

A Prospective Evaluation of Infant Cerebellar-Cerebral Functional Connectivity in Relation to Behavioral Development in Autism

Supplementary Information

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Infant Brain Imaging Study.

The Infant Brain Imaging Study (IBIS) is a longitudinal, multisite study of brain and behavioral development in infants at high and low familial risk for ASD (high risk: at least one older sibling with ASD; low-risk: no first- or second-degree relatives with ASD; refer to *Methods and Materials: Participants*). Neuroimaging (fcMRI, structural MRI, diffusion tensor imaging), eye-tracking, and behavioral (clinician-administered assessments and parent-reports) data were collected at 6, 12, and 24 months. A subset of infants also provided eye-tracking and behavioral data at 9 and 15 months. Given rapid development during the first two years of life, assessment and survey batteries necessarily varied over time; hence, we focused analyses on primary (6, 12, 24 month) rather than secondary (9, 12 month) data collection timepoints. Specifically, we evaluated 6-month fcMRI in relation to 12- and 24-month behaviors and/or outcomes because we were interested in the question of presymptomatic risk, and evidence suggests that early (first year of life) brain biomarkers precede the emergence of later (second year of life) ASD-associated behaviors (1). fcMRI was well-suited to our goals because it can be acquired in sleeping infants with and without ASD, and networks derived from fcMRI data provide insights into the large-scale organization of brain function (2–4).

Functional MRI processing.

Functional MRI processing, as previously described (5,6), included slice-dependent time shift adjustment, head movement quantification for spatial realignment within and across runs, whole brain image intensity normalization to a mode of 1000 (7), and registration to standardized 3-mm isotropic atlas space through affine transformation. In addition, the following updates were implemented to improve data quality: atlas registration was optimized, averaged functional volumes were generated for registration using all movement-censored frames, and calculated field map distortion correction (8) was implemented (<https://4dfp.readthedocs.io/>).

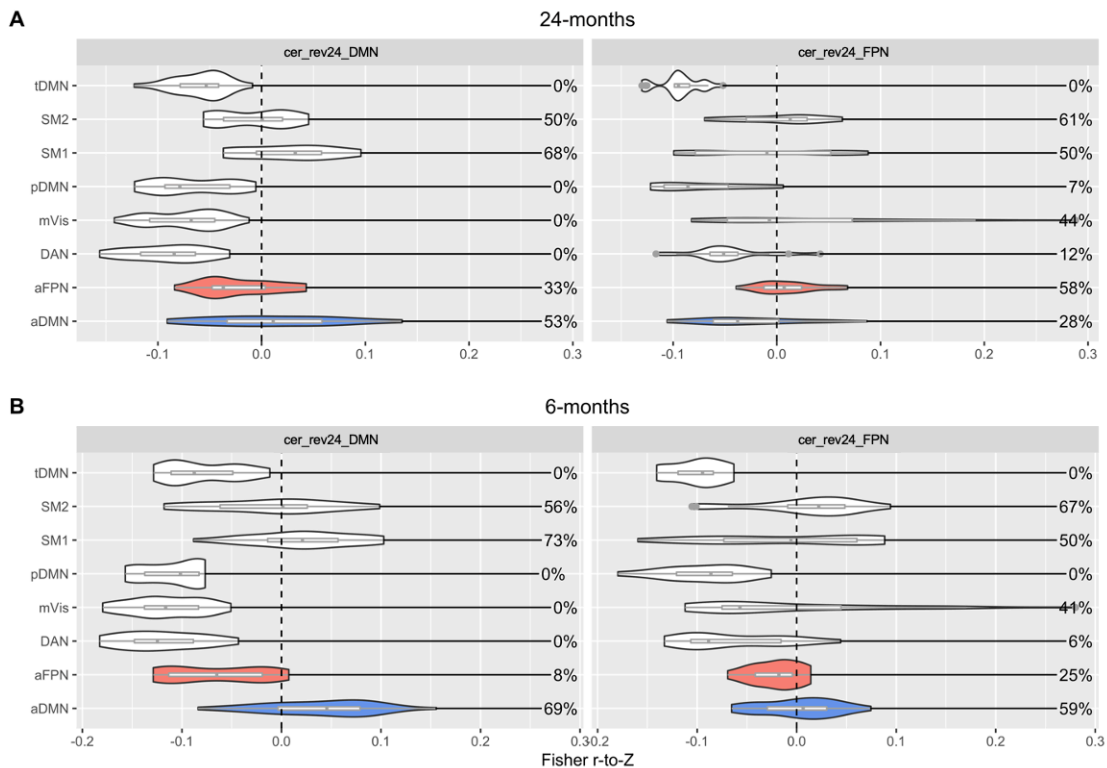
ROI placement.

I. Expanded methods. Given the longitudinal nature of our research questions, average FPN and DMN timeseries were computed using ROIs for which network assignments were stable across development, evaluated using toddler (5), adult (9), and validated in-house (combined 6- and 12-month) network solutions. Stable FPN ROIs ($n = 12$) were localized to anterior regions of the brain; likewise, we also restricted stable DMN ROIs ($n = 16$) to anterior regions of the brain. ROI placement was determined in an independent, mixed (HR+, HR-, LR-) sample of 24-month children to avoid biasing results, and it was visually inspected in 6-month infant data to ensure blood oxygen level dependent (BOLD) signal capture. Visual inspection identified 24 ROIs with insufficient BOLD signal capture (low-signal ROIs), distributed across 21 subjects (HR+ = 1, HR- = 12, LR- = 8). In univariate analyses, low-signal ROIs were filtered from subject-level datasets using case-wise deletion, and regression coefficients were obtained using maximum likelihood estimation. In multivariate and enrichment analyses, low-signal ROIs were retained to maximize sample size. Functional connectivity values derived from low-signal ROIs (0.2% of data) were normally distributed, with mean and standard deviation ($\bar{x} = -0.003$, $sd = 0.038$) comparable to high-signal ROIs ($\bar{x} = 0.013$, $sd = 0.023$).

II. Clarifications, considerations, and rationale. As described above, new cerebellar ROIs were centered on voxels that exhibited maximal correlations with average FPN or DMN timeseries in an independent, mixed (HR+, HR-, and LR-) 24-month sample. This approach does not identify regions that exhibit preferential connectivity with one (and only one) cortical network, nor does it determine network assignment (instead, see *Methods: Network derivation*).

Placing ROIs in an independent sample represents a strength of the current work because it mitigates biases expected to arise if ROIs were optimized in relation to non-generalizable characteristics of the analysis sample. Further, stipulating that cortical FPN and DMN ROIs exhibit stable network assignments across development (refer to section I., *Expanded methods*) allowed us to leverage what is known about network functionality in adults, an important design consideration given that task-based functional neuroimaging is extremely difficult in infants. Nonetheless, we acknowledge that new cerebellar ROIs were not derived from prior studies linking cerebellar connectivity to ASD, nor were they derived in a 6-month sample. Future research is necessary to develop and validate infant cerebellar functional parcellations, which may be used to further improve ROI placement.

III. Additional data. Plots depict distributions of group-average cerebellar-cerebral correlations (x-axis), organized by cerebellar ROI (vertical panels) and cortical network (y-axis). Cortical ROIs were required to exhibit stable network assignments across development. Shading (salmon and blue) identifies FPN and DMN ROIs that were used to reverse-seed the cerebellum; as described above, all were in anterior regions of the brain. Percentages (at right in plots) indicate the proportion of positive correlations. Correlation magnitude is consistent with the literature (10). (A) At 24-months, cerebellar ROIs show hypothesized patterns of connectivity ($\text{cer_rev24_DMN-aDMN} > \text{cer_rev24_DMN-aFPN}$; $\text{cer_rev24_FPN-aDMN} < \text{cer_rev24_FPN-aFPN}$).¹ (B) At 6-months, cerebellar ROIs placed in relation to the DMN show hypothesized patterns of connectivity ($\text{cer_rev24_DMN-aDMN} > \text{cer_rev24_DMN-aFPN}$), but cerebellar ROIs placed in relation to the FPN do not ($\text{cer_rev24_FPN-aFPN} < \text{cer_rev24_FPN-aDMN}$). The latter may be expected if infant cerebellar-FPN connectivity reflects presymptomatic risk for ASD: correlations will be attenuated if they are moderated by ASD risk and/or outcome. Alternatively, attenuated $\text{cer_rev24_FPN-aFPN}$ correlations may index developmental changes in network structure and/or function (11).



