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Changes in dynamic functional connections with aging

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ABSTRACT

Despite numerous studies on age-related changes in static functional connections (FCs), the available literature on the changes in dynamic FCs with aging is lacking. This study investigated the changes in dynamic FCs with aging based on resting state fMRI data of 61 healthy adults aged 30–85 years. The time-resolved FCs among 160 pre-defined regions of interest (ROIs) were first estimated using sliding-window correlation. Based on the dynamic FC matrices, we then analyzed the dynamic switches between different FC states using k-means clustering, and correlated age with the dwell time of each FC state across subjects. The elderly were observed to spend more time in an FC state characterized by weak interactions throughout the brain and less time in an FC state characterized by strong interactions within the sensory-motor network and the cognitive control network. These results may reflect an overall weakening of connections in the elderly, which support less efficient information transfer in them. Based on the dynamic FC matrices, we also evaluated the variability and amplitude of FC time-series, which measure the relative (to mean) and absolute strength of FC fluctuations, respectively, and correlated age with the two measures across subjects. Relatively weak age-vs-variability correlations were observed, but we did observe significant negative age-vs-amplitude correlations at both the global and regional level. These results indicate that amplitude may be another effective metric for assessing FC fluctuations, in addition to the widely-used variability metric. Moreover, the observed declines in the amplitude of FC fluctuations in the elderly may support the assumption that it should be the weakening of absolute interactions between brain regions, rather than toggling between positive and negative correlations, that causes the repeatedly reported widespread (static) FC decreases with aging. Overall, the present results not only reflect an overall weakening of connections in the elderly, but indicate the potential of dynamic FC analyses in studies of age-related psychiatric and neurological disorders.

Introduction

Numerous resting state fMRI (RS-fMRI) studies have been performed on aging of the human brain (Ferreira and Busatto, 2013; Sala-Llonch et al., 2015). The majority of these studies analyzed age-related changes in functional connections (FCs), which are expected to reflect functional interactions between brain regions. According to these studies, the networks associated with primary functions (e.g., the somatosensory network and the motor network) are largely intact, while higher-level processing networks (e.g., the default mode network [DMN] and the fronto-parietal network [FPN]) often degenerate in the elderly (Naik et al., 2017). In these studies, FCs were evaluated in a time-averaged sense, based on the assumption that FCs are temporally stationary in the resting brain. The assumption of temporal stationarity provided a convenient framework with which to examine the average interactions among brain regions.

Recent investigations provided compelling evidence challenging the "stationary" assumption of resting state FCs (Deco et al., 2015; Ekman et al., 2012; Vanrullen et al., 2011). In light of this, an increasing number of recent studies analyzed the complex dynamic characteristics of FCs, rather than static FCs, based on resting state fMRI (Allen et al., 2014; Chang and Glover, 2010; Gonzalez-Castillo et al., 2015; Hutchison and Morton, 2015; Hutchison et al., 2013; Ma et al., 2014; Marusak et al., 2017; Shen et al., 2016; Shine et al., 2016; Suk et al., 2016; Yu et al., 2015; Zalesky and Breakspear, 2015; Zalesky et al., 2014). According to these studies, dynamic FCs are reproducible across time and subjects (Allen et al., 2014; Gonzalez-Castillo et al., 2015), and alter with maturation (Hutchison and Morton, 2015; Marusak et al., 2017), long-term training (Shen et al., 2016) and disease (Ma et al., 2014; Suk et al., 2016; Yu et al., 2015). In a recent review by Naik et al. (2017), it was pointed out that "the dynamic nature of FC is often not acknowledged by the current theories of aging." Naik et al. suggest that "age-related

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dynamic changes need to be quantified in terms of FC dynamics … to understand the dynamics of the aging brain better". Consistent with this suggestion, the purpose of this study was to investigate the changes in dynamic FCs with aging.

Dynamic FCs have traditionally been analyzed from two perspectives, namely, dynamic switches between FC states, and temporal fluctuations in FC time-series. Specifically, once the time-resolved FCs were mapped based on resting state fMRI (e.g., using a sliding-window approach), a time-series of FC matrices (time × regions × regions) could be obtained. FC states could then be obtained by clustering the FC matrices (regions × regions) into several network patterns that repeatedly occur across time and subjects. Considering the close link to EEG microstates, FC states have been suggested to reflect the coordination of large-scale neural assemblies supporting various cognitive processes (Allen et al., 2014). Individuals' dwell time in FC states has been reported to vary with maturation (Hutchison and Morton, 2015; Marusak et al., 2017), long-term training (Shen et al., 2016) and disease (Ma et al., 2014; Suk et al., 2016; Yu et al., 2015). Based on their "metastability" hypothesis, Naik et al. (2017) expected "slow switching between network states" and/or "higher dwell-time in a particular network state" in the elderly, while "the literature on aging lacks characterization of such 'switching dynamics'". To enrich our knowledge regarding changes in dynamic switches between FC states with healthy aging, we associated age with the dwell time of FC states in this study.

