Dynamic Resting-State Connectivity Differences in Eyes Open Versus Eyes Closed Conditions

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Abstract

Introduction: Previous studies have shown significant conditional differences between eyes open, fixated at an image (EO) and eyes closed (EC) in the acquired resting-state functional magnetic resonance imaging (rs-fMRI) data.

Aim: We recently showed significant functional network connectivity (FNC) differences between EO and EC across a variety of networks. In this study, we aim at further evaluating differences in dynamic FNC (dFNC) between EO and EC.

Materials and Methods: Rs-fMRI were collected from adolescents aged 9–15 years old during both EO and EC conditions, and dFNC was calculated by using the independent component analysis framework.

Results: We found that out of five states (clusters), state 1 was observed to be more dominant in the EO condition, whereas state 2 was observed to be more dominant in the EC condition. States 1 and 2 showed significant differences in the mean dwell time based on false discovery rate, and states 1, 2, 3, and 4 differed in the frequency of occurrences. These results are consistent with our previous study of static connectivity in suggesting that EO and EC differences not only are relatively strong but also importantly reveal that these differences vary over time, especially in one particularly transient connectivity pattern.

Conclusion: Our results manifest as changes in the proportion of time spent in unique functional connectivity patterns, and they show unique transient functional connectivity patterns in a subset of identified states. Overall, our findings indicate that both static and dynamic rs-fMRI connectivity patterns are strongly impacted by basic conditional differences such as EO and EC.

Keywords: dynamic functional network connectivity; eyes closed; eyes open; independent component analysis; resting state

Impact Statement

Our findings not only suggest that eyes open, fixated at an image (EO) and eyes closed (EC) condition-related resting state functional magnetic resonance imaging differences are relatively strong, but they also reveal an important attribute of these conditions that these differences vary over time, especially in one particularly transient connectivity pattern. Our results manifest as changes in the proportion of time spent in unique functional connectivity patterns, and they show unique transient functional connectivity patterns in a subset of identified states. We believe there is benefit in having the EO/EC as a contrast of interest in future studies, if time allows.

Introduction

RESTING-STATE FUNCTIONAL magnetic resonance imaging (rs-fMRI) is commonly collected under one or more of three conditions, eyes closed (EC), eyes open with-

out fixation, and eyes open, fixated on an image (EO). In the EC case, participants are asked to keep their EC and not to fall asleep during scanning. In the EO case, participants are asked to keep their eyes open during scanning. For the EO case, there is a target, usually a crosshair, and subjects are

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asked to keep their eyes fixated on that target. Several studies, including our own, showed that the conditions of the eyes can affect the resting-state connectivity patterns (Agcaoglu et al., 2019; Liu et al., 2013; Patriat et al., 2013; Van Dijk et al., 2010; Wei et al., 2018; Yan et al., 2009).

Liu et al. (2013) found reproducible patterns of EO and EC differences in three metrics, including amplitude of low-frequency fluctuations, regional homogeneity, and seed-based correlation by using a dataset containing 48 college students (aged 19–31 years, 24 females). Wei et al. (2018) found significantly greater brain activity in attentional system areas in the EO condition, but significantly lower brain activity in sensorimotor system areas compared with the EC condition by using the same dataset with Liu et al. (2013).

A few studies analyzed brain connectivity via group independent component analysis (gICA). Wu et al. (2010) found high functional network connectivity (FNC) and widespread alpha hemodynamic responses in EC condition, whereas for the EO condition they reported lowered hemodynamic responses and decreased FNC by using a dataset of 25 participants (age 29 ± 8 years, 17 males). The previous studies used relatively small datasets: Patriat et al. (2013) used a dataset consisting of 25 participants (age 35.5 ± 17.7 years, 10 females), Van Dijk et al. (2010) used a dataset of 30 participants for the EO–EC analysis (aging from 19 to 29 years), and Yan et al. (2009) used a dataset comprising 20 participants (10 females, age 18–24 years).

Recently, we investigated static FNC (sFNC) differences between EO and EC conditions by using a large fMRI dataset collected from adolescents aged 9–15 years old (i.e., the same data used in this work; Agcaoglu et al., 2019).

Dynamic FNC (dFNC) is an extension of FNC that utilizes a sliding window approach to estimate inter-network connectivity in each time window. This enables one to track connectivity changes during scanning, rather than obtaining an average estimate of the whole scan. Sakoglu et al. (2010) used a sliding window approach to show that multiple networks identified via whole-brain FNC dynamically change during scanning. Chang and Glover (2010) highlighted time-frequency dynamics within a set of regions of interest selected from several of the well-known resting networks and evaluated voxel-wise variability over time.

