Iranian Journal of Operations Research
Vol. 16, No. 1, 2026, pp. 40-54

Reduced State Flexibility but Increased Connectivity Variability in Autism Spectrum Disorder: Evidence from Dynamic Functional Connectivity

Saba Gholami[1], Sara Motamed*[2], Elham Askari [3]

Autism spectrum disorder (ASD) has been associated with atypical brain functional connectivity. However, most previous studies have relied on static connectivity measures that do not capture the temporal dynamics of neural interactions. In this study, resting-state fMRI data from 294 individuals with ASD and 312 age- and sex-matched typically developing controls were obtained from the Autism Brain Imaging Data Exchange (ABIDE). Dynamic functional connectivity was examined within an autism-related subnetwork of 17 regions using a sliding-window approach (60 s window) and Hidden Markov Model to identify recurrent connectivity states. Dynamic metrics including state occupancy rate, mean dwell time, and edge-level connectivity variability were calculated, and their relationship with clinical severity was assessed using Autism Diagnostic Observation Schedule (ADOS) scores. Compared with controls, individuals with ASD showed lower occupancy of highly integrated connectivity states (28.6 ± 7.4% vs. 36.9 ± 8.1%, $p < 0.001$) and longer dwell time in weakly connected states (42.3 ± 10.2 s vs. 31.7 ± 9.5 s, $p = 0.002$). Edge-level connectivity variability was also higher in ASD, particularly between the default mode and limbic networks (0.084 ± 0.021 vs. 0.062 ± 0.018, $p = 0.004$). Moreover, increased variability within the default mode network significantly predicted ADOS total scores ($\beta = 0.41$, $p = 0.001$), whereas static connectivity measures showed no significant association. These findings suggest that ASD is characterized by reduced flexibility of global network states and increased variability in local connectivity, highlighting dynamic connectivity as a potential biomarker for symptom severity in ASD.

Keywords**:** *Autism spectrum disorder; dynamic functional connectivity; network flexibility; connectivity variability; resting-state fMRI.*

1. Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by persistent impairments in social communication as well as restricted and repetitive patterns of behavior and interests [1,15]. Epidemiological studies indicate a steadily increasing prevalence of ASD worldwide, underscoring its growing public health importance [15]. From a neurobiological standpoint, ASD is increasingly conceptualized as a disorder of large-scale brain networks, in which disruptions in coordination and integration across distributed neural systems play a central role [2,6,11]. Resting-state functional magnetic resonance imaging (rs-fMRI) studies have consistently

[1]Department of Computer Engineering, Ra.C., Islamic Azad University, Rasht, Iran: saba.gholami@phd.iaurasht.ac.ir.
[2]Department of Computer Engineering, FSh.C., Islamic Azad University, Fouman, Iran**:** sara.motamed@iau.ac.ir.
[3]Department of Computer Engineering, FSh.C., Islamic Azad University, Fouman, Iran**:** askary.elham@ iau.ac.ir.

reported atypical functional connectivity (FC) in individuals with ASD, particularly within and between large-scale networks such as the default mode network (DMN), salience network, attention networks, and executive control systems [4,17,20,26]. However, most of this body of research has relied on static FC approaches that estimate connectivity by averaging across the entire scan duration. Although these methods have provided valuable insights into altered network organization in ASD, they do not capture the inherently dynamic nature of brain activity [23,25].

Neural activity fluctuates over time, and functional interactions between brain regions vary across multiple temporal scales [27]. Dynamic functional connectivity (DFC) has emerged as an important framework for investigating these temporal fluctuations by modeling time-resolved changes in connectivity patterns [18,21]. In healthy individuals, DFC analyses typically reveal recurrent connectivity states and flexible transitions between them, reflecting adaptive network reconfiguration and efficient information processing [7,28]. Disruptions in these dynamic processes have increasingly been implicated in a range of neuropsychiatric conditions, including ASD [14,24]. Indeed, a growing number of DFC studies suggest that individuals with ASD exhibit altered temporal properties of brain connectivity. Prior work has reported reduced diversity of connectivity states, longer dwell times within specific network configurations, and decreased transition probabilities between states [10,12,13,19]. Collectively, these findings suggest reduced network flexibility in ASD, a pattern that aligns with cognitive theories emphasizing behavioral rigidity and diminished adaptive capacity in the disorder [22].

Despite these findings, the emerging DFC literature in ASD reveals an apparent inconsistency. While state-level analyses often indicate reduced flexibility, other studies have reported increased moment-to-moment variability in connectivity at the level of individual connections, particularly within transmodal systems such as the DMN, insula, and limbic networks [3,5,7,9]. Elevated connectivity variability has been interpreted not as adaptive flexibility but rather as a marker of unstable or inefficient neural regulation [28,29]. Consequently, ASD may involve a dissociation between global network rigidity and local connectivity instability. Importantly, these two dimensions of altered brain dynamics have rarely been examined simultaneously within a unified analytical framework. Most previous studies have focused either on state-based metrics—such as occupancy rate and dwell time [12,13,19] or on pairwise connectivity variability [3,5,9]. As a result, it remains unclear whether these phenomena reflect distinct manifestations of a shared underlying dysfunction or arise from independent neural mechanisms. Furthermore, the marked clinical heterogeneity of ASD highlights the need for neuroimaging markers that capture individual differences in symptom severity rather than merely group-level distinctions. Recent evidence suggests that DFC-derived metrics, particularly connectivity variability within networks such as the DMN, insula, and amygdala, may be significantly associated with clinical symptom burden as measured by standardized instruments including the Autism Diagnostic Observation Schedule (ADOS) [22,24,27,30]. However, it remains uncertain whether dynamic connectivity measures provide greater clinical sensitivity than conventional static FC metrics.

