Aberrant Time-varying Cross-Network Interactions in Children With Attention-Deficit/Hyperactivity Disorder and Its Relation to Attention Deficits

Supplemental Information

Supplemental Methods and Materials

Dataset access and participant selection

Behavioral and brain imaging data acquired by researchers at New York University (Primary cohort) and Peking University (Replication cohort), and made available through the ADHD-200 consortium (1) were used in this study. Based on our stated study objective and recommendations on rsfMRI data characteristics, we used the following cohort/site inclusion criteria (i) Number of participants matched on age, gender, handedness, IQ and head motion and with good quality rsfMRI data in each group > 20, (ii) Number of rsfMRI scan volumes > 105 (~3.5 mins scan with TR = 2s), and (iii) TR <= 2s. Out of the eight independent sites who participated in the ADHD-200 collection, only NYU and PKU site conformed to our inclusion criteria and were therefore included in the present study. In light of concerns about the potential influence of head motion on functional connectivity findings (2-4), participants whose head motion range in each of six dimensions (three in translation and three in rotation) exceeded 1 voxel were excluded. We used an in-house group-matching algorithm (5, 6) to select subsets of ADHD and TD children from each dataset such that the two groups were well-matched on age, gender, IQ, handedness, and head motion. The algorithm identified a well-matched subset of 40 ADHD and 40 TD in the Primary cohort and 30 ADHD and 30 TD in the Replication cohort.

Detailed participant demographic information is reported in **Table 1**. Conners' Parent Rating Scale-Revised, Long Version and the ADHD Rating Scale were used to evaluate ADHD symptoms in the Primary and Replication cohorts, respectively

Description of Primary and Replication cohorts

Primary cohort: The Schedule of Affective Disorders and Schizophrenia for Children – Present and Lifetime Version (KSADS-PL) was used for ADHD diagnosis. The Conners' Parent Rating Scale-Revised, Long Version (CPRS-LV) was used for dimensional measures of ADHD symptoms. The Wechsler Abbreviated Scale of Intelligence (WASI) was used for IQ evaluation. The fMRI protocol had a repetition time (TR) of 2000ms, an echo time (TE) of 15ms, and 176 volumes in each session. Since not all participants had two rsfMRI sessions, only the first session was used in the analysis.

Replication cohort: The KSADS-PL was used for ADHD diagnosis. The ADHD Rating Scale (ADHD-RS) was used for dimensional measures of ADHD symptoms. The Wechsler Intelligence Scale for Chinese Children-Revised (WISCC-R) was used for IQ evaluation. The fMRI protocol had a TR of 2000ms, a TE of 30ms, and 236 volumes in one session.

Time-averaged cross-network interaction

We computed a network interaction index (NII) (7) to assess cross-network interactions among the three networks based on the hypothesized role of the SN in switching interactions with the CEN and DMN (8, 9). NII has the advantage of capturing interactions simultaneously among all three networks. Specifically, NII was computed as the

difference in correlation between SN and CEN time series and correlation between SN and DMN. The rationale here is that SN and CEN are typically co-activated during cognitively demanding tasks, while SN and DMN are typically anti-correlated (8, 10). NII thus captures the extent to which SN can temporally integrate itself with CEN and dissociate itself from DMN.

$$NII = f\left(CC_{SN,CEN}\right) - f(CC_{SN,DMN})$$

where

$$f(CC) = \frac{1}{2}\ln(\frac{1+CC}{1-CC})$$

CC is Pearson's correlation between the time series of two component networks, e.g., $CC_{\text{SN, DMN}}$ refers to correlation between the time series of SN and DMN. $f\left(CC_{SN,LCEN}\right)$ and $f\left(CC_{SN,RCEN}\right)$ were computed separately and then their average was used as $f\left(CC_{SN,CEN}\right)$. Larger NII values reflect more segregated cross-network interactions between the SN-CEN and SN-DMN systems in the context of the triple-network model. We then compared NII between ADHD and TD groups in each cohort using *t*-tests. Tests of NII normality are described below. Outliers were determined using median absolute deviation (11). Cutoffs were set at 3 absolute deviations greater or smaller than the median. Two TD participants from the Primary cohort, and one TD and two ADHD participants from the Replication cohort were excluded from further analyses.