After that, several studies further examined these changes and showed that dFNC is a useful tool to investigate brain connectivity. For example, several studies (Damaraju et al., 2014; Espinoza et al., 2019; Rashid et al., 2014, 2018) found significant differences in dFNC between healthy controls and patients with schizophrenia, bipolar disorder, autism spectrum disorder, and Huntington's disease. A recent study from our group investigated the effect of EO versus EC using the dFNC framework (Allen et al., 2018) and the same dataset as Wu et al. (2010) and found an association between dynamic connectivity in concurrently collected electroencephalography (EEG) and fMRI data, as well as a large effect of vigilance on FNC.

In this study, we extend our previous sFNC work (Agcaoglu et al., 2019) by utilizing a dFNC framework to analyze connectivity differences over time between EO and EC conditions. Our study (173 subjects, age 11.95 ± 1.78 years) is different than the previous dFNC study comparing EC and EO (Allen et al., 2018; 20 subjects, age 29 ± 8 years) in both the targeted age range and richness of the dataset.

In our study, we investigate the EO and EC differences in a cohort of 9- to 15-year-old adolescents, an age range that is generally considered to be a time of rapid physical and mental development. These data also use a more modern pulse sequence with a shorter repetition time (TR) (460 ms vs. 2 sec). Yang et al. (2007) found that a shorter TR revealed higher EO–EC differences in their study of amplitude of low-frequency fluctuations by using a dataset containing 15 participants (9 females, aged 22–27 years). We hypothesized that dFNC values will be strongly associated with age and gender, especially in cognitive control networks, with more association in the EO cases as reported in our sFNC study (Agcaoglu et al., 2019).

Materials and Methods

The study was approved and monitored by the Advarra Institutional Review Board for New Mexico and the Institutional Review Board of the University of Nebraska Medical Center for Nebraska. All participants provided signed informed consent before study procedures.

Participants

In this study, we utilized an existing dataset reported in a previous study (Agcaoglu et al., 2019). The rs-fMRI scans were collected from 182 adolescents at two different sites (Mind Research Network [MRN]/New Mexico and University of Nebraska Medical Center/Nebraska) under both EO and EC conditions as part of the National Science Foundation (NSF) supported DevCog project. Participants were instructed to close their eyes but remain awake during the EC condition and were asked to stare at a fixation cross during the EO scan. Initially, 358 different scans were used in the gICA state; for further analysis, we removed participants who did not have both EO and EC scans available.

Final analysis included 346 EO and EC scans from 173 participants (age range from 9.1 to 15.5 years, 11.95 ± 1.78). The retained participants had a maximum mean frame displacement (MFD) of 0.3 mm, and the mean of MFD was 0.074 mm with a standard deviation of 0.03 mm. Demographic information of the participants is presented in Table 1.

TABLE 1. DEMOGRAPHIC INFORMATION OF THE PARTICIPANTS, THE DATASET INCLUDED 173 SUBJECTS (89 MALES) WITH AGE RANGE 9.1–15.5 YEARS OLD

	No. of subjects		%	
Gender	173		100	
Male	89		51.45	
Female	84		48.55	
Handedness				
Left	15		9	
Right	151		91	
Age (years)	Mean	SD	Min	Max
Male	12.03	1.88	9.1	15.5
Female	11.89	1.66	9.2	15.1
Barratt simplified measure of social status	43.70	12.80	11.50	66

We used the Barratt simplified measure of social status, which was available for 153 subjects to measure socioeconomic status. Max, maximum; Min, minimum; SD, standard deviation.

Imaging parameters

Imaging data were collected at the MRN site on a 3T Siemens TIM Trio scanner and at the Nebraska site on a 3T Siemens Skyra scanner. A total of 650 volumes of multiband echo planar imaging blood oxygen level dependent (BOLD) data (a length of 4 min and 59 sec) were collected per condition and participant with TR of 0.46 sec, echo time = 29 ms, flip angle = 44°, and a slice thickness of 3 mm with no gap. Rs-fMRI scans were acquired by using a standard gradient-echo planar imaging paradigm; MRN site: field of view (FOV) of 246 × 246 mm (82 × 82 matrix), 56 sequential axial slices; Nebraska: FOV of 268 × 268 mm (82 × 82 matrix), and 48 sequential axial slices. The scanning order of the EO and EC sessions was counter-balanced across participants at each site. Eyes were monitored via an eye-tracker during the EO condition to ensure compliance with instructions.