¹ We describe cerebellar ROIs using nomenclature (e.g., cer_rev24_DMN) intended to emphasize the process by which they were derived; namely, by reverse-seeding cortical FPN or DMN networks at 24-months. It does not follow that cerebellar ROIs must belong to the network they were seeded from, nor does it follow that they will exhibit maximal connectivity with that network (refer to section II., *Clarifications, considerations, and rationale*).

Cross-validated behavioral prediction.

Secondary validation analyses were conducted to verify that functional connections from enriched ($p < .01$) network pairs could be leveraged to predict behaviors relevant to ASD. Though circular (enrichment identified networks based on behavior; enrichment-derived networks were used to predict behavior), this approach nonetheless provided an important test of multimethod convergence and corroborated the behavioral significance of enriched network pairs. Poisson and linear regression were used to predict behaviors (as indicated by distributions), with principal component analysis (PCA) for feature reduction. Feature reduction (n components: [1, 10]), hyperparameter tuning (Poisson α : [0.0001, 3.2], logistic C : [0.0001, 10.0]), training, and testing were performed within 5-fold cross-validation. Empirical p -values were computed by comparing mean cross-validated prediction error of the best estimator (refit to full dataset) in real and randomized data ($n = 500$ runs).

Re-analysis of univariate associations.

Our primary analyses of univariate associations between 6-month cerebellar-cerebral (FPN, DMN) functional connections and later dimensional behaviors entailed FDR correction for $n=252$ comparisons (9 cerebellar ROIs x 28 FPN and DMN ROIs). Below, we describe the rationale for this decision, as well as the rationale motivating post-hoc tests. We will refer to cerebellar ROIs ($n=4$) placed in relation to the FPN and DMN as cer_FPN and cer_DMN ROIs, respectively. We note that primary analyses were adequately powered (Figure S3), and post-hoc tests were conceived after we observed null results in primary analyses. In both primary and post-hoc analyses, we adjusted for multiple comparisons within (rather than across) behaviors, thus lowering the bar for statistical significance.

Primary analyses. (1) Aggregation of significant results among cer_FPN-FPN connections would provide support for EBL theories of ASD. (2) Aggregation of significant results among cer_FPN-FPN and cer_DMN-DMN connections would suggest generalized cerebellar disruption in the context of intact functional organization; such results *may* be consistent with EBL theories of ASD *if* EBL is primarily cerebellar-mediated. (3) Aggregation of significant results among cer_DMN-DMN connections would suggest the importance of considering the cerebellum in the context of DMN disruption in ASD (12–15); however, such patterning (cer_DMN-DMN *but not* cer_FPN-FPN) is inconsistent with EBL theories of ASD. (4) Significant results distributed across cerebellar-cerebral connections (cer_FPN-DMN, cer_DMN-FPN) would suggest generalized cerebellar disruption unrelated to functional organization (perhaps due to ongoing network development), providing a base rate against which to evaluate aggregation in (1)-(3).

Post-hoc tests. To guard against false negatives, we re-analyzed data under conditions that provided more targeted tests of EBL and entailed less severe multiple comparison correction. First, we re-analyzed data using only cer_FPN-FPN and cer_DMN-DMN connections ($n=52$). Prior to FDR correction, 7% of univariate tests were significant at empirical $p < .05$; however, no results remained after FDR correction at $q < .05$. Next, we re-analyzed data using only cer_FPN-FPN connections ($n=24$). Prior to FDR correction, 6% of univariate tests were significant at empirical $p < .05$; once again, no results remained after FDR correction at $q < .05$.

Table S1. Empirically-supported relationships among ASD-associated behaviors and error-based learning (EBL). We focus our review on oculomotor tasks of EBL (eye-blink conditioning, saccade accuracy and adaptation) because they are extensively researched, with well-described cerebellar circuitry (16–19). For a comprehensive review of other tasks used to study cerebellar-mediated EBL, refer to Kelly *et al.* (19). As evident below, multiple studies link EBL impairment to ASD-associated motor and social behaviors. Multiple studies also report EBL impairments in samples with ASD diagnoses and/or ASD symptoms. There is less support for a relationship between EBL and ASD-associated restricted and repetitive behavior (RRB). In the context of EBL, it has been suggested that RRB may serve a compensatory function to make the immediate environment more predictable (20,21). Our selection of behavioral measures (column 5) was designed to capture variation along axes of development relevant to ASD diagnosis and/or strongly related to EBL. However, these measures have not previously been directly associated with EBL. Future research is necessary to test associations among behavioral measures used in the present study and EBL.

Task	Citation + reference number	Ref. #	Associated outcome	Associated behavioral measure
Eye-blink conditioning	Piochon, Kloth, Grasselli, Titley, Nakayama, Hashimoto, <i>et al.</i> (2014)	(22)	Social behavior (in mice)	CSBS-IJA, ADOS SA
	Reeb-Sutherland, Levitt, and Fox (2012)	(23)	Social behavior (in infants)	CSBS-IJA, ADOS SA
	Oristaglio, Hyman West, Ghaffari, Lech, Verma, Harvey, <i>et al.</i> (2013)	(24)	ASD diagnosis	ADOS Total
Saccade accuracy and adaptation	Mosconi, Luna, Kay-Stacey, Nowinski, Rubin, Scudder, <i>et al.</i> (2013)	(18)	Motor functioning	MSEL Fine & Gross Motor
	Johnson, Rinehart, White, Millist, and Fielding (2013)	(25)	ASD diagnosis	ADOS Total
	Connolly, Rinehart, & Fielding (2016)	(26)	Motor functioning (in ADHD)	MSEL Fine & Gross Motor
	Unruh, McKinney, Bojanek, Fleming, Sweeney, and Mosconi (2021)	(27)	Social & motor behaviors	CSBS-IJA, ADOS SA, MSEL Fine & Gross Motor

** Unless specified, sample is assumed to be children and/or adults with and without ASD. Ref. # = reference number, CSBS = Communication and Symbolic Behavior Scales, IJA = initiation of joint attention, ADOS = Autism Diagnostic Observation Schedule, RRB = restricted interests and repetitive behaviors, SA = social affect, MSEL = Mullen Scales of Early Learning*

Table S2. Descriptive statistics (sample size, mean, standard deviation) segmented by outcome variable (far left column) and group (HR-, HR+, LR-). Sample size varied slightly across analyses in relation to outcome data availability.