Relation of time-averaged NII measures to clinical symptoms

The relation between time-averaged NII and individual clinical scores was investigated using Pearson's correlation and its significance was examined using a permutation testing procedure because of non-normal distribution of clinical scores in our samples (**Supplementary Figure S1**) (12). Specifically, in each permutation, individual clinical scores were randomly shuffled and a correlation coefficient was computed across participants. Correlation coefficients from 500 permutations were used to construct the empirical null distribution from which a *p* value was obtained. To further test whether NII is the best predictor against other potential predictors, a multiple linear regression analysis was conducted to model symptom severity from the effects of NII, age, IQ and scan-to-scan head motion.

Motion analysis

We conducted several additional analyses to rule out motion-related confounds. First, we examined whether time-averaged NII and variability of dynamic time-varying NII are correlated with head motion as assessed using scan-to-scan movement. Second, we used partial correlation to examine whether the correlation between both time-invariant NII and variability of dynamic time-varying NII and inattention scores holds even after removing the potential influence of head motion as assessed using scan-to-scan movement.

Controlling for confounding variables

We conducted regression analyses to investigate whether time-averaged NII and variability of dynamic time-varying NII values differ between the ADHD and TD groups after controlling for age, gender, handedness, IQ as well as scan-to-scan movement.

Examining the influence of sliding window shape on findings

We conducted additional analysis to test the robustness of our findings using an exponentially decaying sliding window (13, 14). Exponentially decaying weights were applied to each time point within a window as described in previous studies (13, 14). All other procedures remain unchanged (**Supplementary Figure S4**).

Supplemental Results

Normal distribution of time-averaged NII in each cohort

To examine whether time-averaged NII values are normally distributed, we conducted Shapiro-Wilk normality test (15) on time-averaged NII values in each cohort and found no rejection of the null hypothesis (normal distribution) in each cohort (Primary cohort: p=0.44; Replication cohort: p=0.15),

Differences in CEN- and DMN-centered time-averaged NII measures between ADHD and TD groups

Analogous to the SN-centered time-averaged NII, we computed CEN- and DMN-centered time-averaged NIIs. The CEN-centered time-averaged NII measured the difference

between CEN-SN and CEN-DMN couplings. The ADHD group had significantly smaller CEN-centered time-averaged NII values compared to the TD group in the Primary cohort (p<0.05) but not in the Replication cohort (p>0.6). Similarly, the DMN-centered time-averaged NII measured the difference between DMN-SN and DMN-CEN couplings. The DMN-centered NII was not significantly different between the two groups in any cohort (all ps>0.6). Thus, neither the CEN- nor the DMN-centered time-averaged NII was consistently different between the groups across the two cohort.

Ruling out potential confounds on between-group comparisons

Time-averaged NII values were significantly different between ADHD and TD groups in each cohort (all ps<0.05, **Supplementary Table S1**), even after controlling for the potential confounding effects of age, gender, handedness, IQ, range and movement parameter.

Variability of dynamic time-varying NII values were significantly different between ADHD and TD groups in each cohort (all ps<0.001, **Supplementary Table S2**), even after controlling for the potential confounding effects of age, gender, handedness, IQ, range and movement parameter.

NII in medication-naïve and non-naïve children with ADHD

Using the limited and inconsistent information available on medication status in the ADHD-200 cohort (see main text), we examined whether medication-naïve and non-naïve children with ADHD have different time-invariance and time-varying cross-network interactions in each cohort. In a well-matched subset of the ADHD group in the Primary

cohort, 11 participants were medication naïve, 8 participants were medication non-naive and the other participants did not have their medical status recorded. There was no significant difference in time-averaged NII between the medication-naïve and non-naive subgroups (t=1.7, p=0.11) and no significant difference in temporal variability of time-varying NII between the medication–naïve and non-naïve subgroups (t=0.81, p=0.43). In a well-matched subset of the ADHD group in the Replication cohort, 17 participants were medication naïve and 13 participants were medication non-naïve. There was no significant difference in NII scores between the medication-naïve and non-naïve subgroups (t=0.27, p=0.79) and no significant difference in temporal variability of time-varying NII between the medication–naïve and non-naïve subgroups (t=0.24, p=0.81).

Ruling out motion-related confound on brain-behavior relationship

We conducted several additional analyses to rule out motion-related confounds. We found no significant correlation between time-averaged NII values and head motion in each cohort (all ps>0.2). Next, partial correlation analysis revealed that the relation between NII values and inattention scores was preserved in each cohort even after controlling for head motion (Primary cohort: r=-0.23, p=0.04; Replication cohort: r=-0.27, p=0.04).