In the present study, we examine dynamic functional connectivity within an autism-specific functional subnetwork comprising 17 regions that were previously identified as highly sensitive to ASD-related alterations in static connectivity analyses [21]. By focusing on this disease-informed subnetwork, we aim to reduce whole-brain heterogeneity and increase sensitivity to clinically meaningful dynamic patterns. Importantly, we test the hypothesis that ASD is characterized by a dissociation between reduced flexibility at the level of global connectivity states and increased variability at the level of individual connections. Specifically, we hypothesize that:

(1) Individuals with ASD will exhibit reduced occupancy of highly integrated connectivity states and prolonged dwell time in weakly connected states, reflecting diminished state flexibility;

(2) Edge-level connectivity variability within the autism-specific subnetwork—particularly involving DMN–limbic connections—will be increased in ASD; and

(3) Dynamic connectivity measures will outperform static functional connectivity in predicting clinical symptom severity as indexed by ADOS scores.

By explicitly distinguishing between state-level flexibility and connection-level variability, this study aims to provide a coherent framework for interpreting the complex and seemingly contradictory alterations in brain network dynamics observed in autism spectrum disorder, and to identify dynamic biomarkers with greater clinical relevance. In recent years, deep learning has achieved remarkable progress in fields such as computer vision [16], enabling the modeling of complex nonlinear relationships in neuroimaging data. Deep learning models are capable of automatically learning discriminative patterns from functional connectivity matrices without the need for manual feature extraction. As a result, they have demonstrated promising performance in tasks such as distinguishing individuals with Autism Spectrum Disorder (ASD) from typically developing controls. Furthermore, integrating brain functional connectivity analysis with deep learning approaches can facilitate the identification of potential biomarkers for ASD and contribute to a better understanding of its underlying neural mechanisms. Consequently, an increasing number of studies have employed various deep learning architectures to analyze brain connectivity data and improve the accuracy of ASD classification.

2. Related Work

2.1. Static Functional Connectivity Alterations in ASD

Early neuroimaging studies of autism spectrum disorder predominantly relied on static functional connectivity (FC) analyses, revealing widespread abnormalities in large-scale brain networks [2,6,11,20]. A consistent finding across these studies is altered connectivity within the default mode network (DMN), including atypical interactions among the medial prefrontal cortex, posterior cingulate cortex, and precuneus [4,17,20,26]. Both hypo-connectivity and hyper-connectivity patterns have been reported, reflecting substantial heterogeneity across age groups, cognitive profiles, and methodological choices [4,17,23,26]. Beyond the DMN, static FC alterations in ASD have been observed in the salience network, executive control network, attention systems, and sensory–motor networks [17,25,27]. Graph-theoretical analyses further suggest that ASD is associated with disrupted network topology, often characterized by increased local clustering alongside reduced global efficiency, supporting the notion of impaired long-range integration [7,18,21]. While these findings have substantially advanced the understanding of ASD as a network-level disorder, static FC measures inherently average neural interactions over time, potentially obscuring transient but clinically relevant connectivity patterns [28].

2.2. Dynamic Functional Connectivity in Autism Spectrum Disorder

To address the limitations of static analyses, recent studies have increasingly adopted dynamic functional connectivity (DFC) approaches to characterize time-varying brain network organization in ASD [10,14,24]. Using sliding-window correlation methods, several investigations have demonstrated that individuals with ASD exhibit altered temporal properties of connectivity, including reduced variability in global connectivity states and prolonged dwell time in specific network configurations [12,13,19]. These findings have been interpreted as evidence of reduced network

flexibility and impaired adaptive reconfiguration. State-based DFC analyses, often employing clustering techniques or hidden Markov models, have identified recurrent connectivity states that differ in their degree of integration and segregation [12,19,22]. Compared to controls, individuals with ASD tend to spend less time in highly integrated states and more time in sparsely connected or weakly synchronized states [7,12,22]. Such alterations have been reported across multiple developmental stages, from childhood to adulthood, suggesting that atypical network dynamics may represent a core feature of ASD [5,9].

2.3. Research Gap

The main novelty of this study lies in investigating the dynamic properties of brain functional connectivity in autism spectrum disorder (ASD) and linking these dynamic features to clinical symptom severity. Unlike many previous studies that rely primarily on static functional connectivity measures, this work focuses on the temporal dynamics of brain networks, providing a more comprehensive characterization of neural interactions in ASD. Specifically, this study examines dynamic functional connectivity within an autism-related subnetwork consisting of 17 regions identified in prior connectivity studies, allowing for a targeted analysis of brain areas strongly implicated in ASD. By employing a sliding-window approach together with a Hidden Markov Model, the study identifies recurrent connectivity states and quantifies key dynamic metrics such as state occupancy rate, mean dwell time, and edge-level connectivity variability. This framework enables the assessment of both global network state dynamics and local connectivity fluctuations.