Despite the heavy dependence on factors such as size of window and extent of overlap (Betzel et al., 2016; Thompson and Fransson, 2015), there has been a surge of interest in analyzing the temporal fluctuations in FC time-series in recent years. Many studies have analyzed the *variability* of temporal fluctuations in FC time-series (Kucyi et al., 2013; Kucyi and Davis, 2014; Laufs et al., 2014), and the measure was reported to be sensitive to maturation (Hutchison and Morton, 2015; Marusak et al., 2017) and disease (Ma et al., 2014; Suk et al., 2016; Yu et al., 2015). The measure "variability" in these studies was used to evaluate the relative strength (relative to the mean) of FC fluctuations. Shen et al. (2016) recently introduced a measure named "amplitude of the low-frequency fluctuation of FC (ALFF-FC)" to assess the absolute strength (relative to zeros) of FC fluctuations. According to Shen et al. (2016), ALFF-FC was not only sensitive to long-term training, but also specific enough to decode individuals' experience in long-term training. We expect that "amplitude" may be an effective measure for connections that toggle between positive and negative correlations, which would reduce to zero in static FC analyses (Zalesky et al., 2014). To deepen our understanding of the changes in brain function with aging, age was also associated with the variability and amplitude of FC fluctuations in this study.

This study was performed on the RS-fMRI data of 61 healthy adults aged 30–85 years extracted from a publicly released dataset. The study was carried out by first evaluating the time-resolved FCs between each pair of 160 regions of interest (ROIs) for each subject using sliding-window correlation. To investigate the changes in dynamic switches between FC states with healthy aging, k-means clustering was then used to capture the FC states, and age was finally correlated with the dwell time of each FC state across subjects. To investigate the changes in temporal fluctuations in FC time-series with aging, age was also correlated with the variability and amplitude of FC fluctuations.

Materials and methods

Dataset

The data used in this study were selected from the publicly released dataset "the Nathan Kline Institute/Rockland Sample (NKI–RS)" (http://fcon_1000.projects.nitrc.org/indi/pro/nki.html) (Nooner et al., 2012), which has been used in several recent studies on age-related changes in brain function (Betzel et al., 2014; Cao et al., 2014; Tian et al., 2016; Yang et al., 2014). The data acquisition was approved by the institutional review board of the Nathan Kline Institute. The initial release of the NKI-RS dataset included 207 participants, each of whom underwent multimodal brain scans and a battery of psychiatric assessments. Subjects that satisfied the following criteria were included in the present study: 1) RS-fMRI data were available; 2) ≥30 years old; 3) with no mental disorder; 4) with no excessive head motions. That is, head motions were ≤2.0 mm displacement in any of the x, y, or z directions and ≤2.0° of any angular motion throughout the scan, and time points with framewise displacement >0.5 mm were less than 20% (Shine et al., 2016). According to the criteria, 61 subjects were included in the study (34 males, aged 30–85 years [mean ± standard deviation = 50.10 ± 14.59]). The ID list of subjects included in this study can be found in Table S1, and a bar plot of the distribution of subjects' ages can be found in Fig. S1.

The MRI data were acquired on a 3.0 T SIEMENS Trio scanner. RS-fMRI images were collected axially using an echo-planar imaging sequence sensitive to blood oxygen level dependent (BOLD) contrast with the following parameters: TR/TE = 2500/30 ms, FA = 80°, FOV = 216 mm, matrix = 64 × 64, slices = 38, thickness = 3.0 mm, 260 volumes. A total of 260 vol of RS-fMRI images were obtained. High-resolution T1-weighted images were acquired using the magnetization-prepared rapid gradient echo (MPRAGE) sequence with the following parameters: TR/TE = 2500/3.5 ms, FA = 8°, thickness = 1.0 mm, slices = 192, matrix = 256 × 256, FOV = 256 mm. Other images not used in the present study will not be described here.