Similarly, we found no significant correlation between variability of dynamic time-varying NII values and head motion in each cohort (all ps>0.2). Partial correlation analysis revealed that the relation between variability of dynamic time-varying NII values and inattention scores was preserved in each cohort even after controlling for head motion (Primary cohort: r=0.41, p<0.001; Replication cohort: r=0.64, p<0.001).

Additional analyses using exponentially decaying sliding window

We conducted additional analyses to examine time-varying cross-network interaction using exponentially decaying sliding window.

Dynamic time-varying cross-network interactions

Analysis of dynamic functional interactions among SN, CEN and DMN revealed two states (temporal clusters) in the TD and five in ADHD group in the Primary cohort and two states in the TD and seven in ADHD group in the Replication cohort (**Supplementary Figure S5A**), reflecting variation in cross-network interactions across time in both groups. Permutation analysis revealed significantly more states in the ADHD than TD groups in the Primary and Replication cohorts (*ps*<0.005).

Next, we compared mean lifetime of dynamic brain states between the two groups. In the Primary cohort, the mean lifetime of state 1 in the TD group was significantly longer than the mean lifetime of any of the five states in ADHD group (*ps*<0.001). The mean lifetime of state 2 in the TD group was longer than the mean lifetime of all the five states in the ADHD group, but their significance did not survive after multiple comparison correction (*ps*<0.05, uncorrected). In the Replication cohort, the mean lifetime of states 1 and 2 in TD group was significantly longer than the mean lifetime of any states but the state 7 in the ADHD group (*ps*<0.05) (**Supplementary Figure S5B**). Bonferroni correction was used for multiple comparisons. These results demonstrate that, compared to TD, children with ADHD show less persistent and more volatile brain states.

We then compared NII of dynamic brain states between the two groups. In the Primary cohort, NII of states 3, 4 and 5 in the ADHD group was significantly lower than

NII of any of the states in the TD group (*ps*<0.05). In the Replication cohort, the NII of states 1, 2 and 5 in the ADHD group was significantly lower than NII of all states in the TD group (*ps*<0.01) and the NII of states 3 and 6 in the ADHD group was significantly higher than NII of states in the TD group (*ps*<0.01) (**Supplementary Figure S5C**). Bonferroni correction was used for multiple comparisons. These results demonstrate an intermittent lack of integration of the SN with the CEN and decoupling of the SN from the DMN in children with ADHD, and that cross-network interactions are more variable in children with ADHD than TD children.

Variability of dynamic time-varying cross-network interactions and its relation to inattention

Compared to TD children, children with ADHD showed greater variability in dynamic NII strength across states in both cohorts (*ps*<0.001) (**Supplementary Figure S6A)**. Additional analyses further confirmed lower NII values in ADHD than TD after controlling for confounds (**Supplementary Table S3**) and dynamic NII values were not correlated with head motion in both cohorts (*ps*>0.32). And there was no significant difference in temporal variability of time-varying NII between the medication–naïve and non-naïve subgroups in both cohorts (*ps*>0.14). Notably, we found that individual inattention scores were positively correlated with variability of time-varying NII measures in the Primary (*r*=0.55*, p*<0.002) and Replication (*r*=0.72*, p*=0.002) cohorts, despite the use of different clinical questionnaires at each cohort (**Supplementary Figure S6B)**. Individual hyperactivity/impulsivity scores were also positively correlated with variability of time-varying NII measures in the Primary (*r*=0.48*, p*<0.002) and Replication cohorts (*r*=0.51*,*

p<0.002) (**Supplementary Figure S6B)**. Multiple linear regression further demonstrated that variability of dynamic time-varying NII outperformed other variables in predicting clinical symptom scores (**Supplementary Table S4**).

Supplemental Discussion

The signs of SN-centered time-averaged NII are different between the two cohorts. The differential neurobiological correlates of (absolute) “positive” and “negative” fMRI-derived intrinsic connectivity values remain poorly understood. We speculate that the difference in signs could be attributed to different study protocols. For instance, in the Primary cohort, participants were verbally instructed to remain still with eye open while the word “Relax” was centrally displace (16), whereas, in the Replication cohort, participants were instructed to keep their eye close (17). It is known that eye open and close conditions differentially impact cerebral blood flow and oxygen consumption (18). Importantly, regardless of such differences, we found consistent reduction of the SN-centered time-averaged NII in ADHD relative to TD in the two cohorts.