	N			Mean frames			SD frames			Mean Score			SD Score		
	HR-	H+	LR-	HR-	H+	LR-	HR-	H+	LR-	HR-	H+	LR-	HR-	H+	LR-
Diagnosis	46	13	35	242.6	273.7	227.1	57.2	69.9	47.7	NA	NA	NA	NA	NA	NA
CSBS IJA	36	11	29	241.6	278.5	229.2	57.5	75.5	46.1	1.5	0.6	1.7	1.3	0.7	1.6
MSEL Fine Motor	39	13	29	243.3	273.7	225.1	56.3	69.9	48.5	55.0	51.8	59.8	7.3	11.4	10.5
MSEL Gross Motor	39	13	29	243.3	273.7	225.1	56.3	69.9	48.5	48.8	44.5	50.5	13.4	13.2	11.6
RBS-R Restricted	39	13	27	240.9	273.7	225.4	56.5	69.9	48.8	0.4	0.5	0.1	0.9	1.2	0.4
RBS-R RitSame	39	13	27	240.9	273.7	225.4	56.5	69.9	48.8	0.5	1.6	0.4	0.9	3.8	0.7
ADOS RRB	46	13	35	242.6	273.7	227.1	57.2	69.9	47.7	2.6	6.1	2.1	2.2	3.1	1.8
ADOS Social	46	13	35	242.6	273.7	227.1	57.2	69.9	47.7	1.8	6.5	1.6	1.0	1.9	0.8
ADOS Total	46	13	34	242.6	273.7	226.5	57.2	69.9	48.3	1.5	6.2	1.3	0.8	2.0	0.6

N = sample size; *SD* = standard deviation; *CSBS* = *Communication and Symbolic Behavior Scales*, *IJA* = *initiation of joint attention*, *ADOS* = *Autism Diagnostic Observation Schedule*, *CSS* = *calibrated severity score*, *RRB* = *restricted interests and repetitive behaviors*, *SA* = *social affect*, *RBS-R* = *Repetitive Behavior Scale—Revised*, *MSEL* = *Mullen Scales of Early Learning*

Table S3. Neuroimaging exclusions by group. HR+, HR-, LR+ = familial risk and diagnostic information were both reported; HR, LR = familial risk was reported, but diagnostic information was unavailable; UNK = familial risk and diagnostic information were both unavailable.

Reason for exclusion	UNK	HR	HR-	HR+	LR	LR-
Excluded or inactive from IBIS	1	3	1	0	5	1
Failed initial image processing	2	1	7	1	0	6
Failed radiological review	1	1	0	0	0	0
Failed T1, T2, or BOLD quality control	2	2	13	4	0	9
Less than 150 motion-scrubbed frames	6	1	26	3	6	8
No dx information	8	9	0	0	9	0
No radiological review at time of analysis	0	0	2	0	0	2
Problems during image acquisition	2	1	12	3	4	6

HR = high risk, LR = low risk, UNK = unknown

Table S4. ROI coordinates (Talairach [X, Y, Z] and MNI [X, Y, Z]) and network assignments across development. Networks were derived in 6-month infants (6M-Net; present results), 12 and 24-month toddlers (12_24M_Net) (5), and adults (Adult_230_Net) (9). Cerebellar ROIs 231 and 234 were placed by reverse seeding the DMN in the right and left hemispheres, respectively; cerebellar ROIs 232 and 233 were placed by reverse seeding the FPN in the right and left hemispheres, respectively. Refer to primary Figure 3B for network names and abbreviations.

Key	Talairach			MNI			Network Solution			CBM_ROI
	X	Y	Z	X	Y	Z	6M	12-24M	Adult	
1	-23.00	-96.00	-15.00	-24.66	-97.84	-12.33	VIS	DAN	US	0
2	26.00	-96.00	-15.00	26.68	-97.30	-13.49	VIS	DAN	US	0
3	23.00	27.00	-12.00	23.96	31.94	-17.78	aFP	aFPC	US	0
4	-53.00	-45.00	-24.00	-56.16	-44.76	-24.23	DAN	DAN	US	0
5	8.00	36.00	-18.00	8.13	41.12	-24.31	aFP	aFPC	US	0
6	-20.00	-24.00	-18.00	-21.38	-22.22	-19.97	mVIS	pcDMN	DMN	0
7	-35.00	-30.00	-24.00	-37.26	-28.80	-25.58	DAN	DAN	US	0
8	62.00	-27.00	-15.00	64.60	-24.41	-18.57	tDMN	pFPC	DMN	0
9	50.00	-36.00	-24.00	51.79	-34.17	-27.23	DAN	DAN	US	0
10	53.00	-33.00	-14.00	55.18	-30.80	-16.93	tDMN	pFPC	US	0
11	32.00	33.00	-6.00	33.55	38.46	-12.03	aFP	Sal	FPC	0
12	-8.00	-54.00	57.00	-7.12	-52.22	60.71	SM1	SMN	Sal	0
13	8.00	-6.00	45.00	9.50	-1.84	44.73	SM1	SMN	Mot	0
14	-8.00	-24.00	63.00	-6.90	-20.59	65.21	SM1	SMN	Mot	0
15	-8.00	-36.00	69.00	-6.79	-33.09	72.27	SM1	SMN	Mot	0
16	-52.00	-25.00	41.00	-53.52	-22.54	43.10	SM2	SMN2	Mot	0
17	8.00	-48.00	69.00	9.94	-45.52	72.63	SM1	SMN	Mot	0
18	-39.00	-22.00	52.00	-39.63	-19.04	54.21	SM2	SMN	Mot	0
19	26.00	-42.00	57.00	28.54	-39.24	59.17	SM2	SMN2	Mot	0
20	47.00	-24.00	42.00	50.24	-20.37	41.74	SM2	SMN2	Mot	0
21	18.00	-32.00	58.00	20.21	-28.80	59.80	SM1	SMN	Mot	0
22	-29.00	-45.00	57.00	-29.10	-43.00	60.66	SM2	SMN2	Mot	0
23	20.00	-45.00	66.00	22.45	-42.29	68.99	SM1	SMN	Mot	0
24	-44.00	-34.00	44.00	-45.10	-31.85	46.63	SM2	SMN2	DAN	0
25	-21.00	-34.00	58.00	-20.66	-31.33	60.85	SM1	SMN	Mot	0
26	39.00	-24.00	54.00	42.14	-20.24	54.59	SM2	SMN2	Mot	0
27	35.00	-21.00	45.00	37.74	-17.30	45.01	SM2	SMN	Mot	0
28	-48.00	-14.00	34.00	-49.47	-11.06	34.95	MotM	CO	MotM	0
29	34.00	-13.00	16.00	36.04	-9.44	13.95	MotM	CO	MotM	0
30	48.00	-10.00	34.00	51.14	-5.80	32.42	MotM	CO	MotM	0
31	-51.00	-13.00	24.00	-52.84	-10.23	24.41	MotM	CO	MotM	0