Data preprocessing

RS-fMRI data preprocessing was performed by use of FSL (Jenkinson et al., 2012; Smith et al., 2004) (http://www.fmrib.ox.ac.uk/fsl). The following processing steps were applied to the RS-fMRI data of each subject: 1) removing the first 5 vol; 2) correcting for head motion with MCFLIRT; 3) removing the non-brain tissues with BET; 4) spatial smoothing using a Gaussian kernel of full width at half maximum 5 mm; 5) high-pass temporal filtering to remove slow drift (cut-off frequency = 0.01 Hz); 6) registering the subject's RS-fMRI data to his/her high-resolution structural image, then to Montreal Neurological Institute 152 standard space using FLIRT and FNIRT tools, and resampling the subject's registered RS-fMRI data to 2 × 2 × 2 mm resolution; 7) regressing out nuisance including white matter, cerebrospinal fluid, and global signals and their derivatives, in addition to 24 movement regressors derived by Volterra expansion (Power et al., 2014; Shine et al., 2016); 8) band-pass filtering (0.01 < f < 0.1 Hz) of the time-series of each voxel.

Analysis of dynamic FCs

We defined the 160 ROIs by setting ten-mm-diameter spheres centered at the meta-analysis-based activity peaks reported in the study by Dosenbach et al. (2010). The mean time-series of each ROI was obtained by averaging the signals of all voxels within the ROI. For display convenience, the ROIs were divided into four networks following the same strategy as that in the study by Dosenbach et al. (2010). The four networks are the cognitive control network (CCN), the DMN, the sensori-motor network (SMN) and the occipital-cerebellum network (OCN).

The time-resolved FCs were mapped based on the mean time-series of 160 ROIs using a sliding window approach. Specifically, we calculated the Pearson's correlation between each pair of ROI time-series using a sliding temporal window of 45 s (18-point Tukey window, with the ratio of the length of taper section to the total length of the window set to 0.5, slid in steps of 1 TR [2.5 s]) (Rashid et al., 2016). According to Zalesky and Breakspear (2015), this window length ensures the detection of non-stationary fluctuations in FC while controlling false positives. The correlation coefficients were finally transformed into z-scores using Fisher's r-to-z transformation to improve normality. These analyses produced a time-series of FC matrices (238 windows × 160 × 160) for each subject, and later analyses were based on these matrices.

Relating age to functional connectivity states

K-means clustering was used in this study to capture the FC states (Allen et al., 2014; Shen et al., 2016). For this purpose, each of the 61 × 238 (samples × windows) windowed FC matrices was regarded as one observation, and each element of a 160 × 160 windowed FC matrix was regarded as one variable. The cityblock distance was used as the distance measure in this study (Allen et al., 2014; Shen et al., 2016). Two critical but challenging problems for k-means clustering are determining the k-value (number of clusters) and setting the initial cluster centroids. In this study, k-means clustering with varying k-values (3–10) was performed to avoid possible bias caused from the use of an inappropriate k-value. The initial cluster centroids were set by performing preliminary clustering based on 10% of observations (1464, out of 61 × 238) at random, and the preliminary phase itself was initialized using k observations at random. Considering randomness may occur in the preliminary phase, the k-means clustering was repeated ten times based on each k-value.

Three cluster centroids, which are referred to as FC states below, were observed to repeatedly occur in all 80 clustering analyses (8 k-values × 10 rounds) by visual inspection. In the following analyses, the three FC states were the primary focus, and are referred to as State 1, State 2 and State 3, respectively, in this section. To evaluate the consistencies of the results based on the 80 clustering analyses, the three FC states obtained in the first round of clustering based on k = 3 (shown in Fig. 1 [A-C]) were set as reference states. States 1, 2 and 3 in each of the other 79 cases were designated as the one whose centroid exhibited the strongest correlation with that of reference states 1, 2 and 3, respectively. The reoccurring frequency of each FC state was evaluated by its dwell time, which was calculated as the number of temporal windows belonging to the FC state.

To investigate whether there were significant changes in switching dynamics with healthy aging, age was correlated with the dwell time of each FC state across subjects. The individuals' gender, brain volume, mean framewise displacement and the number of time points whose framewise displacement was >0.5 mm were used as covariates. The statistical significance of the correlation between age and the dwell time of each FC state was evaluated using permutation analysis. Unlike parametric tests, which rely on certain assumptions of data distribution (e.g., normal or uniform distribution), permutation analysis is a non-parametric method and requires only exchangeability of the data (Winkler et al., 2014). With the use of permutation analysis, possible biases that may occur in parametric tests (i.e., because of the non-uniformity of the subjects' ages) can largely be avoided (Winkler et al., 2014). Using 10,000 permutations, we estimated how likely we were to observe the same correlation by chance. The permutation analyses were performed by randomly permuting the ages of the subjects 10,000 times (randomly assigning the age of one subject to another), and the dwell time of the FC state was correlated with the permuted ages. The p-values representing the probability of observing the reported correlation by chance were defined as:

$$P = \frac{1 + \mathrm{N}_{Stronger\ \ Correlations}}{1 + \mathrm{N}}$$

where N is the number of permutations, and here $N = 10,000$; $\mathrm{N}_{Stronger\ \ Correlations}$ is the number of stronger correlations (as compared to that based on non-permuted ages).