Supplementary Table S1. Multiple linear regression revealed that, after controlling for all potential confounds, time-averaged NII were still significantly different between the TD and ADHD groups in the two cohorts.

	Primary cohort		Replication cohort	
	t	*p*	t	*p*
Group	-2.91	**0.005**	-2.19	**0.03**
Age	-0.1	0.91	-0.23	0.82
Gender	1.99	0.05		
Handedness	-1.05	0.29	0.93	0.36
IQ	0.7	0.49	-0.32	0.76
Scan-to-Scan Motion	-0.66	0.51	0.84	0.41

Supplementary Table S2. Multiple linear regression revealed that, after controlling for all potential confounds, variability of dynamic time-varying NII were still significantly different between the TD and ADHD groups in the two cohorts.

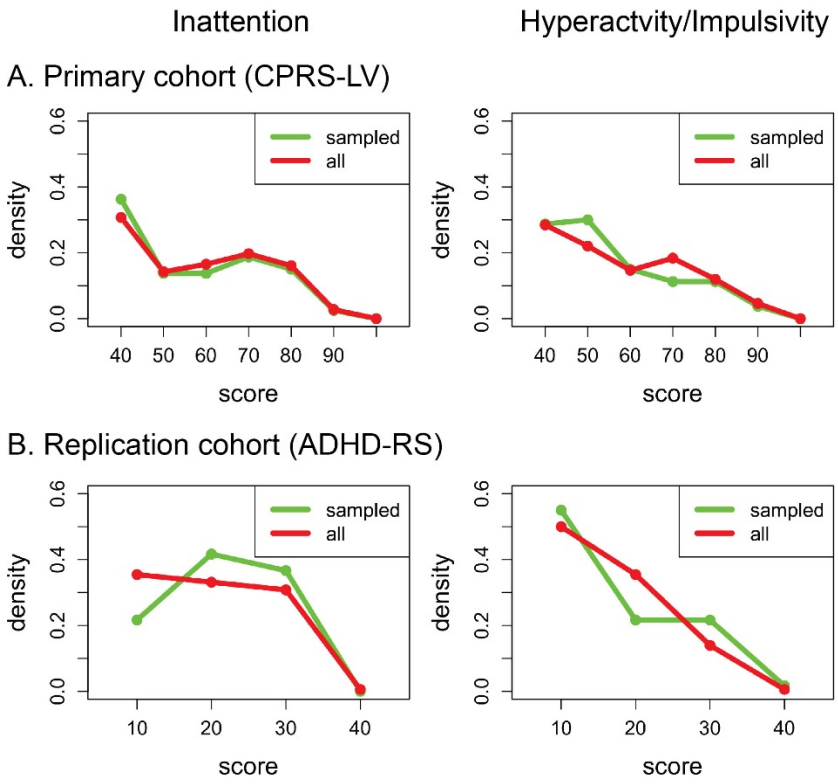
	Primary cohort		Replication cohort	
	t	*p*	t	*p*
Group	4.08	**0.001**	7.6	**0.001**
Age	-1.01	0.32	0.16	0.87
Gender	0.37	0.71		
Handedness	-0.34	0.74	0.57	0.57
IQ	0.77	0.45	-0.04	0.97
Scan-to-Scan Motion	-1.46	0.15	0.47	0.64

Supplementary Table S3. In the additional analyses using exponentially decaying window, multiple linear regression revealed that, after controlling for all potential confounds, variability of dynamic time-varying NII were still significantly different between the TD and ADHD groups in the two cohorts.