32	62.00	-12.00	27.00	65.64	-7.88	24.83	MotM	CO	MotM	0
33	-4.00	-2.00	53.00	-2.88	2.38	53.21	CO	SMN	Mot	0
34	51.00	-31.00	34.00	54.22	-27.83	33.64	SM2	SMN2	CO	0
35	17.00	-12.00	63.00	19.33	-7.71	63.88	SM1	SMN	Mot	0
36	-11.00	-6.00	42.00	-10.48	-2.10	42.02	SM1	SMN	Mot	0
37	35.00	-3.00	0.00	36.73	0.78	-3.57	CO	CO	Mot	0
38	5.00	3.00	51.00	6.52	7.69	50.58	CO	SMN	Mot	0
39	-43.00	-3.00	10.00	-44.76	0.10	8.83	CO	CO	Mot	0
40	47.00	4.00	3.00	49.40	8.32	-1.12	CO	CO	Mot	0
41	-33.00	0.00	6.00	-34.37	3.29	4.19	CO	CO	Mot	0
42	-6.00	13.00	36.00	-5.33	17.80	34.41	CO	SMN	Sal	0
43	34.00	6.00	5.00	35.83	10.32	1.18	CO	CO	Sal	0
44	62.00	-36.00	21.00	65.43	-33.20	19.97	SM2	pCO	CO	0
45	55.00	-19.00	10.00	57.88	-15.62	7.49	pCO	pCO	CO	0
46	-37.00	-35.00	16.00	-38.43	-33.34	16.98	pCO	pCO	CO	0
47	-58.00	-27.00	13.00	-60.48	-25.22	13.82	pCO	pCO	CO	0
48	-47.00	-28.00	5.00	-49.14	-26.30	5.18	tDMN	tDMN	CO	0
49	41.00	-26.00	21.00	43.45	-22.93	19.85	SM2	pCO	Mot	0
50	-48.00	-36.00	24.00	-49.77	-34.36	25.74	SM2	pCO	CO	0
51	-51.00	-24.00	22.00	-52.92	-21.83	22.97	SM2	SMN2	Mot	0
52	-53.00	-12.00	12.00	-55.22	-9.42	11.73	MotM	CO	CO	0
53	53.00	-9.00	16.00	55.96	-5.03	13.25	MotM	CO	CO	0
54	56.00	-21.00	30.00	59.40	-17.34	28.69	SM2	SMN2	Mot	0
55	-29.00	-29.00	12.00	-30.12	-27.02	12.20	SM1	pCO	SubCtx	0
56	-39.00	-75.00	22.00	-40.50	-75.27	25.80	pDMN	pcDMN	DMN	0
57	5.00	60.00	3.00	5.55	66.69	-3.55	aDMN	aFPC	DMN	0
58	8.00	42.00	-9.00	8.36	47.59	-15.18	aDMN	aDMN	DMN	0
59	-17.00	57.00	-3.00	-17.65	63.19	-9.17	aFP	aFPC	US	0
60	-44.00	-61.00	18.00	-45.79	-60.69	20.85	tDMN	tDMN	DMN	0
61	41.00	-73.00	26.00	43.43	-72.21	28.00	pDMN	pcDMN	DMN	0
62	-41.00	9.00	-30.00	-43.58	11.99	-34.15	tDMN	tDMN	DMN	0
63	44.00	12.00	-24.00	45.64	16.20	-30.02	tDMN	tDMN	DMN	0
64	-55.00	-27.00	-14.00	-57.97	-25.69	-14.73	tDMN	pFPC	US	0
65	26.00	12.00	-12.00	27.06	16.22	-16.93	aDMN	aDMN	DMN	0
66	-43.00	-65.00	31.00	-44.45	-64.64	34.78	pDMN	pcDMN	DMN	0
67	-7.00	-56.00	25.00	-6.84	-54.90	27.05	pDMN	pcDMN	DMN	0
68	5.00	-60.00	33.00	5.91	-58.82	35.45	pDMN	pcDMN	DMN	0
69	-11.00	-57.00	14.00	-11.29	-56.20	15.60	pDMN	pcDMN	DMN	0
70	-3.00	-50.00	12.00	-2.94	-48.79	12.87	pDMN	pcDMN	DMN	0
71	7.00	-50.00	29.00	7.94	-48.37	30.57	pDMN	tDMN	DMN	0
72	14.00	-64.00	24.00	15.12	-63.09	25.98	pDMN	pcDMN	DMN	0

73	-3.00	-39.00	42.00	-2.20	-36.68	43.85	pDMN	pcDMN	DMN	0
74	10.00	-55.00	16.00	10.77	-53.83	17.09	pDMN	pcDMN	DMN	0
75	49.00	-61.00	34.00	52.04	-59.37	35.52	tDMN	pFPC	DMN	0
76	21.00	27.00	50.00	23.33	33.07	47.68	aDMN	aDMN	DMN	0
77	-17.00	23.00	54.00	-16.40	28.52	53.05	aDMN	aDMN	DMN	0
78	20.00	33.00	42.00	22.11	39.21	38.90	aDMN	aDMN	DMN	0
79	-20.00	39.00	42.00	-19.78	45.07	39.48	aDMN	aDMN	DMN	0
80	5.00	48.00	21.00	5.94	54.42	16.18	aDMN	aDMN	DMN	0
81	-7.00	45.00	4.00	-7.04	50.82	-1.29	aDMN	aDMN	DMN	0
82	8.00	48.00	9.00	8.80	54.23	3.45	aDMN	aDMN	DMN	0
83	-3.00	39.00	-4.00	-3.06	44.41	-9.46	aDMN	aDMN	DMN	0
84	7.00	37.00	0.00	7.51	42.49	-5.35	aDMN	aDMN	DMN	0
85	-11.00	39.00	12.00	-11.06	44.62	7.61	aDMN	aDMN	DMN	0
86	-3.00	32.00	39.00	-2.06	37.85	36.34	aDMN	aDMN	FPC	0
87	-3.00	36.00	20.00	-2.50	41.70	16.05	aDMN	aDMN	DMN	0
88	-8.00	42.00	27.00	-7.55	48.08	23.18	aDMN	aDMN	DMN	0
89	62.00	-15.00	-15.00	64.64	-11.80	-19.30	tDMN	tDMN	DMN	0
90	-53.00	-15.00	-9.00	-55.72	-12.96	-10.24	tDMN	tDMN	DMN	0
91	-55.00	-31.00	-4.00	-57.75	-29.70	-3.94	tDMN	tDMN	DMN	0
92	62.00	-33.00	-6.00	64.80	-30.55	-8.70	tDMN	pFPC	FPC	0
93	11.00	30.00	24.00	12.25	35.63	20.30	aDMN	aDMN	DMN	0
94	50.00	-6.00	-12.00	52.16	-2.43	-16.40	tDMN	tDMN	DMN	0
95	-25.00	-41.00	-8.00	-26.44	-39.95	-8.26	mVIS	pcDMN	DMN	0
96	26.00	-39.00	-11.00	26.94	-37.34	-12.76	mVIS	pcDMN	DMN	0
97	-32.00	-39.00	-15.00	-33.93	-38.06	-15.60	mVIS	pcDMN	DMN	0
98	28.00	-76.00	-31.00	28.46	-76.56	-31.64	VIS	CO	DMN	1
99	50.00	3.00	-24.00	51.90	6.81	-29.61	tDMN	tDMN	DMN	0
100	-50.00	0.00	-24.00	-52.89	2.55	-27.06	tDMN	tDMN	DMN	0
101	44.00	-52.00	28.00	46.68	-50.08	28.76	pDMN	tDMN	DMN	0
102	-47.00	-43.00	0.00	-49.30	-42.15	0.83	tDMN	tDMN	DMN	0
103	-29.00	15.00	-15.00	-30.63	18.71	-18.98	aDMN	aDMN	DMN	0
104	-3.00	-37.00	30.00	-2.47	-34.80	31.07	pDMN	pcDMN	DMN	0
105	-7.00	-72.00	38.00	-6.58	-71.47	41.74	pDMN	pcDMN	DMN	0
106	10.00	-67.00	39.00	11.27	-66.01	42.09	pDMN	pFPC	DMN	0
107	3.00	-50.00	48.00	4.20	-48.06	50.71	pDMN	pcDMN	DMN	0
108	-44.00	27.00	-9.00	-46.17	31.26	-13.03	aFP	Sal	DMN	0
109	47.00	30.00	-6.00	49.26	35.47	-12.20	aFP	Sal	DMN	0
110	8.00	-90.00	-9.00	7.98	-91.08	-7.10	VIS	DAN	Vis	0
111	17.00	-90.00	-15.00	17.27	-91.09	-13.64	VIS	DAN	US	0
112	-11.00	-93.00	-15.00	-12.08	-94.56	-12.80	VIS	DAN	US	0
113	17.00	-48.00	-9.00	17.53	-46.86	-9.88	mVIS	Vis	Vis	0