Preprocessing

The data were preprocessed by using a combination of toolboxes. Images were distortion corrected by using FSL's *applytopup*; then, images were re-aligned to the single-band reference image (SBref) by using analysis of functional neuro-images (AFNI's) *align_epi_anat.py* (<https://afni.nimh.nih.gov>). Motion parameters were estimated relative to SBref. Next, data were registered to the MNI template by using AFNI's *3dNwarpApply*, as estimated by AFNI's *auto warp.py*. The first 4 volumes of each session were discarded to account for the T1 equilibrium effect. Because participants consisted of children with an age range of 9.1–15.5 years, we re-warped the data to a study-specific template computed as the average of the first time point from each scan. Next, we smoothed the data to 6 mm full-width at half maximum.

Group independent component analysis

The preprocessed functional data were analyzed with gICA implemented in the Group ICA Of fMRI Toolbox (GIFT) software (Calhoun et al., 2001, 2002; Calhoun and Adali, 2012) and decomposed into 150 spatially independent components. Before gICA, a scan-specific principle component analysis (PCA) was utilized to reduce the dimensionality across the 646 time points to 200 maximally variable directions; then, a group PCA was applied to further reduce the dimensionality to 150 (Erhardt et al., 2011). One hundred fifty independent components were estimated by using the infomax algorithm (Bell and Sejnowski, 1995). The ICA algorithm 20 was run times in ICASSO (Himberg et al., 2004) to ensure stability of the estimation, and the most central run was selected from the resulting 20 runs (Ma et al., 2011).

After this group-level estimation, a spatially constrained ICA algorithm, called group information guided ICA as implemented in GIFT software (Du and Fan, 2013; Du et al., 2016; Salman et al., 2019), was used to estimate scan-specific spatial maps and time courses (TCs) from the group maps. Fifty-one components out of 150 were identified as the resting-state networks (RSNs), and these RSNs were grouped based on their anatomical and functional properties including 4 sub-cortical networks (SC), 3 auditory networks (Aud), 8 sensorimotor networks (SM), 18 visual networks (Vis), 4 default-mode networks (DMN), 12 cognitive control networks (CC), and 2

cerebellar networks (Cb). See Agcaoglu et al. (2019) for additional details of the gICA and RSN selection process.

Post-gICA processing and FNC

After the gICA, the subject-specific TCs were post-processed. To do so, subject specific TCs were detrended, motion parameters were regressed (including their derivatives, their squares, and derivatives of their squares) and then despiked, which involved detecting spikes as determined by AFNI's *3dDespike* algorithm and replacing spikes by values obtained from third-order spline fit to neighboring clean portions of the data. The correlation among brain networks is primarily driven by the low-frequency fluctuations in BOLD fMRI data (Cordes et al., 2001); therefore, we low-pass filtered TCs by using a fifth-order Butterworth low-pass filter with a cutoff of 0.15 Hz.

We calculated dFNC by using a sliding window approach with a third-order Gaussian window with 100 timepoints (46 sec). For each window, dFNC was calculated as the pairwise correlation between windowed TCs. We estimated the covariance from the regularized precision matrix or the inverse covariance matrix (Smith et al., 2011). Following the graphical LASSO method of Friedman et al. (2008), we placed a penalty on the L1 norm of the precision matrix to promote sparsity. The regularization parameter λ was optimized separately for each subject by evaluating the log-likelihood of the unseen data in a cross-validation framework. Finally, dFNC values were Fisher-Z transformed and residualized with respect to age, gender, site, and motion (MFD).

Initially, the main modules of the dFNC matrix were organized similar to Allen et al. (2014) as subcortical, auditory, sensorimotor, visual, default mode, cognitive control, and cerebellar; then, they were re-organized after applying the Louvain algorithm from the brain connectivity toolbox to arrange the RSN ordering within these main modules.

Clustering

We applied *k*-means clustering by using the distance method of correlation. We used five cluster centroids to match previous studies investigating resting condition on resting-state dFNC (Allen et al., 2018). We used a *k*-means clustering approach to investigate FNC changes over time by clustering over time, and each centroid represents a connectivity state. We also compared EO and EC dFNC differences on domains such as the number of subjects entering each state and their percentage of occurrences, the number of subjects in each state over time, and the mean dwell time (MDT). The MDT is the average time a participant remains in each state before changing to another state.

We tested for mean dFNC differences between EO and EC for each state. To do so, first, for each subject and each state, the average of EO and EC dFNC windows belonging to that state was calculated. Because some subjects did not enter some states at all, the number of subject-mean dFNCs for EO and EC was not the same, and we used a two-sample *t*-test and corrected for multiple comparisons by using a false discovery rate (FDR).