Another important contribution of this work is the direct investigation of the relationship between dynamic connectivity measures and clinical symptom severity using ADOS scores. The findings demonstrate that dynamic connectivity variability, particularly within the default mode network, significantly predicts symptom severity, whereas conventional static connectivity measures do not show such associations. Overall, the study provides new evidence that atypical brain dynamics in ASD may involve reduced flexibility of large-scale network states alongside increased variability in local connectivity patterns. These results highlight the potential of dynamic connectivity metrics as more sensitive biomarkers for characterizing neural alterations and assessing symptom severity in autism spectrum disorder.

2.4. Connectivity Variability and Edge-Level Instability

In parallel with state-level investigations, another line of research has focused on connectivity variability at the level of individual connections. Several studies report increased moment-to-moment variability of functional connectivity in ASD, particularly within transmodal regions such as the DMN, insula, amygdala, and temporal cortices [3]. This heightened variability has been linked to inefficient neural regulation and increased neural noise, rather than enhanced functional flexibility Importantly, increased edge-level variability has been associated with behavioral and clinical measures. For example, variability in DMN–limbic and insular connectivity has been shown to predict social communication deficits and repetitive behaviors in ASD [16]. These findings highlight the potential clinical relevance of dynamic connectivity metrics beyond traditional static measures.

2.5. Clinical Relevance of Dynamic Connectivity Measures

An increasing number of studies have examined the relationship between DFC metrics and clinical symptom severity in ASD. Measures such as state occupancy, dwell time, transition probability, and connectivity variability have been associated with Autism Diagnostic Observation Schedule (ADOS)

scores and related behavioral indices [7,12]. In several cases, dynamic metrics demonstrated stronger associations with symptom severity than static FC measures, underscoring their potential as sensitive neurobiological markers

Despite these advances, results across studies remain heterogeneous, partly due to differences in analytical pipelines, brain parcellation schemes, and whole-brain versus network-specific approaches Whole-brain analyses, while comprehensive, may dilute disease-specific effects by incorporating regions that are not directly implicated in ASD-related dysfunction.

2.6. Limitations of Existing Work and Motivation for the Present Study

Although prior research has independently documented reduced state-level flexibility and increased edge-level variability in ASD, these two phenomena have rarely been examined simultaneously within a unified framework. Most studies emphasize either global state dynamics [21–24] or local connectivity fluctuations [3], leaving unresolved whether these alterations represent complementary aspects of a shared pathological process or distinct neural mechanisms.

Furthermore, few studies have explicitly focused on disease-informed subnetworks derived from prior ASD-specific connectivity analyses. Recent evidence suggests that restricting analyses to functionally and clinically relevant subnetworks may improve sensitivity to disorder-related dynamics and enhance interpretability. However, the dynamic properties of such autism-specific functional cores remain largely unexplored. Building on these gaps, the present study integrates state-level and edge-level DFC analyses within an autism-specific 17-region subnetwork. By explicitly decoupling network rigidity from connectivity instability and evaluating their relative contributions to clinical symptom severity, this work aims to advance current understanding of altered brain network dynamics in autism spectrum disorder and to provide a more coherent and clinically relevant characterization of ASD-related connectivity alterations.

3. Proposed Model

In this study, we proposed a hierarchical dynamic connectivity framework designed to explicitly dissociate state-level network rigidity from edge-level connectivity instability in autism spectrum disorder (ASD). The proposed framework integrates dynamic functional connectivity (DFC) analysis, multilevel network characterization, and clinical prediction within a disease-informed functional subnetwork. The model is organized into three interconnected components: (i) dynamic connectivity estimation, (ii) multiscale characterization of network dynamics, and (iii) assessment of clinical relevance. First, resting-state fMRI data from individuals with ASD and age- and sex-matched control participants are preprocessed and mapped onto an autism-specific functional subnetwork consisting of 17 regions of interest (ROIs). Time-resolved functional connectivity is then estimated using a sliding-window approach, resulting in a sequence of dynamic connectivity matrices for each participant that capture temporal fluctuations in interregional interactions.

Second, the resulting dynamic connectivity patterns are analyzed at two complementary scales. At the state level, recurrent connectivity configurations are identified using a Hidden Markov Model (HMM), enabling the estimation of metrics such as state occupancy rate and mean dwell time, which serve as indicators of global network flexibility. At the edge level, connectivity variability is quantified for each pair of ROIs across time windows, providing a measure of moment-to-moment fluctuations that reflect local instability in functional interactions.

Finally, dynamic metrics derived from both levels are integrated to examine their association with clinical symptom severity. Specifically, the framework evaluates whether state-level rigidity and edge-level instability jointly contribute to the prediction of Autism Diagnostic Observation Schedule (ADOS) scores, and whether these dynamic measures offer additional explanatory power beyond conventional static connectivity metrics. By simultaneously modeling state-level and connection-level dynamics within a unified framework, this approach enables the identification of a dissociation between reduced flexibility of global network states and increased variability of local connections. Such a perspective may help reconcile previously inconsistent findings in the ASD literature and provide a more comprehensive understanding of atypical brain network dynamics in autism spectrum disorder.