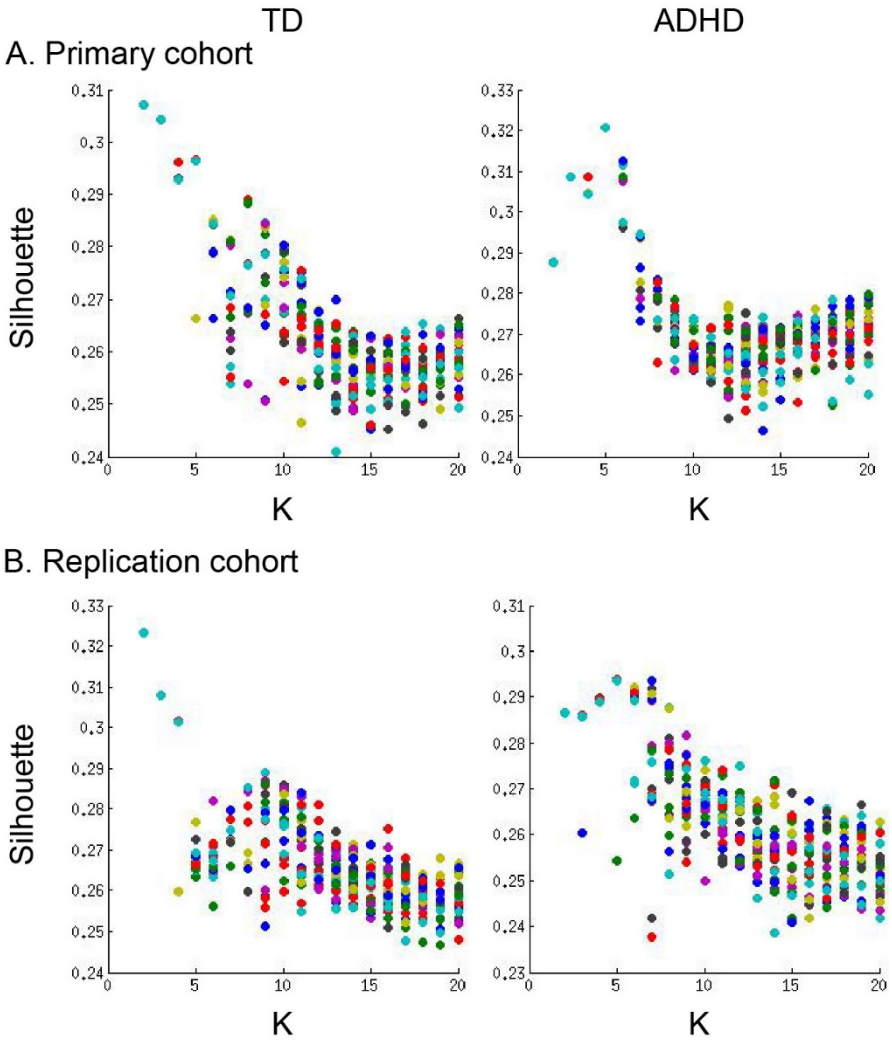
	Primary cohort		Replication cohort	
	t	*p*	t	*p*
Group	4.86	**0.001**	9.38	**0.001**
Age	-0.84	0.4	-0.54	0.59
Gender	0.48	0.63		
Handedness	-0.53	0.6	0.83	0.41
IQ	0.5	0.62	-0.2	0.84
Scan-to-Scan Motion	-1.57	0.12	-0.1	0.92

Supplementary Table S4. In the additional analyses using exponentially decaying window, multiple linear regression revealed that variability of dynamic time-varying NII were the most robust predictor of inattention symptoms in children with ADHD.

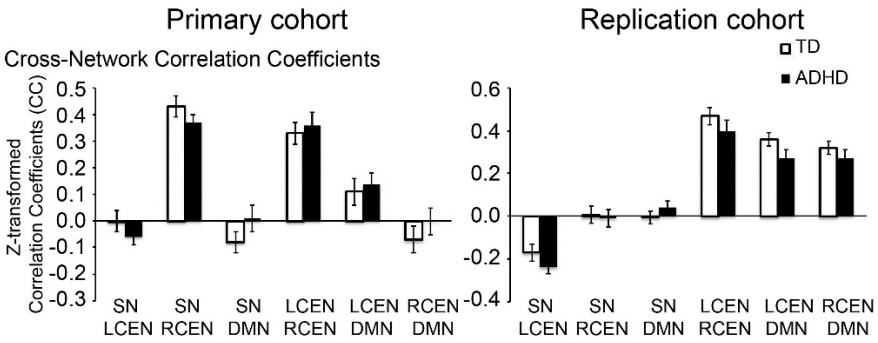
	Inattention			**Hyperactivity / Impulsivity**	
	Beta	*p*		Beta	*p*
Primary cohort					
NII	50.01	0.001		34.21	0.001
Motion	71.06	0.08		67.08	0.08
Age	0.04	0.93		-0.02	0.97
IQ	-0.01	0.52		0.008	0.28
Replication cohort					
NII	28.16	0.001		21.02	0.001
Motion	10.53	0.6		19.13	0.45
Age	-0.22	0.58		-0.78	0.13
IQ	-0.02	0.81		-0.01	0.97