In a similar manner, we also calculated the mean in each connectivity domain, compared mean domain dFNC differences between EO and EC for each state, and corrected for multiple comparisons with FDR. There were no significant differences in motion (*t*-test, $p > 0.05$) between the MFD

computed from the EO and EC scans. We also checked the correlation between age and MFD for EO and EC cases separately. For EO, the association was not significant ($r = -0.138$, $p = 0.069$); whereas for the EC condition, there were some age-related associations ($r = -0.165$, $p = 0.029$). In addition, we repeated the k -means clustering with four and six cluster centroids; the results were generally similar (see Supplementary Data and Supplementary Figures S1–S8 for details).

Results

Resting-state networks

The gICA was applied to resting-state data from both conditions to derive intrinsic RSNs. The selected RSNs were

then grouped based on their anatomical and functional properties, which revealed 4 SC, 3 Aud, 8 SM, 18 Vis, 4 DMN, 12 CC, and 2 Cb. These RSNs are displayed in Figure 1, and the corresponding anatomical regions and their peak locations are detailed in Table 2.

Clustering results

The five dynamic state centers are presented in Figure 2. When we compared these state centers, state 1 showed the highest Vis–Vis connectivity compared with the other states, and it also has a negative correlation with Vis–SM, Vis–Aud, and Vis–SC. State 2 has the highest connectivity among SM–SM and SM–Aud compared with the other states. State 3

FIG. 1. Selected RSN, grouped according to their anatomical and functional location (Agcaoglu et al., 2019), 4 SC, 3 Aud, 8 SM, 18 Vis, 4 DMN, 12 CC, and 2 Cb. Aud, auditory networks; Cb, cerebellar networks; CC, cognitive control networks; DMN, default-mode networks; RSNs, resting-state networks; SC, sub-cortical networks; SM, sensorimotor networks; Vis, visual networks). Color images are available online.

TABLE 2. ANATOMICAL REGIONS CORRESPONDING TO EACH OF THE RESTING-STATE NETWORKS

<i>RSN no.</i>	<i>Nv</i>	<i>Tmax</i>	<i>Coord.</i>	<i>BA</i>
Sub-cortical networks				
85				
Left putamen	1463	141.63	−18 10 4	
Right putamen	735	82.27	20 12 0	
54				
Right putamen	1224	135.54	26 4 − 2	
Left putamen	1197	147.24	−28 0 − 2	
58				
Right thalamus	1033	170.69	2 − 20 6	
68				
Left thalamus	1430	141.33	−4 − 12 12	
Auditory networks				
62				
Left superior temporal gyrus	2267	84.25	−52 − 18 6	22
Right superior temporal gyrus	2029	87.45	60 − 12 0	22
145				
Right superior temporal gyrus	3713	104.6	56 − 44 18	13
Left middle temporal gyrus	1378	51.41	−58 − 54 12	22
125				
Right insula lobe	1908	119.75	42 − 18 12	13
Left superior temporal gyrus	1795	107.6	−46 − 24 12	41
Sensorimotor networks				
9				
Left paracentral lobule	2599	94.33	0 − 24 72	6
8				
Left postcentral gyrus	1828	94.76	−46 − 30 54	2
Right postcentral gyrus	383	37.47	54 − 20 48	1
98				
Left inferior parietal lobule	2438	96.03	−54 − 30 46	2
Right supramarginal gyrus	1621	76.73	60 − 20 40	3
26				
Right postcentral gyrus	2515	96.98	44 − 30 58	2
Left postcentral gyrus	507	37.30	−42 − 38 60	40
2				
Left postcentral gyrus	1080	91.31	−54 − 8 34	6
Right postcentral gyrus	1014	90.70	60 − 6 30	6
73				
Left paracentral lobule	4219	125.8	0 − 24 54	6
Left rolandic operculum	157	40.96	−40 − 26 18	13
124				
Left inferior parietal lobule	1073	76.71	−58 − 42 42	40
Right supramarginal gyrus	873	73.92	60 − 38 40	40
77				
Left SMA	4587	101.23	0 6 52	6
Right insula lobe	516	53.27	48 10 − 2	22
Visual networks				
131				
Left inferior temporal gyrus	2183	74.55	−52 − 50 − 12	37
Right fusiform gyrus	1668	60.82	44 − 30 − 18	20
76				
Right calcarine gyrus	2756	81.86	18 − 102 − 2	18
34				
Left cuneus	3412	82.78	2 − 80 24	18
42				
Right fusiform gyrus	1510	72.64	32 − 78 − 14	19
Left cerebellum	569	36.52	−40 − 68-20	19
71				
Left fusiform gyrus	1920	77.93	−30 − 56 − 14	19
Right fusiform gyrus	1422	69.95	30 − 48 − 18	37
91				
Right lingual gyrus	4008	91.16	24 − 72 − 12	19