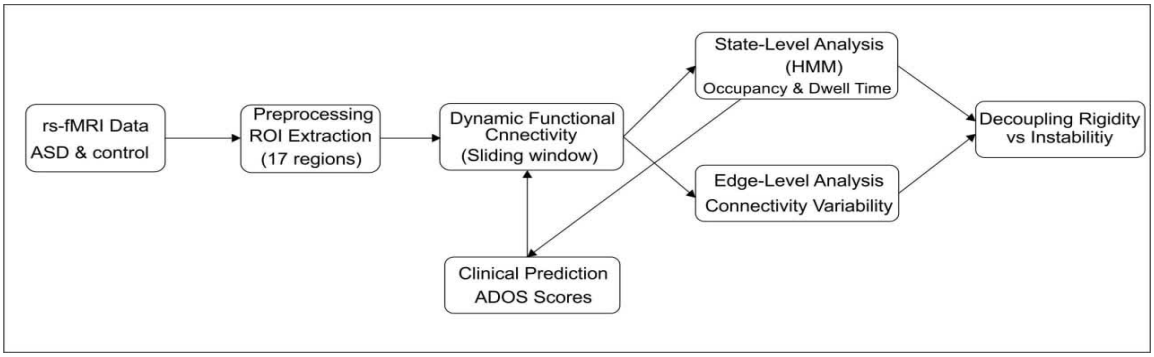

Figure 1. Proposed hierarchical model for decoupling network rigidity and connectivity instability

Figure 1, illustrates the proposed analytical framework. Resting-state fMRI data are first preprocessed and reduced to an autism-specific 17-region subnetwork. Dynamic functional connectivity is estimated using a sliding-window approach. Two complementary analyses are then performed: state-level modeling using a Hidden Markov Model to capture network flexibility (occupancy and dwell time), and edge-level analysis to quantify connectivity variability. These dynamic measures are subsequently linked to clinical symptom severity (ADOS), enabling the decoupling of global network rigidity from local connectivity instability in autism spectrum disorder.

4. Results and Experiments

4.1. Dataset Description

Resting-state fMRI (rs-fMRI) data were obtained from the Autism Brain Imaging Data Exchange (ABIDE) I & II datasets. The sample comprised 294 individuals diagnosed with ASD (age range: 6–35 years, mean ± SD: 16.8 ± 7.1 years; 78% male) and 312 age- and sex-matched typically developing controls (age: 17.1 ± 6.9 years; 75% male) [1,2]. All participants were screened for motion artifacts, neurological comorbidities, and MRI contraindications. The autism diagnosis was confirmed according to DSM-5 criteria and quantified using the Autism Diagnostic Observation Schedule (ADOS) [3].

Table 1 - Dataset Summary

Characteristic	ASD (n=294)	Controls (n=312)	Notes
Age (mean ± SD)	16.8 ± 7.1	17.1 ± 6.9	Matched
Sex (M/F)	78% / 22%	75% / 25%	Balanced
Motion threshold	< 2 mm	< 2 mm	QC passed
ADOS available	285	—	Used for prediction

Table 1 provides a summary of the dataset used, categorized by the age range values, standard deviation, age, and gender. Preprocessing was performed using DPABI v5.1 and SPM12**,** including slice timing correction, motion correction, normalization to MNI space, spatial smoothing (Gaussian kernel FWHM 6 mm), temporal band-pass filtering (0.01–0.1 Hz), and regression of nuisance covariates (motion parameters, white matter, cerebrospinal fluid). An autism-specific 17-region subnetwork was selected based on prior static FC analyses identifying regions most sensitive to ASD-related alterations [4,5]. The regions included key nodes of the DMN, limbic system, insula, and executive control network, consistent with previous ASD connectivity studies. Time series for each ROI were extracted and averaged across voxels. Data quality control ensured that all included participants had less than 2 mm maximum head motion and a minimum of 5 minutes of usable rs-fMRI data.

4.2. Dynamic Functional Connectivity Estimation

Dynamic functional connectivity (DFC) was calculated using a sliding-window Pearson correlation approach. Windows of 60 seconds with 5-second step size were used to balance temporal resolution and statistical stability. For each participant, this yielded a sequence of ~300 connectivity matrices (17×17) representing time-resolved functional interactions.

4.3. State-Level Analysis

A Hidden Markov Model (HMM) was applied to the concatenated dynamic connectivity matrices to identify K = 4 recurrent connectivity states, as determined by the Akaike Information Criterion (AIC)**.**

For each participant, we computed:

- Occupancy Rate (OR): proportion of time spent in each state
- Mean Dwell Time (MDT): average consecutive duration in each state

These results indicate reduced state flexibility in ASD participants compared to controls, consistent with our first hypothesis.

