Data Supplement for Sylvester et al., Cortical Functional Connectivity Evident After Birth and Behavioral Inhibition at Age 2. Am J Psychiatry (doi: 10.1176/appi.ajp.2017.17010018)

SUPPLEMENTARY METHODS

Exclusion and Inclusion Criteria

Infants with chromosomal abnormalities or suspected/proven congenital infection were excluded. Full-term infants had no known history of illicit substance exposure and no evidence of acidosis on cord blood gas. Exclusion criteria for preterm infants included grade III-IV intraventricular hemorrhage, cystic periventricular leukomalacia, moderate-severe cerebellar hemorrhage and/or cortical/deep nuclear gray matter lesions (1). Anatomic MR images were reviewed by a neuroradiologist (J.S.) and a pediatric neurologist (C.D.S.).

Imaging Data Acquisition

MRI was performed without sedation (2) on a Siemens Trio 3-T scanner (Erlangen, Germany) with an infant-specific, quadrature head coil (Advanced Imaging Research, Cleveland, OH). Structural images used a T2-weighted sequence (TR 8600ms; TE 161ms; voxel size 1×1 × 1mm). Resting state functional MRI (fMRI) used a gradient echo, echo-planar-image (EPI) sequence sensitized to T2* BOLD contrast (TR 2910ms; TE 28ms; voxel size 2.4×2.4×2.4mm). Each fMRI run included 200 volumes (9.7 minutes); one or more runs were performed depending upon subject tolerance.

Social Risk and Maternal History of Affective Disorders

Maternal social risk was computed at the two-year follow-up assessment and was a composite index modeled from prior studies (3). The following five characteristics were coded as 1 (present) or 0 (absent) and summed to yield an index: 1) Not a high school graduate, 2) African-American, 3) public insurance at birth, 4) gave birth at age≤18 years, and 5) single parent household. Maternal anxiety and depressive symptoms were assessed via the Hospital Anxiety and Depression scale anxiety subscale (4) and the Beck Depression Inventory-II (5). These scores were dichotomized into none/mild or moderate/severe, based on published cutoffs. Maternal history of depression and anxiety disorders was assessed via self-report with the Family History Assessment Module (6). Mothers who reported a past history of depression or anxiety symptoms (N=6) and/or scored in the moderate/severe range (N=17) on the Hospital Anxiety and Depression scale or Beck Depression Inventory-II were classified as having a history of affective symptoms. These depression and anxiety scores were dichotomized, and mothers in the moderate/severe range were grouped together with mothers reporting a past history of significant affective symptoms in order derive a single variable that captured mothers who were likely to have current or past affective disorders. Because current and past symptoms were relatively low, this procedure provided the most power to assess relations between maternal symptomatology and other variables of interest.

Multiple Comparison Correction

Study-specific auto-correlation parameters were computed on the basis of the original resting state data after preprocessing, using 3dFWHMx from AFNI (7, 8). These auto-correlation parameters were then used in simulations using 3dClustSim from AFNI within the gray matter

mask to derive family-wise cluster-based error rates. To avoid an unacceptable level of false positives, we required each voxel to be significant at $p<0.0027$ ($z=3.0$); 3dClustSim determined that a cluster size of 37 voxels (999 mm^3) at this threshold was required to achieve a cluster-wise error rate of $p<0.05$.

SUPPLEMENTARY RESULTS

Subjects

Subjects were recruited as part of a larger longitudinal study investigating preterm birth (9, 10). For the present study, we considered all subjects who had both imaging data soon after birth and follow-up assessments at age 2 years (total N=89). Forty-four subjects were excluded because of perinatal brain injury (12 subjects), or excessive motion during scan (32 subjects). There were no differences between included and excluded subjects in any variables of interest including behavioral inhibition at age 2 years ($t(87)=0.29$, $p=0.77$), sex ($X^2=0.013$, $p=0.91$), history of preterm birth ($X^2=0.65$, $p=0.42$), social risk score at age 2 years ($t(87)=-0.53$, $p=0.60$), or history of maternal affective disorders ($X^2=0.014$, $p=0.91$). Analyses indicated that term and preterm infants did not demonstrate any differences in behavioral inhibition at age 2 years (see below), and these groups were therefore combined into one sample.