(continued)

TABLE 2. (CONTINUED)

<i>RSN no.</i>	<i>Nv</i>	<i>Tmax</i>	<i>Coord.</i>	<i>BA</i>
111				
Left lingual gyrus	2891	109.15	0 – 78 4	18
69				
Left cerebellum	1662	143.19	–6 – 50 – 2	30
82				
Right cerebellum	1674	142.59	8 – 50 – 2	30
70				
Left lingual gyrus	2152	76.07	–18 – 86 – 18	18
33				
Right calcarine gyrus	3313	115.61	8 – 68 10	30
59				
Right lingual gyrus	2465	127.29	12 – 56 10	30
Left middle occipital gyrus	293	41.29	–42 – 80 30	19
130				
Right middle occipital gyrus	3229	97.50	38 – 84 6	19
Left middle occipital gyrus	3092	82.44	–36 – 86 6	19
100				
Cerebellar vermis	1270	192.57	2 – 42 4	29
129				
Cerebellar vermis	1448	140.21	6 – 56 0	
38				
Left precuneus	3174	79.17	0 – 66 58	7
Right superior frontal gyrus	241	32.07	30 4 60	6
37				
Left posterior cingulate cortex	2019	141.39	0 – 54 30	31
Left angular gyrus	509	45.23	–52 – 68 28	39
27				
Right middle cingulate cortex	2693	88.57	–4 – 24 28	23
Left inferior parietal lobule	207	35.50	–36 – 62 48	7
Default-mode networks				
123				
Right anterior cingulate cortex	4398	118.94	2 42 10	32
Right insula lobe	538	59.55	36 18 – 12	47
49				
Left mid-orbital gyrus	2941	115.46	0 48 – 6	10
Left middle temporal gyrus	253	39.78	–58 – 14 – 18	21
90				
Left angular gyrus	2579	91.98	–52 – 62 30	39
Left middle frontal gyrus	2269	52.91	–42 18 46	8
101				
Right middle frontal gyrus	2450	57.54	30 18 54	8
Right inferior parietal lobule	1892	99.84	54 – 56 40	40
Cognitive control networks				
83				
Left middle temporal gyrus	2105	93.39	–46 6 – 30	21
Right medial temporal pole	1588	96.98	48 10 – 26	21
114				
Left superior medial gyrus	3955	103.29	0 60 22	10
Left temporal pole	733	40.71	–36 22 – 20	47
63				
Right middle frontal gyrus	6987	115.49	32 58 4	10
Right inferior parietal lobule	72	26.70	50 – 50 48	40
48				
Left superior medial gyrus	2744	86.68	0 66 18	10
Right cerebellum	77	31.19	48 – 72 – 38	
120				
Left inferior frontal gyrus (p. Triangularis)	4236	91.65	–48 30 18	46
Right inferior frontal gyrus (p. Triangularis)	855	52.20	50 22 28	46
146				
Right inferior frontal gyrus (p. Opercularis)	7279	114.14	50 18 6	45
Left insula lobe	590	46.17	–34 24 – 2	13

(continued)

TABLE 2. (CONTINUED)

<i>RSN no.</i>	<i>Nv</i>	<i>Tmax</i>	<i>Coord.</i>	<i>BA</i>
119				
Left insula lobe	2186	116.66	−40 18 − 6	47
Right insula lobe	1381	88.71	44 16 − 2	47
96				
Left inferior parietal lobule	3989	78.25	−24 − 72 46	7
Left precentral gyrus	711	47.22	−52 10 34	9
102				
Right inferior parietal lobule	3397	82.40	44 − 42 48	40
Right inferior frontal gyrus (p. Opercularis)	1660	51.33	54 12 30	9
133				
Right rolandic operculum	2745	102.29	54 4 4	22
Left rolandic operculum	766	51.31	−54 0 4	22
55				
Right superior parietal lobule	3916	71.19	18 − 54 66	7
Right cerebellum	106	33.04	26 − 44 − 48	
136				
Left angular gyrus	6603	74.10	−52 − 78 28	39
Right middle occipital gyrus	807	58.53	44 − 78 34	19
Cerebellar networks				
84				
Right cerebellum	4173	150.37	30 − 68 − 38	
110				
Left cerebellum	3906	126.64	−30 − 66 − 38	

Nv, Number of voxels; *Tmax*, maximum *t*-value; *Coord.*, coordinates of the peak; *BA*, Brodmann area number are shown (Agcaoglu et al., 2019). RSNs, resting state networks; SMA, Supplementary motor area.

differs from the other states in that it has all negative correlations between Vis and all CC networks, whereas the other states show more positively correlated networks overall.