Relating age to temporal fluctuations in FC time-series

This study also investigated whether there were general or regional

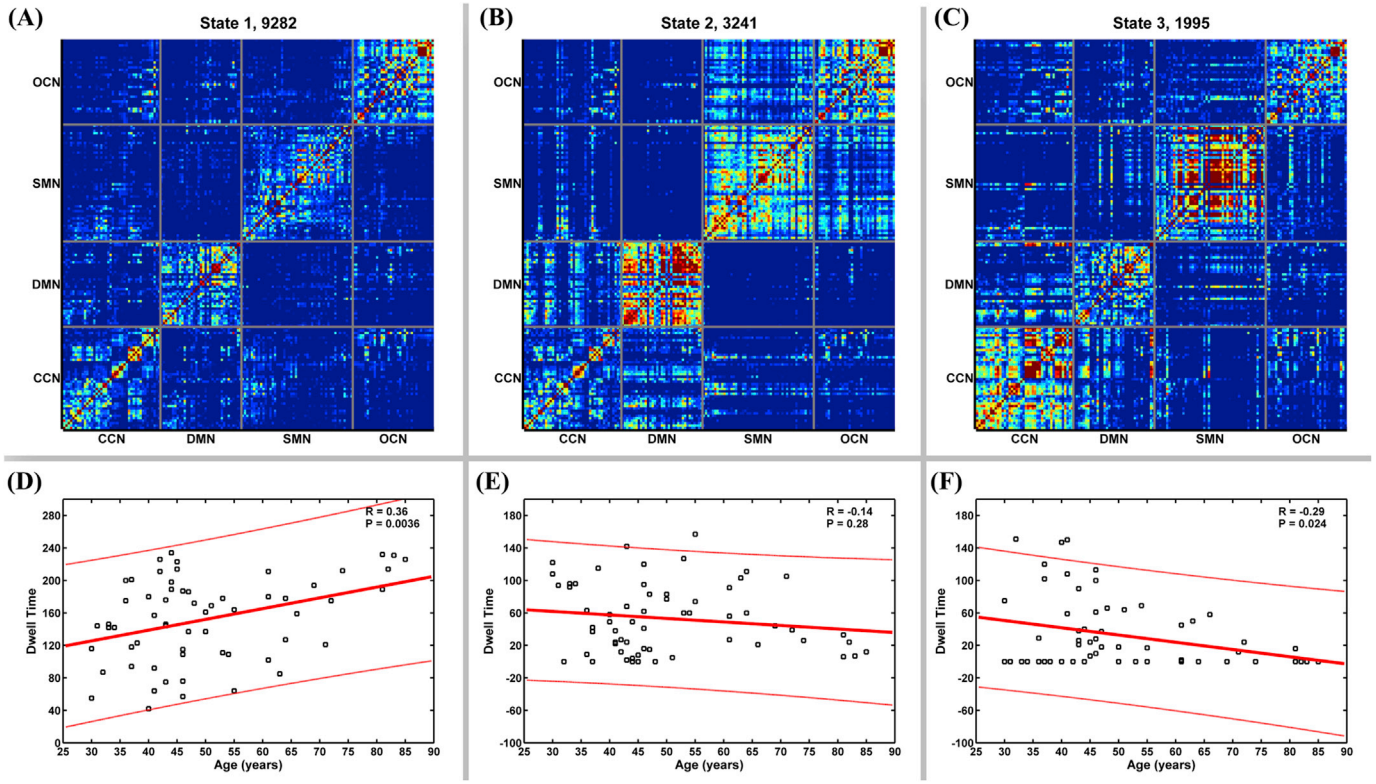

Fig. 1. Representative results regarding functional connectivity states. The results were based on the first round of k-means clustering with k = 3. The centroid of States 1 (the loose interaction state), 2 (the DMN interaction state) and 3 (the SMN-CCN interaction state) are shown in (A), (B) and (C), respectively, with the connectivity matrices arranged based on functional networks. On the top of each cluster centroid, the total number of occurrences is listed. The plots of the dwell time vs age are shown in (D), (E) and (F). CCN - cognitive control network; DMN - default mode network; SMN - sensori-motor network; OCN - occipital-cerebellum network.