Zero-Order Relations Among Variables

We examined all zero-order relations for the included subjects among the variables of interest in this study: sex, history of preterm birth, history of maternal affective disorders, behavioral inhibition at age 2 years, residual motion after functional connectivity processing (framewise displacement), and resting state functional connectivity of the three functional connections derived from the hypothesis-driven analysis (right ventrolateral prefrontal cortex and right temporal parietal junction; medial prefrontal cortex and right superior parietal lobule; medial prefrontal cortex and right inferior parietal lobule). Statistics for all comparisons are fully described in Table S1. As reported in the main text, behavioral inhibition was significantly related to social risk ($r=–0.34$, $p=0.02$) and girls had a nonsignificant increase in behavioral inhibition at age two years compared with boys ($t=2.0$, $p=0.06$). The only other relations detected were that, relative to children born at term, children born prematurely had nonsignificantly higher social risk scores at age 2 years ($X^2=2.90$, $p=0.09$) and had significantly less movement after image processing ($t(43)=-2.8$, $p=0.007$). These relations were expected, as social risk is known to be a potent risk factor for preterm birth, and children born prematurely have lower motor activity. Notably, however, there were no significant relations between resting state functional connectivity of the three connections reported in the main text and the other variables of interest (except for behavioral inhibition at age 2 years, as expected) including residual motion. These results strongly suggest that behavioral inhibition, and not confounding variables, drove the main findings of this report.

Motion

In order to test whether there was any identifiable motion-related artifact remaining in our data after functional connectivity processing, we computed the correlation between postprocessing framewise displacement for each subject and resting state functional connectivity strength of each of the 23,200 region-to-region connections from the exploratory analysis. Of the 23,200 potential connections, the strength of 4.29% (995 connections) were nominally significantly related to residual motion using $p<0.05$. Given that this percentage of connections is at chance

level, this analysis does not suggest that there was any detectable residual motion artifact. As noted above, residual motion was not significantly related to the strength of the three connections detected in the hypothesis-driven analysis, and all analyses included residual motion as a covariate.

Exploratory Analysis

In order to contextualize our findings within the scope of other potential functional brain connections that could have been tested, we completed a fully exploratory analysis relating neonatal functional connectivity among 216 regions (see methods) to behavioral inhibition at age 2 years, covarying for sex, social risk, sex by behavioral inhibition interaction, social risk by behavioral inhibition interaction, and postprocessing framewise displacement. This procedure yielded 23,200 region-region pairs. In Figure S2, we display the standardized beta weights for the relation between connectivity and behavioral inhibition for these models. In order to provide some level of control over false positives and in order to limit the analysis to relations that were as strong as those detected in the main text, we only display beta weights in which $p<0.01$ and in which the effect size was large at 0.11 (indicating that for every standard deviation change in behavioral inhibition, connectivity changed by +/– 0.11, which represents a substantial difference in connectivity strength). Table S3 lists the 32 region pairs that survive these stringent criteria, in rank order of effect size. Notably, the connection between the right ventrolateral prefrontal cortex and right lateral parietal region reported in the confirmatory analysis in the main text had the 2nd highest effect size (–0.136) out of the 23,200 connections detected. The connection between the medial prefrontal cortex and the right superior parietal lobule reported in the main text had the 21st highest effect size (–0.114). Given our strong a priori hypothesis, these data provide compelling support that our analyses were sensitive to some of the strongest brain/behavior relations present in our data set. The connection with the largest effect size (–0.150) was between a region near the right temporal-parietal junction that maps to the salience network in adults (center 52 -47 36) and a region in the right medial temporal lobe that includes the inferior, lateral amygdala (center 32 -15 -30) and is not classified to any particular functional brain network in adults.