We also compared the total number of subjects entering each state at least one time for each task, and the results are presented in Table 3. We found that 154 out of 173 subjects enter state 1 at least one time for EO scans, whereas 85 out of 173 subjects enter state 1 for EC scans. Forty-two subjects enter state 2 for EO, whereas 97 subjects enter this state for the EC condition. State 3 (152 and 150 for EO and EC, respectively), state 4 (129 and 128 for EO and EC, respectively), and state 5 (158 and 143 for EO and EC, respectively) have almost equal number of subjects entering the state across the two conditions. Moreover, we compared the total number of occurrences of each state for each condition. Results are displayed in both Table 2 and as a graph in Figure 3 (top left). State 1 has 33% of occurrences in the EO case, whereas it has only 8% of occurrences in the EC case. The condition is reversed in state 2: EO has only 5% of occurrences in state 2, whereas EC has 22% of occurrences. All states but state 5 showed 0.05 FDR significant differences between EO and EC on occurrences. The MDT for each state and each condition is displayed in Figure 3 (bottom left), and states 1 and 2 differed (FDR <0.05) between EO and EC conditions. Figure 3 (right column) shows the state transition matrix for EO and EC cases, and only one index, translation probability from states 3 to 1, showed significant (FDR <0.05) differences between EO and EC cases. Figure 4 shows the paired *t*-test results between EO and EC state transition matrices.

Figure 5 shows the number of subjects in each state over time. The most remarkable change is in state 2, which shows

an increase in occupancy as time proceeds. This is more significant for the EC condition, but it is also observable in the EO condition. We also observed a decrease in the number of subjects in state 3 over time for the EC condition. State 3 also had a decrease in the number of subjects over time for the EC condition. States 1, 4, and 5 did not show a linear trend (increase or decrease) as time proceeded, although they did show changes over time.

Figure 6 shows the results from our previously published sFNC analysis (Agcaoglu et al., 2019) for comparison with Figure 2, as well as the dynamic state center differences between states 1 and 2. The differences between states 1 and 2 look similar to the sFNC EO versus EC comparison. Overall, we can conclude that state 1 is observed to be dominant in the EO dFNC and we label this an “EO State,” whereas state 2 is observed to be dominant in the EC dFNC and we label this an “EC State.”

Figure 7 shows the results comparing the mean dFNC differences per subject between EO and EC cases for each state. The upper triangular matrix presents the unthresholded results, and the lower triangular matrix presents the results corrected at an FDR of 0.01. All states show a significantly higher correlation between Vis–SM and Vis–Aud, and in most states for Vis–DMN for the EC case. We also generally see higher Vis–Vis correlations. Figure 8 shows the domain average results comparing the mean dFNC window differences per subject between EO and EC cases, the upper triangular matrix presents the unthresholded results, and the lower triangular matrix presents the results corrected with 0.01 FDR.

Finally, we also examined the effect of age, gender, and socioeconomic status scores on MDT, frequency of occurrence,

FIG. 2. Center of the five clusters, states collapsed across both conditions. State 1 has the highest Vis–Vis connectivity compared with the other states and has a negative correlation with Vis–SM, Vis–Aud, and Vis–SC. State 2 has the highest connectivity among SM–SM and SM–Aud compared with the other states. State 3 has a negative correlation between Vis and all CC networks, whereas the other states show a more positive correlation between Vis and CC networks. Color images are available online.

TABLE 3. NUMBER OF SUBJECTS ENTERING EACH STATE AND THEIR PERCENTAGE OF OCCURRENCES FOR EYES OPEN AND EYES CLOSED CASES

No. of subjects entered state	State 1	State 2	State 3	State 4	State 5
EO	154	42	152	129	158
EC	85	97	150	128	143
Total % of occurrences	State 1	State 2	State 3	State 4	State 5
EO	%33	%5	%21	%18	%23
EC	%8	%22	%28	%23	%19

States 1 and 2 show the largest differences for EO and EC cases. For EO case, 154 out of 173 subjects enter the state 1 with an occurrence percentage of 33; for EC case, this reduces to 85 and only %8. For EC case, 97 subjects enter state 2, and only 47 subjects enter that state for the EO case.

EC, eyes closed; EO, eyes open.