114	38.00	-73.00	13.00	39.98	-72.49	14.36	mVIS	pcDMN	Vis	0
115	8.00	-72.00	9.00	8.45	-71.84	10.79	mVIS	Vis	Vis	0
116	-8.00	-80.00	5.00	-8.43	-80.50	7.44	mVIS	Vis	Vis	0
117	-27.00	-79.00	16.00	-28.07	-79.45	19.43	mVIS	DAN	Vis	0
118	19.00	-66.00	1.00	19.81	-65.56	1.72	mVIS	Vis	Vis	0
119	-23.00	-90.00	15.00	-23.94	-90.98	18.96	mVIS	DAN	Vis	0
120	26.00	-60.00	-9.00	26.93	-59.37	-9.36	mVIS	DAN	Vis	0
121	-14.00	-72.00	-9.00	-15.02	-72.42	-7.68	mVIS	Vis	Vis	0
122	-17.00	-68.00	3.00	-17.87	-68.03	4.81	mVIS	Vis	Vis	0
123	41.00	-78.00	-12.00	42.52	-78.17	-11.78	DAN	DAN	Vis	0
124	-44.00	-75.00	-12.00	-46.54	-75.95	-9.95	DAN	DAN	Vis	0
125	-14.00	-90.00	27.00	-14.22	-90.66	31.40	mVIS	Vis	Vis	0
126	14.00	-87.00	33.00	15.27	-87.09	36.89	mVIS	Vis	Vis	0
127	27.00	-77.00	23.00	28.68	-76.62	25.42	mVIS	DAN	Vis	0
128	19.00	-85.00	-4.00	19.64	-85.62	-2.39	VIS	DAN	Vis	0
129	14.00	-77.00	28.00	15.18	-76.68	31.00	mVIS	Vis	Vis	0
130	-15.00	-53.00	-2.00	-15.85	-52.34	-1.43	mVIS	Vis	Vis	0
131	40.00	-66.00	-8.00	41.60	-65.50	-8.27	DAN	DAN	Vis	0
132	23.00	-87.00	21.00	24.41	-87.21	24.01	mVIS	Vis	Vis	0
133	5.00	-72.00	21.00	5.59	-71.65	23.52	mVIS	Vis	Vis	0
134	-40.00	-73.00	-2.00	-42.10	-73.62	0.38	DAN	DAN	DAN	0
135	25.00	-79.00	-16.00	25.66	-79.47	-15.56	mVIS	DAN	Vis	0
136	-16.00	-77.00	30.00	-16.21	-76.97	33.82	mVIS	Vis	Vis	0
137	-3.00	-81.00	18.00	-2.88	-81.25	21.10	mVIS	Vis	Vis	0
138	-38.00	-87.00	-9.00	-40.21	-88.44	-6.19	DAN	DAN	Vis	0
139	35.00	-84.00	11.00	36.76	-84.11	12.99	mVIS	DAN	Vis	0
140	6.00	-81.00	4.00	6.21	-81.41	6.11	mVIS	Vis	Vis	0
141	-25.00	-89.00	0.00	-26.39	-90.23	3.12	VIS	DAN	Vis	0
142	-31.00	-78.00	-15.00	-33.00	-79.02	-13.24	DAN	DAN	Vis	0
143	35.00	-81.00	0.00	36.51	-81.16	1.20	DAN	DAN	Vis	0
144	-43.00	-2.00	45.00	-43.93	1.80	45.70	CO	SMN	FPC	0
145	45.00	19.00	30.00	47.98	24.56	26.50	aFP	aFPC	FPC	0
146	-45.00	7.00	24.00	-46.50	10.85	23.04	aFP	SMN2	FPC	0
147	-51.00	-50.00	39.00	-52.60	-48.83	42.50	pFP	pFPC	FPC	0
148	56.00	-54.00	-12.00	58.31	-52.79	-13.61	DAN	DAN	FPC	0
149	23.00	39.00	-9.00	24.07	44.61	-15.35	aFP	aFPC	FPC	0
150	32.00	48.00	-6.00	33.60	54.22	-12.95	aFP	aFPC	FPC	0
151	17.00	-79.00	-34.00	16.85	-79.89	-34.36	aDMN	SubCtx	FPC	1
152	34.00	-67.00	-33.00	34.72	-67.08	-34.45	aDMN	SubCtx	FPC	1
153	44.00	5.00	35.00	47.01	9.93	32.66	aFP	aFPC	FPC	0
154	-40.00	2.00	33.00	-41.06	5.81	32.72	aFP	aFPC	FPC	0

155	-41.00	33.00	24.00	-42.23	38.21	21.35	aFP	aFPC	FPC	0
156	36.00	37.00	20.00	38.37	43.18	15.06	aFP	aFPC	FPC	0
157	46.00	-45.00	44.00	49.18	-42.41	45.16	pFP	pFPC	FPC	0
158	-28.00	-59.00	44.00	-28.40	-57.93	47.78	pFP	DAN	FPC	0
159	41.00	-55.00	45.00	43.93	-52.95	46.95	pFP	pFPC	FPC	0
160	35.00	-66.00	38.00	37.45	-64.70	40.38	pFP	pFPC	FPC	0
161	-41.00	-56.00	41.00	-42.09	-54.98	44.74	pFP	pFPC	FPC	0
162	37.00	13.00	42.00	39.87	18.39	39.72	aFP	aDMN	FPC	0
163	-33.00	49.00	9.00	-34.16	54.83	4.36	aFP	aFPC	FPC	0
164	-40.00	40.00	2.00	-41.68	45.16	-2.31	aFP	aFPC	FPC	0
165	31.00	-55.00	42.00	33.38	-53.12	44.02	pFP	DAN	FPC	0
166	41.00	43.00	4.00	43.25	49.25	-2.31	aFP	aFPC	FPC	0
167	-41.00	20.00	31.00	-42.10	24.68	29.53	aFP	aFPC	FPC	0
168	-4.00	21.00	46.00	-2.98	26.41	44.42	aDMN	aDMN	FPC	0
169	9.00	-41.00	48.00	10.51	-38.54	50.02	SM1	SMN	Sal	0
170	52.00	-47.00	36.00	55.27	-44.59	36.70	pFP	pFPC	FPC	0
171	39.00	-5.00	48.00	42.05	-0.39	47.10	CO	SMN	Sal	0
172	29.00	27.00	30.00	31.24	32.79	26.39	aFP	aFPC	Sal	0
173	45.00	17.00	14.00	47.60	22.16	9.74	aFP	aFPC	Sal	0
174	-34.00	16.00	3.00	-35.44	20.03	0.07	CO	CO	Sal	0
175	34.00	17.00	7.00	35.91	21.91	2.62	CO	CO	Sal	0
176	35.00	27.00	3.00	36.89	32.35	-2.24	CO	CO	Sal	0
177	32.00	12.00	-3.00	33.56	16.45	-7.58	aDMN	aDMN	Sal	0
178	-2.00	10.00	45.00	-0.94	14.86	43.99	aDMN	aDMN	Sal	0
179	-27.00	46.00	25.00	-27.50	52.04	21.28	aFP	aFPC	Sal	0
180	4.00	18.00	39.00	5.23	23.22	37.03	aDMN	SMN	Sal	0
181	9.00	17.00	30.00	10.26	22.06	27.48	aDMN	aDMN	Sal	0
182	29.00	49.00	20.00	31.07	55.71	14.49	aFP	aFPC	Sal	0
183	24.00	43.00	31.00	26.07	49.56	26.58	aFP	aFPC	Sal	0
184	-10.00	-21.00	8.00	-10.28	-18.48	7.04	SubC	SubCtx	SubCtx	0
185	11.00	-20.00	9.00	11.75	-17.18	7.54	SubC	SubCtx	SubCtx	0
186	-21.00	4.00	-2.00	-21.97	7.48	-4.78	CO	SubCtx	SubCtx	0
187	29.00	-17.00	4.00	30.50	-13.92	1.65	CO	SubCtx	SubCtx	0
188	22.00	6.00	5.00	23.26	10.19	1.46	CO	SubCtx	SubCtx	0
189	27.00	-3.00	7.00	28.52	0.82	4.01	CO	SubCtx	SubCtx	0
190	-30.00	-14.00	1.00	-31.38	-11.48	-0.30	MotM	SubCtx	SubCtx	0
191	51.00	-45.00	22.00	53.90	-42.76	21.83	tDMN	tDMN	Van	0
192	-54.00	-51.00	8.00	-56.47	-50.48	9.92	tDMN	tDMN	Van	0
193	-53.00	-41.00	12.00	-55.30	-39.89	13.51	tDMN	tDMN	Van	0
194	49.00	-35.00	9.00	51.52	-32.52	7.55	tDMN	tDMN	Van	0
195	49.00	-31.00	-2.00	51.28	-28.52	-4.30	tDMN	tDMN	DMN	0