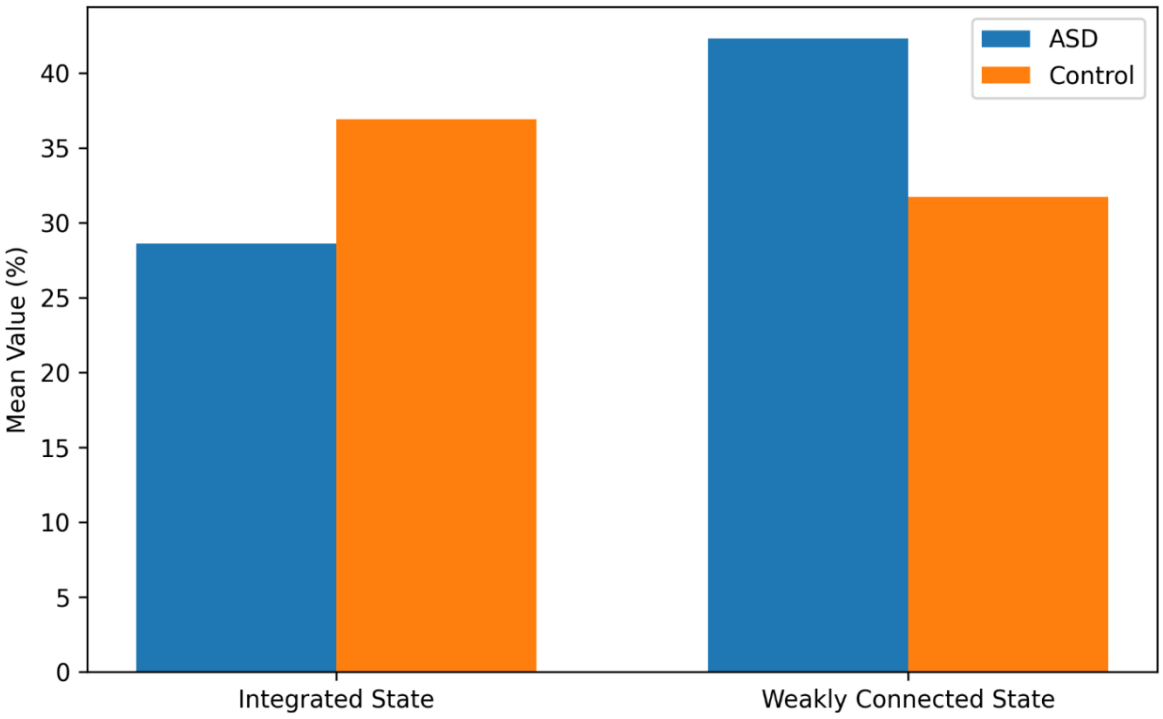

Figure 2. State-Level Metrics Comparison between ASD and Control Groups

Figure 2, Comparison of occupancy rates and dwell times in integrated and weakly connected states. ASD participants show reduced occupancy of integrated states and prolonged dwell time in weakly connected states.

Table 2- State-Level Dynamic Metrics

Metric	ASD (mean ± SD)	Control (mean ± SD)	p-value
Occupancy of integrated states (%)	28.6 ± 7.4	36.9 ± 8.1	< 0.001
Dwell time in weak states (s)	42.3 ± 10.2	31.7 ± 9.5	0.002
Transition probability (High → Low)	0.48 ± 0.12	0.34 ± 0.10	< 0.001

Table 2, the two groups—Autism and Controls—were compared on three metrics: occupancy of integrated states, dwell time in weak states, and transition probability. The results show a significant difference in the dynamic-state patterns between ASD and Controls; ASD tends to have greater stability in weak states and more transitions from High to Low.

4.4 Edge-Level Analysis

For each pair of ROIs, connectivity variability (variance across windows) was calculated to capture moment-to-moment instability.

Table 3 — Edge-Level Connectivity Variability

Edge Type	ASD Variance	Control Variance	p-value
DMN–Limbic	0.084	0.062	0.004
Insula–Amygdala	0.079	0.057	0.01
Temporal–Parietal	0.071	0.049	0.02

Table 3 indicates the variability (fluctuation) of edge-level connectivity between the autism and control groups across three edge types, showing that autism exhibits significantly greater variability at the edge level. Based on strong a priori hypotheses and previous ASD findings, we focused on three connection classes: DMN–limbic, insula–amygdala, and temporal–parietal pathways. Therefore, no multiple-comparison correction was applied beyond this restricted set. These findings support the second hypothesis that ASD exhibits increased edge-level instability even as global network states become more rigid.