changes in temporal fluctuations in FC time-series with aging. The FC fluctuations were evaluated by their variability and amplitude. The variability of a connection was defined as the standard deviation of the FC time-series across the 238 windows, and the amplitude of a connection was defined as the average absolute value of the FC time-series across the 238 windows. FC fluctuations at the global level were evaluated by averaging the variability/amplitude over all 12,720 (C_{160}^2) connections, and FC fluctuations at the regional level were evaluated by averaging the variability/amplitude over the 159 connections associated with each ROI. Age was finally correlated with global and regional measures of FC fluctuations, again with individuals' gender, brain volume, mean framewise displacement, and the number of time points whose framewise displacement was >0.5 mm used as covariates. A total of 10,000 permutations were performed to estimate how likely we were to observe the correlations by chance, and regional-level correlations were thresholded at $p \leq .05$ (FDR corrected).

Results

K-means clustering was used in this study to capture the FC states that reoccur over time and present in multiple subjects. For the consideration of reliability, 80 clustering analyses (8 k-values × 10 rounds) were performed. The centroids obtained in the first round of clustering based on k = 3 are displayed in Fig. 1 (A, B, C). State 1 was characterized by weak interactions throughout the brain, referred to as a "loose interaction state" below; State 2 was characterized by strong interactions within the DMN, referred to as a "DMN interaction state" below; State 3 was characterized by strong interactions within the SMN and the CCN, referred to as a "SMN-CCN interaction state" below. Despite the fact that there were often states being unique to individual participants when k ≥ 4 (Fig. S2), the three states were repeated not only across different rounds of clustering analyses, but also across different k-values (Figs. 1, S3–S10, Tables 1, 2). Specifically, the correlations between the reference centroids (as displayed in Fig. 1[A, B, C]) and those obtained in the other 79 cases (as displayed in Figs. S3–S10) for the loose interaction state (State 1), the DMN interaction state (State 2) and the SMN-CCN interaction state (State 3) were 0.99 ± 0.0075 (0.97–1.00), 0.95 ± 0.033 (0.87–1.00) and 0.96 ± 0.027 (0.88–1.00), respectively. In addition, the loose interaction state occurred much more frequently than other FC states. For instance, the loose interaction state occurred 9282 times in the first round of clustering based on k = 3, compared with 3241 and 1995 times for the DMN interaction state and the SMN-CCN interaction state, respectively (Fig. 1 [A, B, C]).

The dwell time of the loose interaction state was uniformly positively correlated with age in all 80 cases (8 k-values × 10 rounds), indicating that the elderly spend more time in the loose interaction state. The correlations were significant in 76 cases ($p \leq .05$), and marginally significant in 3 case ($p \leq .1$) (Fig. 1 [D], Tables 1, 2, S2). The dwell time of the SMN-CCN interaction state was uniformly negatively correlated with age. The correlations were significant in 57 cases ($p \leq .05$), and marginally significant in 16 cases ($p \leq .1$) (Fig. 1 [F], Tables 1, 2, S2). Although the dwell time of the DMN interaction state was negatively correlated with age in all 80 cases, none of the correlations were significant (Fig. 1 [E], Tables 1, 2, S2). Notably, as the results were repeatable when the k-value was relatively large (e.g., k = 8, 9, 10), the observed correlations are less likely to stem from the see-saw effects (more time points assigned to one FC state, fewer time points assigned to another FC state).

To investigate whether there were global or regional changes in temporal fluctuations in FC time-series with aging, age was also associated with the variability and amplitude of FC fluctuations. At the global level, age significantly correlated with the amplitude of FC fluctuations ($R = -0.39$, $p = .0018$) (Fig. 2 [B]), but the correlation with the variability of FC fluctuations was less significant ($R = -0.26$, $p = .044$) (Fig. 2 [A]). At the regional level, age was significantly negatively correlated with the amplitude of fluctuations in FCs associated with eight ROIs ($p \leq .05$, FDR corrected) (Fig. 3). Two of these ROIs were critical components of the salience network (SN) (Fig. 3 [B, H]), five were components of the FPN (Fig. 3 [A, D-G]) and one was in the cerebellum (Fig. 3 [C]). No significant age-vs-variability correlation was observed at the regional level.