196	53.00	-48.00	12.00	55.75	-46.07	11.42	tDMN	tDMN	Van	0
197	50.00	27.00	6.00	52.68	32.58	0.57	aFP	Sal	Van	0
198	-47.00	21.00	2.00	-49.07	25.13	-0.98	aFP	Sal	Van	0
199	22.00	-58.00	-22.00	22.43	-57.55	-23.11	mVIS	SubCtx	US	1
200	1.00	-62.00	-18.00	0.51	-61.91	-18.14	SubC	SubCtx	US	1
201	32.00	-15.00	-30.00	32.85	-12.41	-34.41	UNA	DAN	US	0
202	-29.00	-12.00	-33.00	-31.13	-9.99	-36.32	UNA	DAN	US	0
203	47.00	-6.00	-33.00	48.52	-2.85	-38.49	tDMN	tDMN	US	0
204	-47.00	-9.00	-36.00	-50.06	-7.09	-39.24	tDMN	tDMN	US	0
205	8.00	-63.00	57.00	9.61	-61.50	60.88	UNA	SMN	DAN	0
206	-50.00	-63.00	3.00	-52.44	-63.14	5.29	tDMN	DAN	DAN	0
207	-44.00	-51.00	-21.00	-46.68	-50.91	-20.91	DAN	DAN	DAN	0
208	44.00	-48.00	-15.00	45.68	-46.67	-16.85	DAN	DAN	DAN	0
209	44.00	-33.00	48.00	47.21	-29.75	48.70	SM2	SMN2	DAN	0
210	20.00	-66.00	45.00	21.90	-64.74	48.12	pFP	DAN	DAN	0
211	44.00	-60.00	4.00	46.09	-58.93	3.93	DAN	DAN	DAN	0
212	23.00	-60.00	57.00	25.34	-58.18	60.34	SM2	DAN	DAN	0
213	-32.00	-48.00	44.00	-32.56	-46.42	47.20	DAN	DAN	DAN	0
214	-26.00	-71.00	33.00	-26.60	-70.72	36.86	pFP	DAN	FPC	0
215	-32.00	-5.00	53.00	-32.23	-1.08	54.06	CO	SMN	DAN	0
216	-40.00	-60.00	-10.00	-42.26	-60.12	-8.85	DAN	DAN	DAN	0
217	-17.00	-60.00	60.00	-16.50	-58.57	64.46	pFP	DAN	DAN	0
218	26.00	-9.00	54.00	28.56	-4.62	53.99	CO	SMN2	DAN	0
219	48.00	10.00	22.00	50.91	14.99	18.54	aFP	SMN2	FPC	0
220	26.00	4.00	-4.00	27.23	7.96	-8.00	CO	SubCtx	SubCtx	0
221	-8.00	-12.00	58.00	-6.98	-8.08	59.20	SM1	SMN	Mot	0
222	-9.00	10.00	10.00	-9.10	14.14	7.23	aDMN	SubCtx	SubCtx	0
223	-48.00	-66.00	-8.00	-50.61	-66.47	-6.18	DAN	DAN	DAN	0
224	-28.00	42.00	-8.00	-29.34	47.21	-13.27	aFP	aFPC	FPC	0
225	-20.00	2.00	52.00	-19.65	6.39	52.29	aDMN	SMN2	FPC	0
226	20.00	-70.00	-9.00	20.61	-69.94	-8.61	mVIS	Vis	Vis	0
227	12.00	-78.00	38.00	13.31	-77.56	41.66	UNA	Vis	DMN	0
228	56.00	-8.00	-2.00	58.68	-4.28	-5.87	tDMN	tDMN	Van	0
229	39.00	-39.00	-20.00	40.35	-37.36	-22.56	DAN	DAN	DAN	0
230	-20.00	-22.00	64.00	-19.44	-18.61	66.42	SM1	SMN	Mot	0
231	34.00	-84.00	-39.00	34.53	-85.05	-39.74	aDMN	NA	NA	1
232	31.00	-75.00	-54.00	31.06	-75.90	-56.04	CO	NA	NA	1
233	-14.00	-78.00	-24.00	-15.39	-79.00	-23.14	VIS	NA	NA	1
234	-35.00	-51.00	-45.00	-37.82	-51.25	-46.45	aDMN	NA	NA	1

Figure S1. Spring-embedded graphs (28) visualize ROI affiliations across a range (2%-10%) of edge densities. Cortical ROIs are spherical, cerebellar ROIs are square, and colors denote consensus network assignment (see Figure 3B).