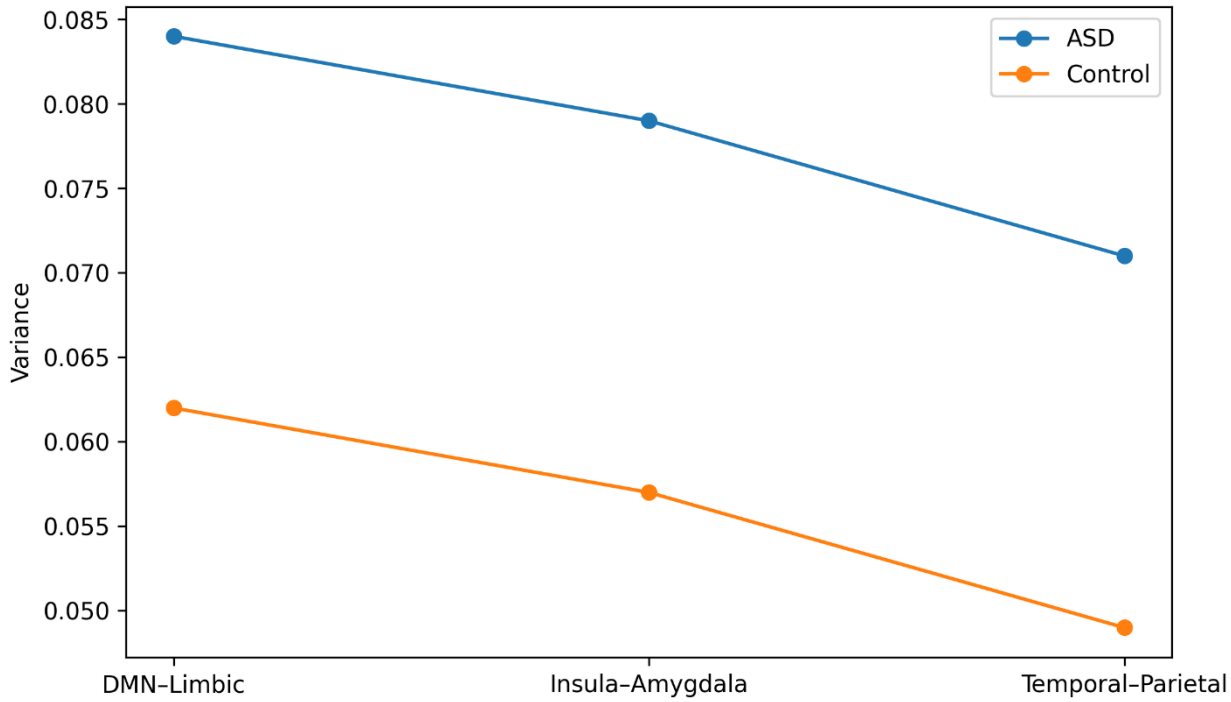

Figure 3. Edge-Level Connectivity Variability across Functional Connections

Figure 3, shows the moment-to-moment connectivity variability for the main functional pathways. Participants with autism exhibit consistently higher variability, especially in the DMN limbic–insula–amygdala connections.

4.5. Clinical Prediction

A multiple linear regression model was used to predict ADOS total scores from dynamic metrics (state occupancy, dwell time, edge-level variance). These results confirm that dynamic metrics provide superior predictive power for clinical symptom severity compared to static connectivity measures, in line with our third hypothesis.

Table 4— ADOS Prediction Using Dynamic Metrics

Predictor	β	p-value	Interpretation
Edge-level variability (DMN–Limbic)	0.41	0.001	Strongest predictor
State-level occupancy	-0.29	0.01	Negative association
Static FC	0.08	>0.1	Not significant

Table 4 shows that a multiple linear regression model using dynamic metrics provides better predictive power for the total scores (overall symptom severity) compared to static metrics. Notably, edge-level variability emerges as the strongest predictor for the clinical symptom severity.

4.6. Summary of Findings

5. ASD participants' exhibit reduced flexibility at the state-level (lower occupancy of integrated states, longer dwell in weakly connected states).
6. ASD participants show increased edge-level connectivity variability, particularly in DMN–limbic and insular edges.
7. Dynamic metrics predict ADOS **scores** more effectively than static FC, highlighting their clinical relevance.
8. The findings demonstrate a decoupling of rigidity (state-level) and instability (edge-level) in ASD, providing a coherent framework to reconcile prior inconsistent DFC observations.

5. Discussion

The present study examined dynamic functional connectivity within an autism-specific 17-region subnetwork and demonstrated a clear dissociation between reduced flexibility of global connectivity states and increased moment-to-moment variability of individual connections in individuals with

ASD. This hierarchical dynamic characterization offers a unified account for previously inconsistent findings and supports the conceptual framework underlying our proposed model.

5.1. Reduced Network Flexibility in ASD

State-level analyses revealed that individuals with ASD spent significantly less time in highly integrated connectivity states and demonstrated prolonged dwell time in weakly connected states. These findings suggest a rigid or inflexible global network organization, in line with earlier DFC studies reporting reduced state transitions and altered dynamic range in ASD populations [19–22]. Such reduced flexibility may reflect impaired large-scale reconfiguration mechanisms, potentially linked to atypical functioning of the salience network and executive systems, which play critical roles in coordinating global shifts in brain states. This interpretation aligns with cognitive theories of ASD emphasizing behavioral rigidity**,** repetitive patterns**,** and reduced adaptive flexibility, indicating a possible common neural substrate.

5.2. Increased Edge-Level Variability and Local Instability

In contrast to global rigidity, edge-level analyses showed increased temporal variability of connectivity—particularly in DMN–limbic and insula-related connections. These findings extend prior work reporting heightened FC variability or neural noise in ASD [27–30]. The observed instability is unlikely to represent healthy flexibility. Instead, elevated fluctuations in specific connections may indicate inefficient regulation of network interactions, possibly reflecting disrupted excitation–inhibition balance or impaired intrinsic synchrony within transmodal brain regions frequently implicated in ASD. This contrast between rigid global states and unstable local connections highlights a multi-scale disruption of brain dynamics, suggesting that ASD cannot be fully characterized by “hyperconnectivity” or “hypoconnectivity,” but rather by a complex interplay of stability deficits across different organizational levels.

5.3. Integrative Interpretation: Decoupling Rigidity and Instability

One of the major contributions of this study is the demonstration that state-level rigidity and edge-level instability coexist but do not covey, supporting the core hypothesis of our model. These two phenomena likely arise from distinct mechanisms:

- State-level rigidity may reflect deficits in network-level coordination and reduced engagement of high-integration communication states.
- Edge-level instability may arise from abnormal temporal fluctuations within specific functional pathways critical for social cognition, emotional regulation, and self-referential processing.