SUPPLEMENTARY REFERENCES

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FIGURE S1. Neonatal resting state functional connectivity of the ventrolateral prefrontal cortex (VLPFC), dorsal anterior cingulate (dACC), and medial prefrontal cortex (mPFC) seeds used in the hypothesis-driven analysis. Maps are averaged across the subjects. R: right; VAN: ventral attention network; SN: salience network; DMN: default mode network.

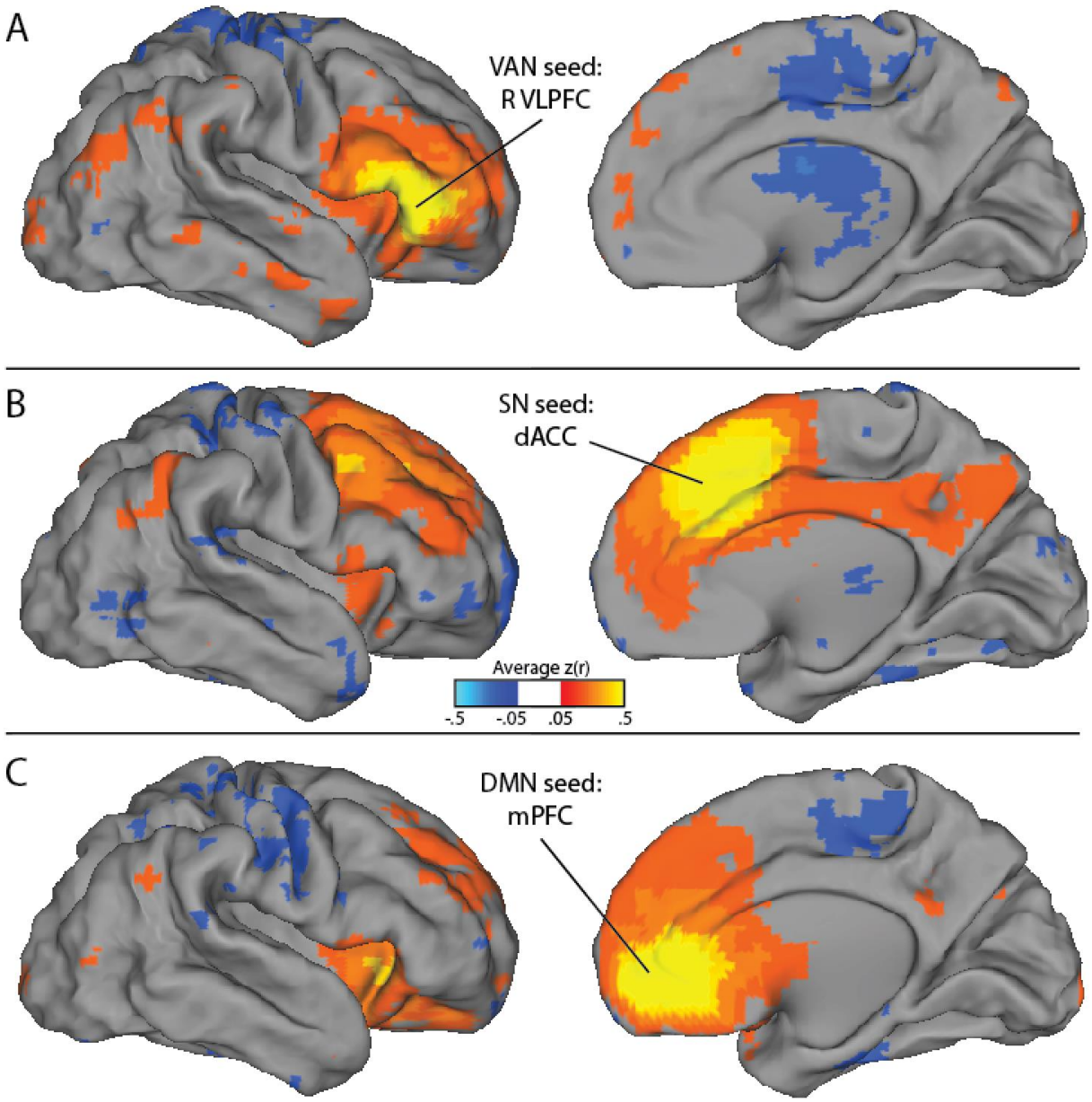

FIGURE S2. Exploratory analysis relating functional connectivity among 216 regions-of-interest at term equivalent to behavioral inhibition at age 2 years. The color of each square in the 216×216 matrix represents the standardized beta weight, for a particular region-region pair, of the relation between neonatal resting state functional connectivity and behavioral inhibition at age 2 years, controlling for sex, social risk, postprocessing residual motion (average framewise displacement), sex by BI interaction, and social risk by BI interaction. Only beta weights in which $p<0.01$, uncorrected, and effect size>0.11 are depicted. Numbers next to arrows indicate the rank order of the effect size among all depicted relations. Arrows indicate a functional connection between regions near the right temporal-parietal junction and the right lateral, inferior amygdala (arrow 1, effect size -0.150), between the right ventrolateral prefrontal cortex and right lateral parietal regions reported in the confirmatory analysis in the main text (arrow 2, effect size -0.136), and between the medial prefrontal cortex and right superior parietal lobule regions reported in the confirmatory analysis in the main text (arrow 21, effect size -0.111). All connections displayed in this matrix are listed in Table S3, in rank order by effect size. For clarity, only network identities, and not region centers, are presented. Regions are ordered from top to bottom on the y-axis and from left to right on the x-axis, in the same order as regions are listed in Table S2.