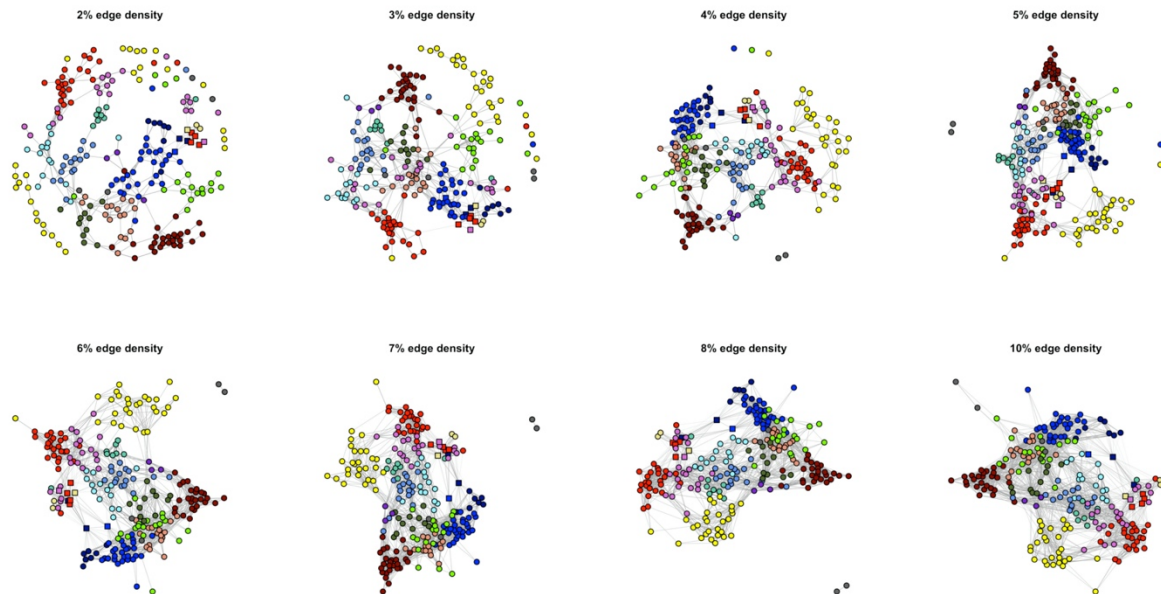
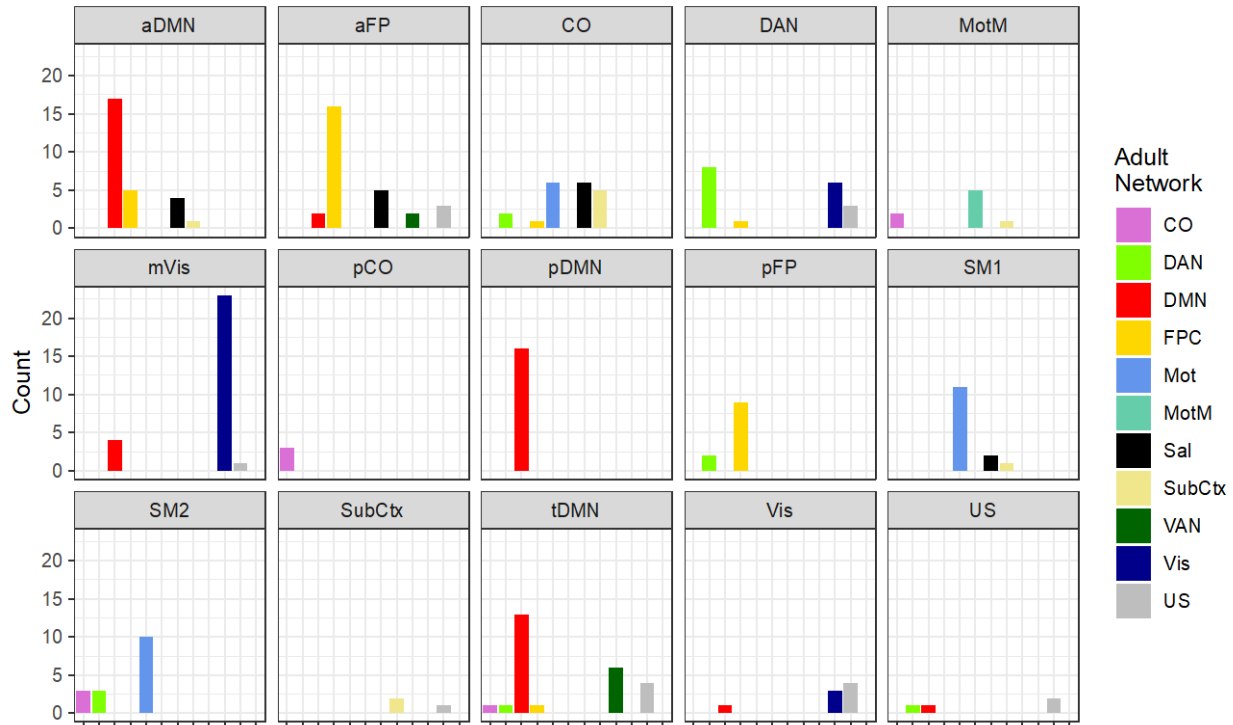
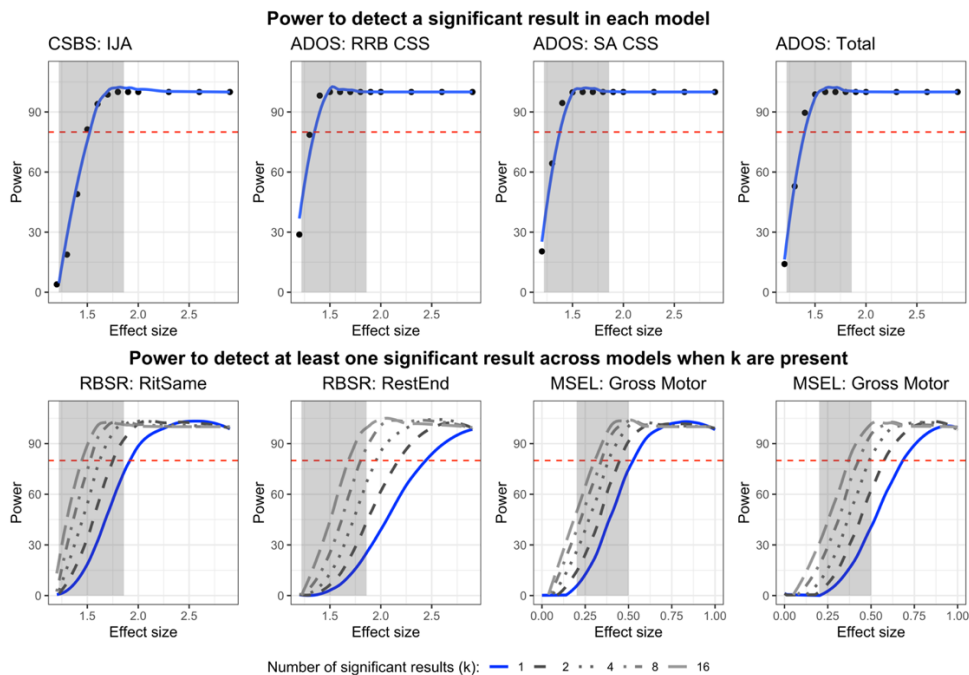


Figure S2. Histograms depict the relationship between adult (9) and infant network assignments for the original set of $n = 230$ ROIs (5). Counts reflect the number of ROIs from a given adult network (indicated by color) that sorted into a given infant network (indicated by subplot title).



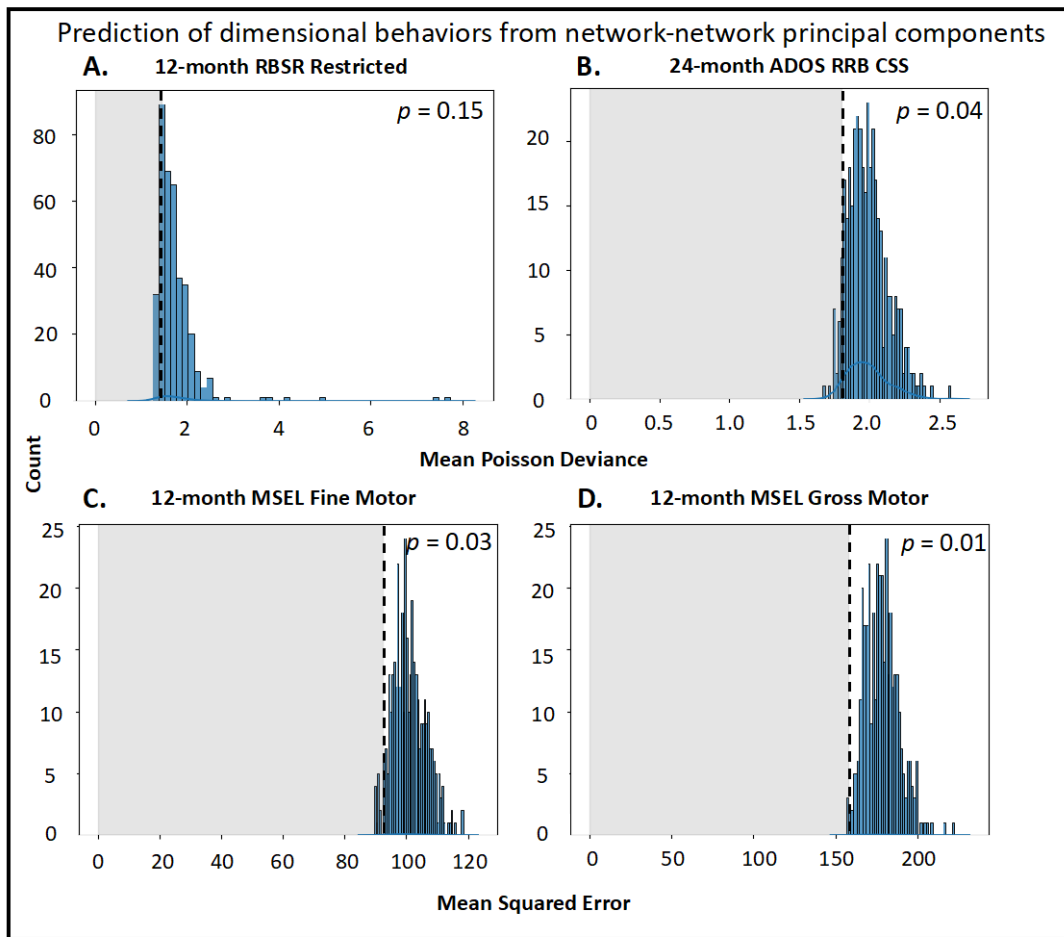
aDMN = anterior default mode network, *aFP* = anterior frontoparietal network, *CO* = cingulo-
 opercular network, *DAN* = dorsal attention network, *MotM* = motor-mouth network, *mVis* =
 medial visual network, *pCO* = posterior cingulo-*opercular* network, *pDMN* = posterior default
 mode network, *pFP* = posterior frontoparietal network, *SM1* = somatomotor network 1, *SM2* =
 somatomotor network 2, *SubCtx* = subcortical network, *tDMN* = temporal default mode network,
Vis = visual network, *FPC* = frontoparietal control network, *Mot* = motor, *Sal* = salience, *VAN* =
 ventral attention network, *US* = unspecified