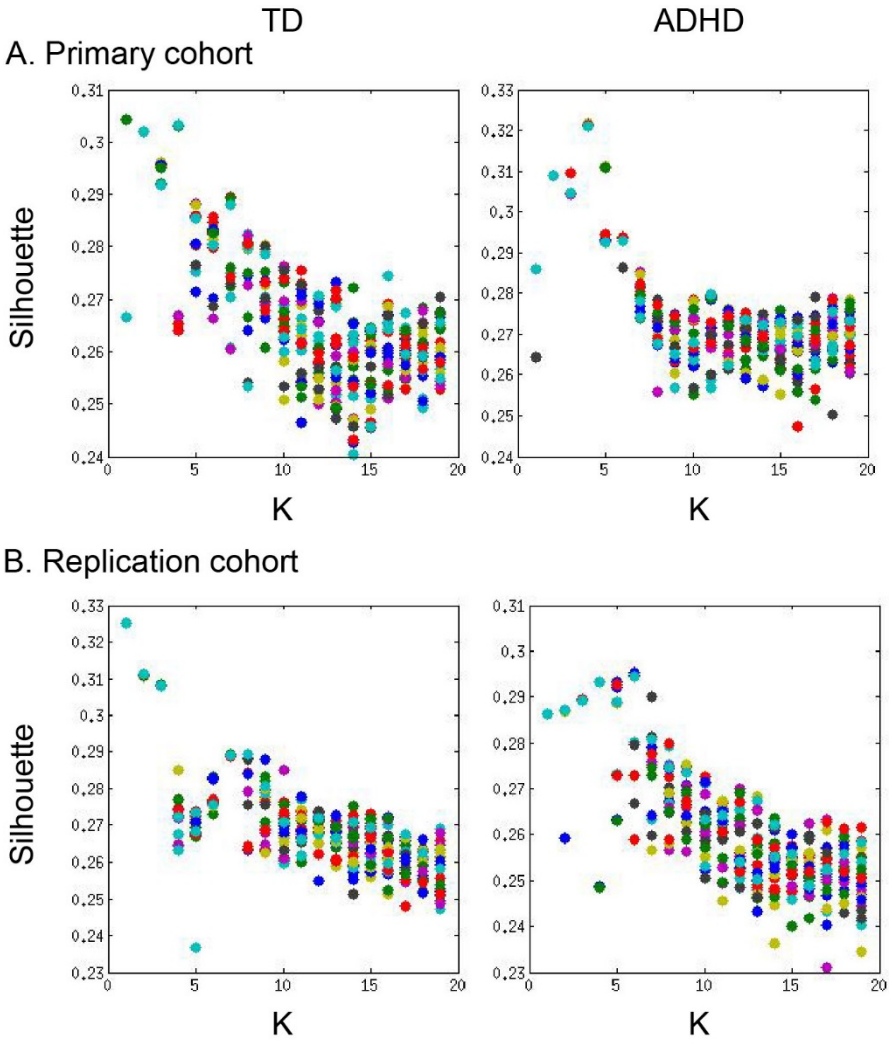
Supplementary Figure S1: Distribution of ADHD symptom (i.e., inattention and hyperactivity/impulsivity) severity in the sampled group (using a case-control strategy) matches well the distribution in the original group in the Primary and Replication cohorts.