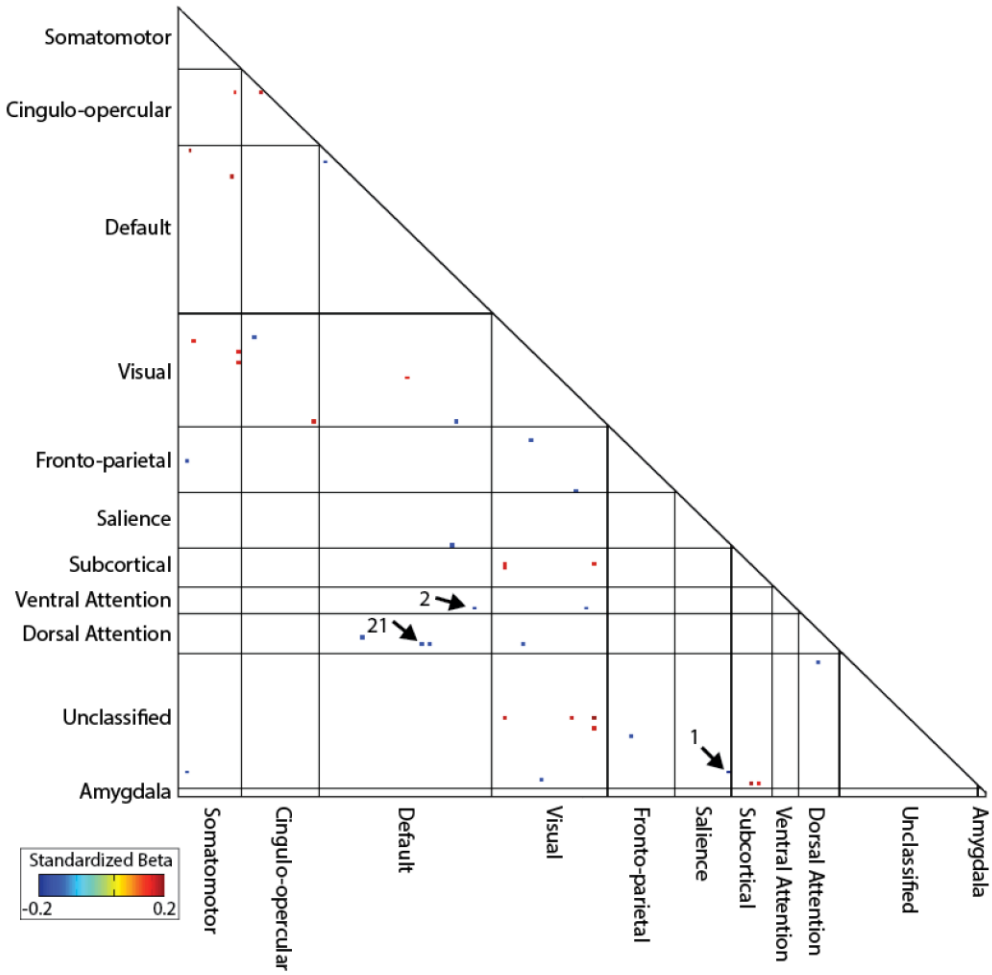

TABLE S1. Zero-order relations among variables of interest in this study including postprocessing residual scan motion and resting state functional connectivity values from the three connections reported in the hypothesis-driven analysis. Relations with p<0.10 are indicated with bolded text. The relations between behavioral inhibition and functional connectivity of the three connections are not computed because the connections were derived from whole brain analysis that specially looked for this relation; effect sizes calculated would be circular and inflated. BI=behavioral inhibition; FD=framewise displacement; R VLPFC=right ventrolateral prefrontal cortex; R TPJ=right temporal parietal junction; MPFC=medial prefrontal cortex; R SPL=right superior parietal lobule; L IPL=left inferior parietal lobule; rs-fc=resting state functional connectivity.

	Preterm	Social Risk	Maternal Affective	BI	FD	R VLPFC / R TPJ rs-fc	MPFC / R SPL rs-fc	MPFC / L Occ rs-fc
Female Sex	X^2=0.13, p=0.71	t=–0.34, p=0.74	X^2=0.13, p=0.71	**t=2.0, p=0.06**	t=–0.27, p=0.79	t=–0.97, p=0.34	t=0.42, p=0.67	t=0.09, p=0.93
Preterm		t=1.5, p=0.14	**X^2=2.90, p=0.09**	t=0.16, p=0.88	**t=–2.8, p=0.007**	t=0.73, p=0.47	t=1.01, p=0.32	t=0.89, p=0.38