FIG. 3. Top left; occurrence frequency of each state for the EO and EC cases shows FDR significant differences for states 1, 2, 3, and 4 between EO and EC cases. Bottom left, mean dwell time of each state for EO and EC cases; state 1 and 2 show significant differences between EO and EC conditions. Right column shows the state transition matrix in $-\log_{10}$ format; transition probability from states 3 to 1 shows FDR significant difference between EO and EC cases (marked with 'o'). The asterisk (*) shows those have FDR significant difference. EC, eyes closed; EO, eyes open; FDR, false discovery rate. Color images are available online.

Paired *t*-test TM differences between EO and EC

FIG. 4. Paired *t*-test differences for the state transition matrix, EO and EC differences (EO–EC), are displayed as $-\text{sign}(t\text{-stats}) \times \log_{10}(p)$. Transition from states 3 to 1 shows FDR significant differences with resting condition (marked with ‘o’). Color images are available online.

and transition matrix by using a regression model separately for EO and EC conditions and corrected for multiple comparison ($p < 0.05$), but no FDR significant results were found.

Discussion

In this study, we analyzed dFNC differences between EO and EC conditions in a relatively large dataset collected from 173 adolescents aged 9.1–15.5 years old. Results supported widespread resting functional connectivity differences between eyes open/EC, consistent with our previous study of static connectivity (Agcaoglu et al., 2019); importantly, here we additionally show that these differences vary over time with the largest changes localized to one particular transient connectivity pattern. We found that among five dynamic states, state 1 was observed to be dominant during the EO condition. This state showed the highest Vis–Vis connectivity and the lowest Vis–SM and Vis–Aud connectivity. State 2 was observed to be dominant during the EC condition and showed the highest SM–SM, Aud–Aud, SM–Aud, Vis–SM, and Vis–Aud connectivity. Also, state 2 showed a high anticorrelation between the SC–Aud and SC–SM networks.

States 1 and 2 showed FDR significant differences in the dwell time and states 1, 2, 3, and 4 showed FDR significant differences in the frequency of occurrence between EO and EC conditions.

The centroid of state 1 (observed to be dominant during the EO condition) resembled the average sFNC results for the EO condition, and the centroid of state 2 (observed to be dominant during the EC condition) resembled the average sFNC results for the EC condition. This tells us that the previous static results, though accurate, are not providing a complete picture. The fact that different states capture changes across time tells us that these differences are manifested as

time-varying changes in connectivity (i.e., they are large changes that are visible in the static results, but not occurring with the same temporal signature). These results, thus, provide evidence that having eyes open versus EC during resting-state scanning has a significant impact on the connectivity dynamics.

An important finding of this study is that the number of subjects in each state shows a linear increase or decrease for the EC condition in states 2 and 3. These distinctive linear trends do not occur for any states in the EO condition, although they did fluctuate over time. State 2 was unique in that it showed high anticorrelation between the SC–Aud and SC–SM networks, especially RSN 58 and 68 (right and left thalamus) showed anti-correlation to Aud and SM.

Changing from positive correlation to anti-correlation in thalamus to cortical connectivity has been identified as a feature of the transition from vigilance to sleep (Allen et al., 2018). State 2 in our study resembles state 4 in Allen et al. (2018), which was associated with a decrease in vigilance, an increase in delta and theta power, and a decrease in alpha power within the simultaneously collected EEG. However, in our study, state 2 showed a high increase in occurrences over time in the EC case and a slight increase in occurrences in the EO case, whereas state 4 in Allen et al. (2018) showed a high increase in the EO case and a slight increase in the EC case. Allen et al. (2018) used a dataset from an adult cohort (age 29 ± 8.8 years), whereas our dataset consists of adolescents, which may explain the difference, but further studies are needed.

This is an example of how the current dFNC analysis provides additional information beyond an sFNC analysis; a dFNC analysis enables us to track the changes in brain connectivity during scanning. Also, it provides the opportunity to examine patterns of inter-state changes (i.e., determine the state that follows or precedes another state; the state

FIG. 5. Number of subjects in each state over time. We observe an increase in the number of subjects in state 2 as time increases. This is not only more significant for the EC condition but also observable in the EO condition. State 3 also shows a decrease in the number of subjects over time for the EC condition. Color images are available online.

transition matrix), which appears to be sensitive to conditional differences such as those studied here, as we observed significant differences between EO and EC in the transition from states 3 to 1.

Our results further suggest that brain connectivity for the EC condition changes during scanning, which may be due to changes in the default or idling brain condition, and our results show interesting differences between Vis–Vis, Vis–SM, and Vis–Aud, and between EC and EO.