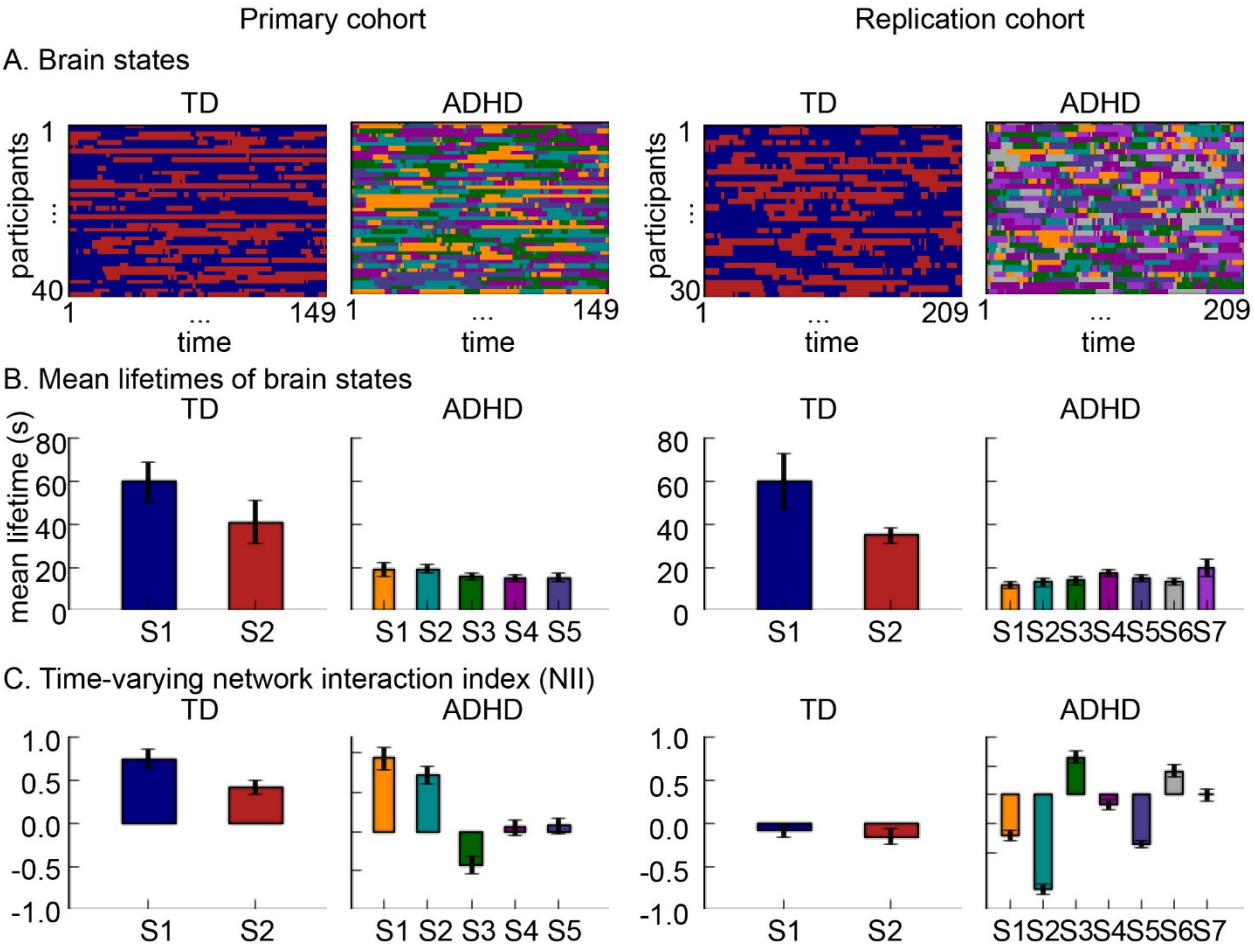
Supplementary Figure S2. The optimal number of temporal clusters was determined using the maximal silhouette obtained across multiple iterations in the TD and ADHD groups. In both the Primary and Replication cohorts, the TD group had maximal silhouette value for two clusters solution whereas the ADHD group had maximal silhouette value for five clusters solution. Silhouette is a measure for validating clustering, which evaluates how similar a data point is to its own cluster compared to other clusters. Each color represents a k-mean clustering performance with a random initialization (the number of clusters ranges from 2 to 20).