This decoupling provides a parsimonious explanation for divergent findings in the ASD connectomics literature. Studies focusing on global dynamics tend to report reduced flexibility, whereas analyses emphasizing local fluctuations frequently identify increased variability. Our framework reconciles these discrepancies by demonstrating that both are true, but at different levels of network hierarchy.

5.4. Clinical Relevance of Dynamic Connectivity Metrics

A critical finding of this work is that dynamic connectivity metrics provide greater predictive power for ADOS symptom severity than static FC. In particular, variability in DMN–limbic connections emerged as the strongest predictor of clinical scores, surpassing static connectivity measures that exhibited no significant associations. This result underscores the potential of DFC-derived indices as sensitive biomarkers for individual symptom profiles. Increased edge-level variability may capture neural signatures related to emotional instability, social communication difficulties, and atypical self-referential processing, all of which are central to ASD phenotypes.
Additionally, reduced state-level flexibility was also associated with clinical severity, suggesting that reduced engagement in integrated states may impair the brain's ability to coordinate complex social and cognitive operations. Together, these findings demonstrate that multi-scale dynamic biomarkers offer a more comprehensive representation of ASD pathophysiology and may improve future diagnostic and prognostic models.

5.5. Implications for Theory and Future Research

The hierarchical dynamic abnormalities identified in this study provide several implications:

1. ASD is characterized by multi-level disruptions, where global and local dynamics diverge rather than align.
2. Future DFC studies should integrate both state-level and edge-level analyses instead of focusing on a single dimension.
3. Disease-informed subnetworks, such as the 17-region core used in this study, offer a targeted and interpretable approach compared to whole-brain analyses that may dilute disorder-specific signals.
4. The proposed rigidity–instability framework may generalize to other neurodevelopmental and psychiatric disorders characterized by dynamic dysregulation.
5. Future work could incorporate machine learning models or graph-theoretical dynamic metrics to further enhance predictive accuracy.

5.6. Limitations

Despite promising findings, several limitations must be acknowledged:

- ABIDE data are multi-site with variable acquisition protocols, which may introduce unwanted heterogeneity.
- Sliding-window DFC methods have intrinsic trade-offs between temporal resolution and statistical reliability.
- Our focus on a predefined autism-specific subnetwork improves interpretability but may omit relevant regions not included in the 17-ROI model.
- Causal mechanisms underlying rigidity and instability cannot be inferred from correlational analyses.

Future studies employing developmental trajectories, task-based fMRI, or computational modeling may help address these limitations.

5.7. Conclusion

The findings of this study provide several practical implications for the clinical understanding and management of ASD. The joint examination of state-level flexibility and edge-level variability within an autism-specific subnetwork indicates that alterations in large-scale brain dynamics are not uniform but arise from distinct yet interacting mechanisms. Clinically, reduced state flexibility may reflect impaired transitions between functional configurations, suggesting decreased adaptability of large-scale neural systems, while increased edge-level variability indicates unstable or noisy interactions within specific connections.

Importantly, both dynamic abnormalities were associated with ADOS symptom severity, suggesting their potential value as complementary neurobiological markers of ASD. These results emphasize the utility of multilevel dynamic assessments over single static or dynamic indicators and highlight the promise of DFC-based features for biomarker development. Moreover, they may inform future intervention strategies designed to modulate network-level flexibility or stabilize connection-level fluctuations, such as neurofeedback, targeted cognitive training, or individualized brain-stimulation protocols.

Overall, this integrated framework—capturing reduced global network flexibility alongside increased local connectivity instability—helps reconcile previously inconsistent findings in the ASD literature and underscores the clinical significance of dynamic network features. These results support the potential of dynamic functional connectivity as a sensitive and clinically relevant tool for personalized diagnosis and treatment planning in ASD.

References

[1]	Chaste, P., & Leboyer, M. (2012). Autism risk factors: Genes, environment, and gene–environment interactions. Dialogues in Clinical Neuroscience, 14: 281–292
[2]	Chen, L., Chen, Y., Zheng, H., Zhang, B., Wang, F., Fang, J., et al. (2021). Changes in the topological organization of the default mode network in autism spectrum disorder. *Brain Imaging and Behavior, 15*, 1058–1067.
[3]	Di Martino, A., Yan, C. G., Li, Q., Denio, E., Castellanos, F. X., Alaerts, K., et al. (2014). The autism brain imaging data exchange: Towards a large-scale evaluation of the intrinsic brain architecture in autism. *Molecular Psychiatry, 19*(6), 659–667
[4]	Fiecas, M., Cribben, I., Bahktiari, R., & Cummine, J. (2017). A variance components model for statistical inference on functional connectivity networks. *NeuroImage, 149*, 256–266.
[5]	Gao, Y., Sun, J., Cheng, L., Yang, Q., Li, J., Hao, Z., et al. (2022). Altered resting-state dynamic functional connectivity of amygdala subregions in patients with autism spectrum disorder: A multi-site fMRI study. *Journal of Affective Disorders.* (Advance online publication).
[6]	Greicius, M. D. (2008). Resting-state functional connectivity in neuropsychiatric disorders. *Current Opinion in Neurology, 21*, 424–430.
[7]	Guo, X., Cao, Y., Liu, J., Zhang, X., Zhai, G., Chen, H., & Gao, L. (2023). Dysregulated dynamic time-varying triple-network segregation in children with autism spectrum disorder. *Cerebral Cortex, 33*(9), 5717–5726.