Finally, in this study, although the main focus was to compare EO/EC differences in dFNC, we were also expecting some development (age) effect on dFNC. However, we did not find any significant association between dFNC and age in our cohort of 9- to 15-year-old adolescents. This age range is generally considered to be a time of rapid physical and mental development, as well as the onset of puberty. Such rapid development and individual differences in the

onset of puberty could be a source of high between-subject variability, and this could underlie our finding of no age-related FNC differences between EO and EC. Nevertheless, further studies are needed to address this, and a future study of longitudinal changes would especially help inform the field regarding the relationship between developmental changes and dynamic brain connectivity.

Limitations

Before concluding, it is important to acknowledge some limitations of this study. First, although the sliding window technique is widely used and accepted in the field, there are some controversies regarding the method, such as the choice of the window size. Our method used a relatively larger window size, which may result in a loss of information if the change in FNC is fast (Faghiri et al., 2020). Another

FIG. 6. Top row, sFNC results for EO and EC cases (Agcaoglu et al., 2019). States 1 and 2 resemble these results with small differences. Middle row shows the paired t -test results between EO and EC. Bottom row shows the differences between state center 1 and state center 2. The state center differences also resemble the paired t -test results between EO and EC sFNCs. sFNC, static functional network connectivity. Color images are available online.

FIG. 7. Two-sample t -test difference between EO and EC samples (EO-EC) in each state; upper triangular matrix shows the unthresholded results, and lower triangular matrix displays 0.01 FDR-corrected results as $-\text{sign}(t\text{-statistics}) \times \log_{10}(p)$. All states show FDR significant differences between EO and EC conditions; the differences in states 1 and 2 are very modular and affect a wide range of areas. States 2 and 4 show fewer differences compared with other states. Color images are available online.

FIG. 8. Two-sample t -test difference between EO and EC domain averages (EO-EC) in each state; upper triangular matrix shows the un-thresholded results, and lower triangular matrix displays 0.01 FDR-corrected results as $-\text{sign}(t\text{-statistics}) \times \log_{10}(p)$. Color images are available online.

controversial point is about the design of appropriate null models. Miller et al. (2018) showed that windowed FNC can be challenging to simulate via null models. Another debated point is the degree to which fluctuations between windows are driven by factors such as task, arousal, or spontaneous thought. Much of these are reviewed in a recent collaborative paper (Lurie et al., 2020). There are also some newer approaches that can potentially be used to avoid selecting a specific window (Faghiri et al., 2020; Yaesoubi et al., 2018).

Second, we used a group ICA model order of 150, but examining the dFNC with lower and higher gICA model orders would also be interesting. Based on our (and others) extensive previous work on ICA analysis showing the replicability of brain networks at different model orders, we know that the primary impact of adding more components or removing some from the model can mainly provide an opportunity to study brain connectivity at different spatial scales. Although a multi-scale approach would be interesting, given the large number of results, we believed that a follow-up study focusing specifically on this question would be most appropriate.

Third, we did not have a measure of pubertal status on our participants. Future developmental studies in this area should implement a pubertal scale or measure hormones such as testosterone to estimate pubertal stage across their sample. Also, there was an association between age and MFD for the EC case.

Finally, though the ICA approach has, by design, identified maximally spatially independent networks, spatial similarities or independence of the selected RSNs with previous studies were not precisely checked. A detailed spatial analysis of each of the networks would be very interesting and future studies should directly investigate this. We are currently also working on auto-labeling tools that may help automate the selection process in the future.

Conclusion

In conclusion, our study indicated that there are significant differences in dFNC between EO and EC rs-fMRI, consistent with our previous study of static connectivity (Agcaoglu et al., 2019). Also, our analysis revealed that these differences vary over time, especially in one particularly transient connectivity pattern. Overall, our findings demonstrate that both static and dynamic rs-fMRI connectivity patterns are strongly impacted by basic condition differences such as EO and EC. One key advantage of EO is that subjects are less likely to fall asleep; however, given the substantial differences in this study, which are unlikely to be due to sleep based on post-scan information, we believe there is benefit in having the EO/EC as a contrast of interest in future studies, if time allows.

Controlling basic condition may also reduce the variability reported across resting-state studies and, in turn, may reduce the overall time required to obtain stable state metrics, which is especially important for studies in children. We also did not find any significant effect of age, suggesting that in these rapidly developing ages of adolescence a high between-subject variability occurs.

Authors' Contributions

V.D.C., J.M.S., T.W.W., and Y.P.W. designed the study, wrote the protocol, and collected the rs-fMRI data; O.A. and V.D.C. processed the data and designed the experiment;

and O.A. ran the analysis and wrote the first draft of the article. All authors contributed to and approved the final version of the article.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5
Supplementary Figure S6
Supplementary Figure S7
Supplementary Figure S8

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