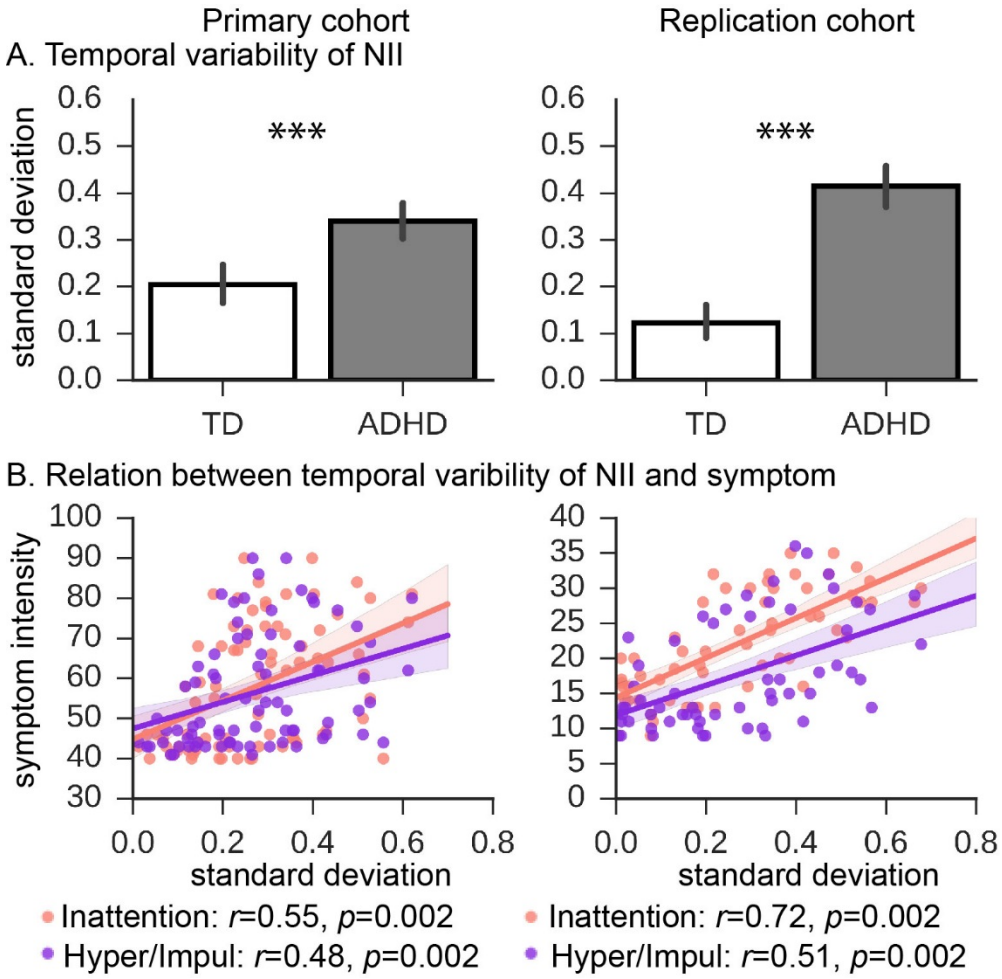
Supplementary Figure S3. Cross-network interactions between network pairs. Cross-network correlation coefficients between SN, LCEN, RCEN and DMN did not exhibit a consistent pattern of between-group differences across the two cohorts.



Supplementary Figure S4. Using exponentially decaying sliding window, the optimal number of temporal clusters was determined using the maximal silhouette obtained across multiple iterations in the TD and ADHD groups. In both the Primary and Replication cohorts, the TD group had maximal silhouette value for two clusters solution whereas the ADHD group had maximal silhouette value for five clusters solution in the Primary cohort and seven clusters solution in the Replication cohort. Silhouette is a measure for validating clustering, which evaluates how similar a data point is to its own cluster compared to other clusters. Each color represents a k-mean clustering performance with a random initialization (the number of clusters ranges from 2 to 20).



Supplementary Figure S5. Dynamic time-varying cross-network interactions among the SN, CEN, and DMN in children with ADHD and TD children in the additional analyses, using exponentially decaying sliding window. **(A)** In both the Primary and Replication cohorts, children with ADHD showed significantly higher number of states than TD children. Color codes distinct states in each participant. **(B)** Mean lifetimes of dynamic brain states were shorter in children with ADHD, compared to TD children in both cohorts. **(C)** NII of dynamic brain states shows intermittently weaker, and more variable, SN-centered cross-network interaction in children with ADHD compared to TD children in both cohorts.



Supplementary Figure S6. Variability of dynamic cross-network interactions among the SN, CEN and DMN in children with ADHD and TD children, and relation to ADHD symptoms children in the additional analyses, using exponentially decaying sliding window. **(A)** Temporal variability of dynamic cross-network interaction, assessed using standard deviation of dynamic NIIs across states, was significantly higher in ADHD, compared to TD children, in both cohorts. **(B)** Temporal variability of dynamic NIIs was strongly positively correlated with inattention and hyperactivity/impulsivity symptoms of ADHD in both cohorts. The Conners' Parent Rating Scale-Revised, Long Version and the ADHD Rating Scale were used as dimensional measures of ADHD symptoms in the Primary and Replication cohorts, respectively. ***, $p<0.001$.

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