Discussion

In this study, we investigated the changes in dynamic FCs with aging based on resting state fMRI data of 61 healthy adults aged 30–85 years. We found that the elderly spent more time in an FC state characterized by weak interactions throughout the brain (the loose interaction state), and less time in an FC state characterized by strong interactions within the SMN and the CCN (the SMN-CCN interaction state). Global and regional declines in the amplitude of FC fluctuations were also observed. The current results demonstrate how the "switching dynamics" changed with aging, and indicate that the repeatedly reported widespread (static) FC decreases in the elderly may largely be caused by the weakening of absolute interactions between brain regions, rather than toggling between positive and negative correlations.

Changes in the dwell time of FC states with aging

The loose interaction state, which was characterized by weak FCs throughout the brain, occurred more frequently than other FC states in all 80 cases in this study (Figs. 1, S2–S10). This finding is consistent with those previously reported by Allen et al. (2014) and Marusak et al. (2017). In the study by Allen et al. (2014), the state characterized by weak interactions occurred in 33% of all windows (in contrast to ≤15% for the other six FC states). In the study by Marusak et al. (2017), the state occurred in 34% of all windows (in contrast to ≤18% for other five FC states). Resting state FCs have been suggested to support efficient information transfer between brain regions (Fox and Raichle, 2007; Petersen and Sporns, 2015). According to this suggestion, weak FCs throughout the brain may indicate relatively less efficient information transfer. Correspondingly, greater occurrence of the loose interaction state in these studies may indicate that the human brain switches to a state supporting less efficient information transfer more frequently than

Table 1
Repeatability of the results regarding the three FC states across ten rounds of clustering with k = 3.

Round No		1	2	3	4	5	6	7	8	9	10
State 1	R′	1.00	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00
	R	0.36	0.30	0.31	0.31	0.36	0.35	0.35	0.35	0.31	0.29
	P	0.0036	0.020	0.017	0.014	0.0046	0.0063	0.0050	0.0054	0.014	0.017
State 2	R′	1.00	0.98	0.98	0.98	1.00	1.00	1.00	1.00	0.99	0.98
	R	−0.14	−0.12	−0.14	−0.15	−0.15	−0.15	−0.15	−0.15	−0.13	−0.13
	P	0.28	0.34	0.27	0.27	0.24	0.26	0.25	0.25	0.30	0.33
State 3	R′	1.00	0.96	0.96	0.96	1.00	1.00	1.00	1.00	0.97	0.96
	R	−0.29	−0.24	−0.24	−0.24	−0.29	−0.29	−0.29	−0.29	−0.26	−0.24
	P	0.024	0.057	0.059	0.061	0.021	0.022	0.022	0.022	0.045	0.057

R′ indicates the correlation with the corresponding reference centroid as was displayed in Fig. 1; R indicates Pearson's correlation between its dwell time and age, and P indicates the corresponding p-value (uncorrected).

Table 2
Repeatability of the results regarding the three FC states with k-values ranging from 4 to 10.

k-value		4	5	6	7	8	9	10
State 1	R′	1.00	0.99	0.99	1.00	0.99	0.99	0.98
	R	0.31	0.38	0.38	0.38	0.35	0.38	0.40
	P	0.013	0.0029	0.0028	0.0026	0.0070	0.0017	0.0011
State 2	R′	1.00	0.98	0.97	1.00	0.98	0.95	0.93
	R	−0.11	−0.10	−0.13	−0.14	−0.10	−0.090	−0.090
	P	0.37	0.47	0.30	0.28	0.44	0.48	0.47
State 3	R′	0.99	0.99	0.99	1.00	0.99	0.99	0.97
	R	−0.28	−0.28	−0.29	−0.27	−0.31	−0.29	−0.26
	P	0.028	0.034	0.026	0.033	0.013	0.021	0.040

For each k-value, results based on the first round of clustering were listed here. R′ indicates the correlation with the corresponding reference centroid as was displayed in Fig. 1; R indicates Pearson's correlation between its dwell time and age, and P indicates the corresponding p-value (uncorrected).