Social Risk			t=1.11, p=0.27	**r=–0.34, p=0.02**	r=0.19, p=0.21	r=–0.06, p=0.71	r=–0.21, p=0.16	r=–0.07, p=0.64
Maternal Affective				t=0.32, p=0.76	t=0.83, p=0.41	t=0.30, p=0.77	t=0.27, p=0.79	t=1.31, p=0.20
BI					r=–0.10, p=0.49	***	***	***
FD						r=0.05, p=0.75	r=–0.11, p=0.48	r=–0.17, p=0.27

TABLE S2. Regions-of-interest used in this study. Region centers and associated functional brain network names (according to adult assignments) are derived from the Power 264 set (11) and represent all regions from this set that accurately map to the neonatal brain. Regions are listed in the same order as the order of regions in Supplementary Figure 2. Asterisks indicate the three regions used in the hypothesis-driven analysis. Bolded regions are the 18 regions included in the confirmatory analysis in the main text. All 216 regions listed above were used in the exploratory analysis. Coordinates are not listed for the left and right amygdala regions because they were individualized for each subject. vSMG: ventral supramarginal gyrus; VLPFC: ventrolateral prefrontal cortex; pSTG: posterior superior temporal gyrus; FEF: frontal eye field; SPL: superior parietal lobule; mPFC: medial prefrontal cortex; Precun: precuneus; LP: lateral parietal; dACC: dorsal anterior cingulate cortex; DLPFC: dorsolateral prefrontal cortex; IPL: inferior parietal lobule.

