75th percentile):", Q3)
23 print("IQR (Interquartile Range):", IQR)
24 print("Lower Bound (Q1 - 1.5 * IQR):", lower_bound)
25 print("Upper Bound (Q3 + 1.5 * IQR):", upper_bound)
26
27 if outliers:
28     print("\nDetected Outliers:")
29     for outlier in outliers:
30         print(outlier)
31 else:
32     print("\nNo outliers detected in the data.")

```

12.3.16 Python Script used for Calculating the Pearson Correlation Between FMA and the Different Graph Theory Measures. Note: The Same Script was used for Structural and Functional Graph Measures. Only the Input File Changed.

```

1
2 import pandas as pd
3 import numpy as np
4 from scipy import stats
5 import matplotlib.pyplot as plt
6
7 # Read the Excel file
8 df = pd.read_excel('/content/FC.xlsx')
9
10 # Define group mappings
11 group_map = {
12     '1': 'TA',
13     '2': 'TA+PT',
14     '3': 'SA+PT'
15 }
16
17 # Create separate dataframes for each group
18 groups = {}
19 for group_num in ['1', '2', '3']:
20     # Before data (b)

```

```

21     before = df[df['Group'].str.startswith(group_num + 'b')]
22     # After data (a)
23     after = df[df['Group'].str.startswith(group_num + 'a')]
24     groups[group_map[group_num]] = {'before': before, 'after': after}
25
26 # Variables to correlate with FMA
27 variables = ['Cp', 'Lp', 'Sigma', 'Eg', 'Eloc']
28
29 # Colors for plotting (darker shades)
30 before_color = '#4B0082' # Darker purple (Indigo)
31 after_color = '#FF4500' # Darker orange (OrangeRed)
32 fit_line_color = 'grey'
33
34 # Iterate through variables and groups to create separate plots
35 for var in variables:
36     for group_name, group_data in groups.items():
37         # Create a new figure for each plot
38         plt.figure(figsize=(6, 5))
39
40         # Get data
41         before_fma = group_data['before']['FMA']
42         before_var = group_data['before'][var]
43         after_fma = group_data['after']['FMA']
44         after_var = group_data['after'][var]
45
46         # Combine before and after data for correlation
47         all_fma = pd.concat([before_fma, after_fma])
48         all_var = pd.concat([before_var, after_var])
49
50         # Calculate Pearson correlation
51         r, p = stats.pearsonr(all_fma, all_var)
52
53         # Plot scatter points
54         plt.scatter(before_var, before_fma, c=before_color, label='Before', alpha=0.6)
55         plt.scatter(after_var, after_fma, c=after_color, label='After', alpha=0.6)
56
57         # Calculate and plot best fit line
58         m, b = np.polyfit(all_var, all_fma, 1)
59         x = np.linspace(min(all_var), max(all_var), 100)
60         plt.plot(x, m*x + b, c=fit_line_color, linestyle='--')
61
62         # Customize plot
63         plt.xlabel(f'{var} (AUC)', fontweight='bold', fontsize=12)
64         plt.ylabel('FMA', fontweight='bold', fontsize=12)
65         #plt.title(f'{group_name}: FMA vs {var}')
66
67         # Add correlation info
68         plt.text(0.05, 0.95, f'r = {r:.3f}\np = {p:.3f}',
69                 transform=plt.gca().transAxes,
70                 verticalalignment='top')
71
72         # Add legend
73         plt.legend()
74
75         # Adjust layout
76         plt.tight_layout()
77
78         # Save the plot as a PNG file
79         filename = f'{group_name}_FMA_vs_{var}.png'

```

```

80     plt.savefig(filename, dpi=300, bbox_inches='tight')
81
82     # Close the figure to free memory
83     plt.close()
84
85     # Print correlation results
86     print("\nPearson Correlation Results:")
87     for var in variables:
88         print(f"\n{var}:")
89         for group_name in groups.keys():
90             all_fma = pd.concat([groups[group_name]['before']['FMA'],
91                                 groups[group_name]['after']['FMA']])
92             all_var = pd.concat([groups[group_name]['before'][var],
93                                 groups[group_name]['after'][var]])
94             r, p = stats.pearsonr(all_fma, all_var)
95             print(f"{group_name}: r = {r:.3f}, p = {p:.3f}")

```

12.3.17 Create FMA Line Graphs for Three Different Groups.

```

1  import pandas as pd
2  import numpy as np
3  import matplotlib.pyplot as plt
4
5  # Read the Excel file
6  data = pd.read_excel("FMA_winsor.xlsx")
7
8  # Define group mappings
9  group_map = {
10     '1': 'TA',
11     '2': 'TA+PT',
12     '3': 'SA+PT'
13 }
14
15 # Define colors for each group
16 colors = {
17     'TA': {'before': '#D8BFD8', 'after': '#800080'}, # Light purple, Dark purple
18     'TA+PT': {'before': '#FFDAB9', 'after': '#FF8C00'}, # Light orange, Dark orange
19     'SA+PT': {'before': '#98FB98', 'after': '#006400'} # Light green, Dark green
20 }
21
22 # Extract group number and condition from Group column
23 data['Group_Num'] = data['Group'].str[0] # Extract '1', '2', or '3'
24 data['Condition'] = data['Group'].str[1] # Extract 'b' or 'a'
25 data['Group_Name'] = data['Group_Num'].map(group_map) # Map to TA, TA+PT, SA+PT
26
27 # Function to calculate mean and standard error
28 def calc_stats(group_data):
29     mean = group_data['FMA'].mean()
30     std = group_data['FMA'].std()
31     n = len(group_data)
32     se = std / np.sqrt(n) if n > 1 else 0 # Standard error, 0 if n=1
33     return mean, se
34
35 # Create a single figure
36 fig, ax = plt.subplots(figsize=(8, 6))
37
38 # Process each group
39 for group_name in ['TA', 'TA+PT', 'SA+PT']:
40     # Filter data for the group

```

```

41     group_data = data[data['Group_Name'] == group_name]
42
43     # Filter before and after data
44     before_data = group_data[group_data['Condition'] == 'b']
45     after_data = group_data[group_data['Condition'] == 'a']
46
47     # Calculate mean and standard error
48     before_mean, before_se = calc_stats(before_data)
49     after_mean, after_se = calc_stats(after_data)
50
51     # Plot before dot (using dark 'after' color)
52     ax.errorbar(1, before_mean, yerr=before_se, fmt='o', color=colors[group_name]['
        after'],
53                capsizes=5)
54
55     # Plot after dot (using dark 'after' color)
56     ax.errorbar(1.05, after_mean, yerr=after_se, fmt='o', color=colors[group_name]['
        after'],
57                capsizes=5)
58
59     # Connect before and after with a solid line
60     ax.plot([1, 1.05], [before_mean, after_mean], linestyle='-',
61             color=colors[group_name]['after'], label=group_name, linewidth=2)
62
63     # Customize plot
64     ax.set_xticks([1, 1.05])
65     ax.set_xticklabels(['Before', 'After'])
66     ax.set_xlabel('Condition')
67     ax.set_ylabel('Mean FMA Score')
68     ax.set_title('FMA Values Across Groups')
69     ax.legend()
70     ax.grid(True, linestyle='--', alpha=0.7)
71
72     # Save plot
73     plt.tight_layout()
74     plt.savefig('fma_combined_plot.png')
75     plt.close(fig)

```