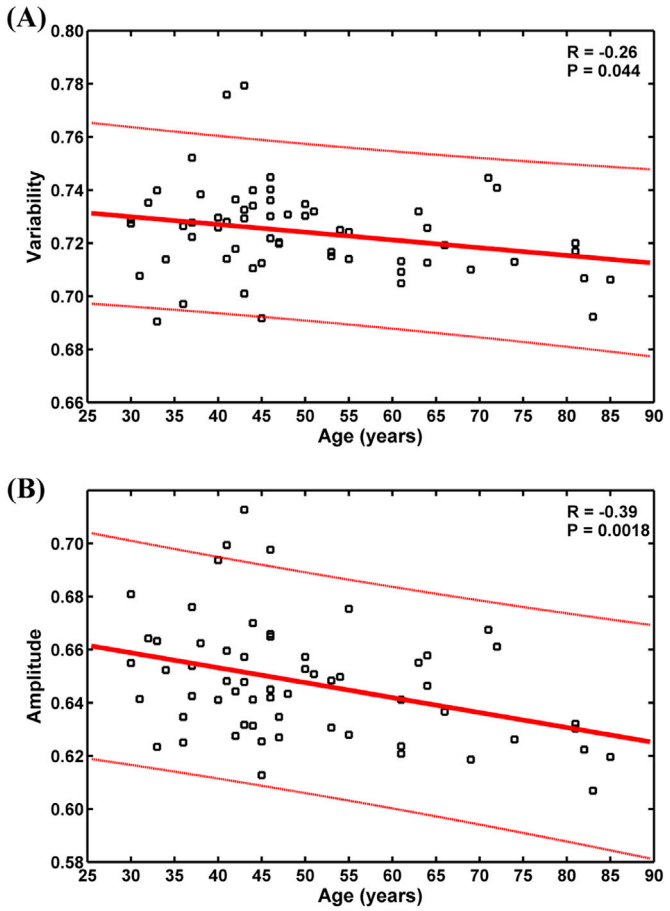

Fig. 2. The plots of the variability (A) and amplitude (B) of FC fluctuations vs age at the global level.

other states.

In this study, the dwell time of the loose interaction state was observed to be positively correlated with age (Fig. 1 [D]). This finding agrees well with the expectations of "higher dwell-time in a particular network state" by Naik et al. (2017). Naik et al. predicted that the working point in the brain of the elderly would shift from the optimal value derived from the young. They suggest that "shifts in the dynamical working point" in the brain of the elderly may "be reflected as slow switching between network states, in other words lower transition probability and/or higher dwell-time in a particular network state." According to the suggestion by Naik et al. (2017), our finding of significant positive correlation between age and the dwell time of the loose interaction state may reflect shifts in the dynamical working point in the brain of the elderly.

Closely related to the above finding, Marusak et al. (2017) reported a significant negative correlation between age and the dwell time of "a state of weak FC" across subjects aged 7–16 years. Based on these two findings, we suggest that the dwell time of the loose interaction state might follow a U-shaped developmental curve throughout life, with children and the elderly spending more time in this state, and the young adults spending less time. Further studies are needed to evaluate this hypothesis.

In addition to positive correlations observed on the loose interaction state, significant (or marginally significant) negative age-vs-dwell-time correlations were observed on the SMN-CCN interaction state (Fig. 1 [F]), which was characterized by strong interactions within the SMN and the CCN (Fig. 1 [C]). In contrast to the loose interaction state, the SMN-CCN interaction state may signify time windows supporting efficient cognitive control and sensory-motor information processing. Taken together, we suggest that the present findings of the elderly spending more time in the loose interaction state, and less time in the SMN-CCN interaction state, may reflect an overall weakening of connections in the elderly, and correspondingly, a less efficient information transfer in them.

Declines in the amplitude of FC fluctuations

In this study, the temporal fluctuations in FC time-series were assessed by their variability and amplitude, which reflect the strength of FC fluctuations relative to the mean and zero, respectively. Changes in the variability of FC fluctuations have been reported in a variety of situations such as maturation (Hutchison and Morton, 2015; Marusak et al., 2017) and disease (Ma et al., 2014; Suk et al., 2016; Yu et al., 2015). However, this study observed only a marginally significant age-vs-variability correlation at the global level (Fig. 2[A]) and no significant age-vs-variability correlation at the regional level. In contrast, the amplitude of FC fluctuations was observed to be significantly correlated with age at both the global (Fig. 2[B]) and regional level (Fig. 3). These results indicate that amplitude may be another effective metric with which to assess FC fluctuations, in addition to variability, which is prevalent in the region.