Somatomotor	Default (con't)	Visual (con't)	Frnt-par (con't)	Dor Atn. (Con't)
–52 –25 41	–44 27 –9	–27 –79 16	**45 19 30 (DLPFC)**	8 –63 57
–44 –34 44	**–43 –65 31 (LP)**	–25 –89 0	46 –45 44	20 –66 45
–39 –22 52	–41 9 –30	–23 –90 15	**Salience**	**23 –60 57 (SPL)**
–29 –45 57	–39 –75 22	–17 –68 3	–51 –50 39	**26 –9 54 (FEF)**
–21 –34 58	–38 –75 39	–16 –77 30	**–34 16 3 (Insula)**	44 –33 48
–11 –6 42	–32 –39 –15	–15 –53 –2	–27 46 25	**Unclassified**
8 –6 45	–25 –41 –8	–14 –72 –9	–11 21 27	–55 –27 –14
18 –32 58	–17 23 54	–14 –90 27	–1 25 30	–53 –45 –24
26 –42 57	–12 –41 1	–8 –80 5	***4 18 39 (dACC)**	–44 –51 –21
35 –21 45	–11 –57 14	–3 –81 18	9 17 30	–35 –30 –24
39 –24 54	–11 39 12	5 –72 21	24 43 31	–30 –55 –25
41 –12 57	–8 42 27	6 –81 4	29 27 30	–29 15 –15
47 –24 42	–7 –56 25	8 –72 9	29 49 20	–29 –12 –33
–51 –13 24	–7 45 4	14 –87 33	32 12 –3	–20 36 –15
–48 –14 34	–3 –50 12	14 –77 28	**34 17 7 (Insula)**	–20 –24 –18
34 –13 16	–3 39 –4	17 –48 –9	35 27 3	–16 –75 –25
48 –10 34	–3 32 39	19 –66 1	45 17 14	–15 –65 –20
Cingulo-operc	–3 36 20	19 –85 –4	52 –47 36	–14 –21 39
–58 –27 13	3 –50 48	23 –87 21	**Subcortical**	–11 –93 –15
–53 –12 12	**5 –60 33 (Precun)**	25 –79 –16	–30 –14 1	–8 –54 57
–51 –24 22	5 48 21	26 –60 –9	–29 –29 12	–7 –72 38
–48 –36 24	7 –50 29	27 –77 23	–21 4 –2	–5 –30 –3
–47 –28 5	***7 37 0 (mPFC)**	35 –84 11	–15 0 10	–3 –37 30
–43 –3 10	8 48 9	35 –81 0	–10 –21 8	–2 –16 13
–37 –35 16	8 42 –9	38 –73 13	8 –7 8	1 –27 30
–33 0 6	10 –55 16	40 –66 –8	11 –20 9	1 –62 –18
–6 13 36	11 30 24	41 –78 –12	14 1 10	6 –26 1
–4 –2 53	14 –64 24	44 –60 4	22 6 5	8 –90 –9
5 3 51	20 33 42	**Fronto-parietal**	27 –3 7	8 36 –18
30 –29 14	21 27 50	–45 7 24	29 –17 4	9 –41 48
34 6 5	26 –39 –11	–41 33 24	**Ventral Attention**	10 –67 39
35 –3 0	37 13 42	**–41 –56 41 (IPL)**	–54 –51 8	17 –90 –15
39 –5 48	41 –73 26	**–41 20 31 (DLPFC)**	–53 –41 12	17 –30 –15
41 –26 21	44 12 –24	–40 2 33	–47 21 2	22 –58 –22

47 4 3	44 –52 28	–40 40 2	–43 –2 45	23 39 –9
51 –31 34	47 30 –6	–33 49 9	**49 –35 9 (pSTG)**	23 27 –12
53 –9 16	**49 –61 34 (LP)**	–28 –59 44	***50 27 6 (VLPFC)**	26 12 –12
55 –19 10	49 –31 –2	–4 21 46	**53 –48 12 (vSMG)**	32 48 –6
56 –21 30	50 –6 –12	29 9 57	**Dorsal Attention**	32 –15 –30
Default	50 3 –24	31 –55 42	–50 –63 3	32 33 –6
–55 –31 –4	51 –45 22	35 –66 38	–40 –60 –10	44 –48 –15
–53 –15 –9	**Visual**	36 37 20	–32 –48 44	47 –6 –33
–50 0 –24	–44 –75 –12	**41 –55 45 (IPL)**	**–32 –5 53 (FEF)**	53 –33 –14
–47 –43 0	–40 –73 –2	41 43 4	–26 –71 33	**Left Amygdala**
–44 –61 18	–31 –78 –15	44 5 35	**-17 –60 60 (SPL)**	**Right Amygdala**