Figure S3. Power to detect significant brain-behavior associations for cerebellar-cerebral connections was calculated in R (*simr* package) (29,30) using Monte Carlo simulation for Poisson and linear regression. Bonferroni correction was applied to account for the number of functional connections per behavior. Effect sizes for Poisson regression (CSBS, RBSR, ADOS) were estimated as incidence rate ratios (IRR), where 1.22, 1.86, and 3.00 represent small, medium, and large effects, respectively (31). Effect sizes for linear regression (MSEL) were estimated using Cohen's conventions, where 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively (32). Small-to-medium effects are shaded. For CSBS and ADOS variables (top), we were well-powered (80%; red line) to detect medium effects in individual models. For RBS-R and MSEL variables (bottom), we were well-powered to detect at least one significant medium effect assuming multiple significant medium effects were present across models, as would be expected if the cerebellum were a major driver of ASD-associated behaviors in early development.



CSBS = Communication and Symbolic Behavior Scales, IJA = initiation of joint attention, ADOS = Autism Diagnostic Observation Schedule, CSS = calibrated severity score, RRB = restricted interests and repetitive behaviors, SA = social affect, RBS-R = Repetitive Behavior Scale—Revised, MSEL = Mullen Scales of Early Learning

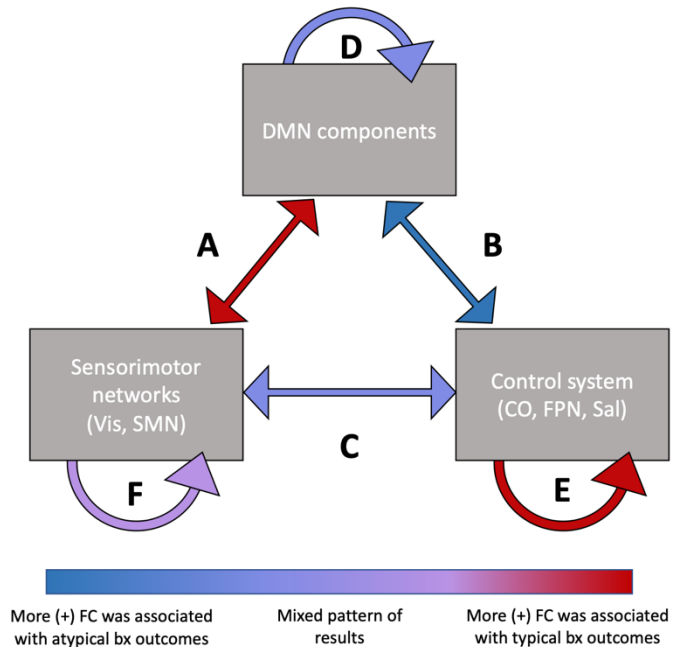
Figure S4. Secondary validation reinforced the predictive utility of three of four enriched network pairs in relation to later ASD-associated behaviors. Dotted lines indicate the mean error ($\bar{x}E$) in real data, and shaded regions identify randomization runs in which $\bar{x}E_{real} > \bar{x}E_{random}$. (A) Although top principal components derived from functional connections between SM1 and tDMN failed to predict 12-month restricted behaviors ($p = 0.15$), top principal components derived from functional connections between (B) pFP-mVis, (C) aFP-pDMN, and (D) CO-aDMN all predicted the dimensional behaviors from which they were derived (24-month RRBs, 12-month fine motor functioning, and 12-month gross motor functioning, respectively) above chance ($p < .05$).



ADOS = Autism Diagnostic Observation Schedule, CSS = calibrated severity score, RRB = restricted interests and repetitive behaviors, RBSR = Repetitive Behavior Scale—Revised, MSEL = Mullen Scales of Early Learning

Figure S5. Summary of primary results* from fcMRI enrichment studies examining fc among control, DMN, and sensorimotor systems in relation to ASD-associated behaviors (5,33–35). *Between networks:* (A) Between sensorimotor and DMN systems, more positive fc was associated with typical bx. (B) Between DMN and control systems, more positive fc was associated with atypical bx. (C) Between control and sensorimotor systems, the relationship between fc and bx was variable. *Within networks:* (D) Within the default mode system, the relationship between fc and bx was variable. (E) Within the control system, more positive fc was associated with typical bx. (F) With the sensorimotor system, the relationship between fc and bx was variable.

Bx	Dir.	Fc-bx	Networks	Age	Reference	Key
IJA	↑	(+)	pDMN-Vis	12M	Eqgebrecht <i>et al.</i>	A
Gross Motor	↑	(+)	tDMN-SMN	12M	Marrus <i>et al.</i>	A
Gross Motor	↑	(+)	aDMN-SMN	12M	Marrus <i>et al.</i>	A
Gross Motor	↑	(+/-)	pDMN-SMN	24M	Marrus <i>et al.</i>	A
Walking	↑	(+)	tDMN-SMN	12M	Marrus <i>et al.</i>	A
Walking	↑	(+)	tDMN-SMN2	12M	Marrus <i>et al.</i>	A
RitSame	↓	(-)	tDMN-Vis	12M	McKinnon <i>et al.</i>	A
Stereotyped	↓	(-)	tDMN-Vis	12M	McKinnon <i>et al.</i>	A
Fine	↑	(-)	aDMN-CO	6M FC	Present results	B
Gross Motor	↑	(-)	pDMN-aFP	6M FC	Present results	B
Gross Motor	↑	(-)	pDMN-pFP	24M	Marrus <i>et al.</i>	B
Walking	↑	(-)	pDMN-pFP	24M	Marrus <i>et al.</i>	B
Restricted	↓	(+)	tDMN-pFP	24M	McKinnon <i>et al.</i>	B
Stereotyped	↓	(+)	tDMN-pFP	24M	McKinnon <i>et al.</i>	B
RRB	↓	(+)	pFP-mVis	6M FC	Present results	C
RitSame	↓	(-)	pFP-Vis	12-24M	McKinnon <i>et al.</i>	C
Aiming-catching	↑	(-)	Sal-Motor	12Y	Wheelock <i>et al.</i>	C
Gross Motor	↑	(-)	tDMN-tDMN	12M	Marrus <i>et al.</i>	D
Walking	↑	(-)	tDMN-tDMN	12M	Marrus <i>et al.</i>	D
Balance	↑	(+)	DMN-DMN	12Y	Wheelock <i>et al.</i>	D
Motor (general)	↑	(+)	FP-FP	12Y	Wheelock <i>et al.</i>	E
Gross Motor	↑	(-)	SMN-SMN2	12M	Marrus <i>et al.</i>	F
Gross Motor	↑	(+)	SMN2-SMN2	12M	Marrus <i>et al.</i>	F
Walking	↑	(+)	SMN2-SMN2	12M	Marrus <i>et al.</i>	F



Fc = functional connectivity, *Bx* = behavior, *Dir.* = higher (↑) vs. lower (↓) scores reflect typical behaviors; *Fc-bx* = sign (positive, negative, mixed) of brain-behavior association; *DMN* = default mode network, *Vis* = visual network, *SMN* = somatomotor network, *CO* = cingulo-opercular network, *FPN* = frontoparietal network, *Sal* = salience network; *M* = months, *Y* = years

*Primary results were defined as described in primary sources. In (5,34,35): network pairs were significantly enriched at both ages (12 and 24 months) or were significantly enriched at one age and significantly different between ages; in the present study: network pairs passed secondary validation testing

Supplementary References

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