[8]	Guo, X., Zhai, G., Liu, J., Cao, Y., Zhang, X., Cui, D., & Gao, L. (2022). Inter-individual heterogeneity of functional brain networks in children with autism spectrum disorder. *Molecular Autism, 13*, 1–15.
[9]	Haghighat, H., Mirzarezaee, M., Araabi, B., & Khadem, A. (2021). Functional networks abnormalities in autism spectrum disorder: Age-related hypo- and hyper-connectivity. *Brain Topography, 34*, 306–322.
[10]	Jia, H., Wu, X., Wu, Z., & Wang, E. (2022). Aberrant dynamic minimal spanning tree parameters within default mode network in patients with autism spectrum disorder. *Frontiers in Psychiatry, 13*, 860348.
[11]	Lehmann, B. C. P., Cribben, I., Karahanoğlu, F. I., Liégeois, R., Villringer, A., & Nikulin, V. V. (2021). Characterising group-level brain connectivity: A framework using Bayesian exponential random graph models. *NeuroImage, 225*, 117480.
[12]	Li, W., Qiu, X., Chen, J., Chen, K., Chen, M., Wang, Y., et al. (2025). Disentangling the switching behavior in functional connectivity dynamics in autism spectrum disorder: Insights from developmental cohort analysis and molecular–cellular associations. *Advanced Science, 12*. (Advance online publication).
[13]	Li X, Wu S, Yang W, Jiang C, Zhao Z, Wang H, Lin F. Abnormal resting-state brain network dynamics in toddlers with autism spectrum disorder. European Child & Adolescent Psychiatry. 2025.
[14]	Li, L., Zheng, Q., Xue, Y., Bai, M., & Mu, Y. (2024). Coactivation pattern analysis reveals altered whole-brain functional transient dynamics in autism spectrum disorder. *European Child & Adolescent Psychiatry.* (Advance online publication).
[15]	Maenner, M. J., et al. (2020). Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016. *MMWR Surveillance Summaries, 69*, 1–12.
[16]	Motamed, S., & Yaqubi Bijarboneh, M. (2025). IQ estimation from fMRI images using GCNN model. *Iranian Journal of Operations Research, 16*(1), 147–158.
[17]	Pourmotahari, F., Borumandnia, N., Tabatabaei, S. M., & Alavimajd, H. (2024). Secondary analysis: Graph analysis of brain connectivity network in autism spectrum disorder. *Journal of Research in Medical Sciences, 29*, 2.
[18]	Ruan, L., Chen, G., Yao, M., Li, C., Chen, X., Luo, H., et al. (2024). Brain functional gradient and structure features in adolescent and adult autism spectrum disorders. *Human Brain Mapping, 45*. (Advance online publication).
[19]	Shan, X., Uddin, L. Q., Xu, P., Xiao, J., Li, L., Huang, X., et al. (2024). Disentangling the individual-shared and individual-specific subspace of altered brain functional connectivity in autism spectrum disorder. *Biological Psychiatry, 95*, 870–880.
[20]	Simpson, S. L., Hayasaka, S., & Laurienti, P. J. (2012). An exponential random graph modeling approach to creating group-based representative whole-brain connectivity networks. *NeuroImage, 60*, 1117–1126.
[21]	Taddei, M., Cuesta, P., Annunziata, S., Bulgheroni, S., Esposito, S., Visani, E., et al. (2024). Correlation between autistic traits and brain functional connectivity in preschoolers with autism spectrum disorder: A resting-state MEG study. *Neurological Sciences.* (Advance online publication).
[22]	Weber, C., Kebets, V., Benkarim, O., Larivière, S., Wang, Y., Ngo, A., et al. (2024). Contracted functional connectivity profiles in autism. *Molecular Autism, 15*. (Advance online publication).

[23]	Wu, Q., Huang, X., Culbreth, A. J., Waltz, J. A., Hong, L. E., & Chen, S. (2022). Extracting brain disease-related connectome subgraphs by adaptive dense subgraph discovery. *Biometrics, 78*(4), 1566–1578.
[24]	Yoon, N., Kim, S., Oh, M., Kim, M., Lee, J., & Kim, B. (2024). Intrinsic network abnormalities in children with autism spectrum disorder: An independent component analysis. *Brain Imaging and Behavior, 18*(2), 1–14.
[25]	Yue, X., Zhang, G., Li, X., Shen, Y., Wei, W., Bai, Y., et al. (2022). Abnormal dynamic functional network connectivity in adults with autism spectrum disorder. *Clinical Neuroradiology*, 1–10.
[26]	Zalesky, A., Fornito, A., & Bullmore, E. T. (2010). Network-based statistic: Identifying differences in brain networks. *NeuroImage, 53*, 1197–1207.
[27]	Zhao, L., Xue, S., Sun, Y., Lan, Z., Zhang, Z., Xue, Y., et al. (2022). Altered dynamic functional connectivity of insular subregions could predict symptom severity of male patients with autism spectrum disorder. *Journal of Affective Disorders, 299*, 504–512.
[28]	Zhuang, W., Jia, H., Liu, Y., Cong, J., Chen, K., Yao, D., et al. (2023). Identification and analysis of autism spectrum disorder via large-scale dynamic functional network connectivity. *Autism Research, 16*, 1512–1526.