TABLE S3. Region pairs from the exploratory analysis in which neonatal resting state functional connectivity was significantly related to behavioral inhibition at age two years. Only relations in which p<0.01 (uncorrected) and the effect size was larger than +/–0.11 are listed; the relations are listed in rank order of effect size. These same results are depicted graphically in Supplementary Figure 2. Each row lists a region pair, including the centers of the two regions, the networks of the regions based on adult classification, and the effect size. The two bolded rows indicate the connections that are very similar to two of the three connections identified in the hypothesis-driven analysis and are identical to the 2 connections from the confirmatory analysis with the largest effect sizes.

Region 1			Region 1 Network	Region 2			Region 2 Network	Effect Size
32	–15	–30	Unclassified	52	–47	36	Salience	–0.1501
50	**27**	**6**	**Ventral Attention**	**49**	**–61**	**34**	**Default**	**–0.1362**
–2	–16	13	Unclassified	38	–73	13	Visual	0.1342
–44	–61	18	Default	–53	–15	–9	Default	–0.1332
29	9	57	Fronto-parietal	–39	–22	52	Somatomotor	–0.1280
47	–6 –33		Unclassified	8	–7	8	Subcortical	0.1270
–53	–15	–9	Default	–29	–45	57	Somatomotor	0.1270
23	–60	57	Dorsal Attention	8	42	–9	Default	–0.1254
–39	–75	22	Default	–48	–14	34	Somatomotor	0.1238
52	–47	36	Salience	26	–39	–11	Default	–0.1220
–44	–51	–21	Unclassified	–17	–60	60	Dorsal Attention	–0.1215
8	36	–18	Unclassified	–33	49	9	Fronto-parietal	–0.1194
–37	–35	16	Cingulo-opercular	–43	–3	10	Cingulo-opercular	0.1190
41	–78	–12	Visual	37	13	42	Default	–0.1189
–2	–16	13	Unclassified	–27	–79	16	Visual	0.1188
–2	–16	13	Unclassified	23	–87	21	Visual	0.1187
44	–48	–15	Unclassified	5	–72	21	Visual	–0.1180
46	–45	44	Fronto-parietal	25	–79	–16	Visual	–0.1176
–41	20	31	Fronto-parietal	–14	–90	27	Visual	–0.1175
6	–26	1	Unclassified	38	–73	13	Visual	0.1174
41	–78	–12	Visual	55	–19	10	Cingulo-opercular	0.1174
23	–60	57	Dorsal Attention	7	37	0	Default	–0.1170
50	27	6	Ventral Attention	35	–84	11	Visual	–0.1163
8	–7	8	Subcortical	–27	–79	16	Visual	0.1143
8	**–63**	**57**	**Dorsal Attention**	**–25**	**–41**	**–8**	**Default**	**–0.1141**
32	–15	–30	Unclassified	–39	–22	52	Somatomotor	–0.1141

–10	–21	8	Subcortical	–27	–79	16	Visual	0.1140
–10	–21	8	Subcortical	38	–73	13	Visual	0.1137
23	–60	57	Dorsal Attention	–15	–53	–2	Visual	–0.1135
5	–72	21	Visual	48	–10	34	Somatomotor	0.1134
–17	–68	3	Visual	–48	–36	24	Cingulo-opercular	–0.1126
–37	–35	16	Cingulo-opercular	34	–13	16	Somatomotor	0.1113
–16	–77	30	Visual	–21	–34	58	Somatomotor	0.1112
–14	–90	27	Visual	48	–10	34	Somatomotor	0.1110
14	–77	28	Visual	3	–50	48	Default	0.1109
47	–6	–33	Unclassified	14	1	10	Subcortical	0.1106