Many RS-fMRI studies on aging have reported widespread (static) FC decreases in the elderly (Ferreira and Busatto, 2013; Sala-Llonch et al., 2015). Theoretically, decreases in static FCs may be caused either by declines in the absolute strength of interactions between brain regions, or by toggling between positive and negative correlations (which were reduced to zero by temporal averaging in static FC analyses). The present finding of general declines in the amplitude of FC fluctuations (Fig. 2 [B]) supports the assumption that it may be the decreases in the absolute strength of interactions between brain regions that drive the repeatedly reported widespread (static) FC decreases in the elderly, as toggling between positive and negative correlations still requires the strong interactions between brain regions. This suggestion is in line with the findings that the elderly exhibit a certain degree of decline in white matter integrity (Sullivan and Pfefferbaum, 2006), which has been reported to shape the resting state FCs (Grandjean et al., 2017). That is,

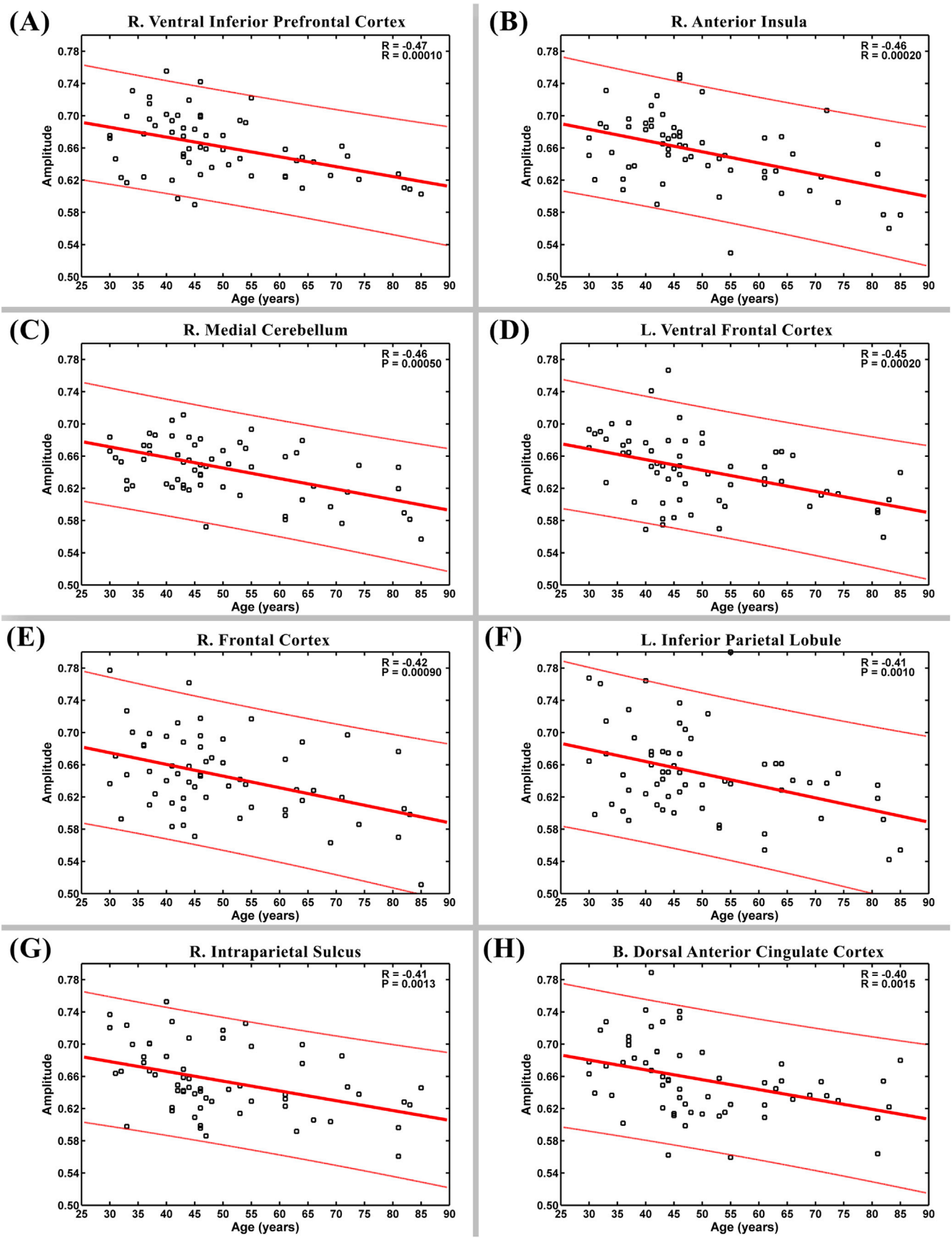

Fig. 3. The plots of the amplitude of FC fluctuations vs age at the regional level. Age was significantly negatively correlated with the amplitude of fluctuations in FCs associated with the ROIs ($p \leq .05$, FDR corrected). R – Right; L – Left; B – Bilateral.

deficits in white matter integrity in the elderly may be the structural basis of declines in the amplitude of FC fluctuations.

At the regional level, age was significantly negatively correlated with the amplitude of FC fluctuations associated with two components of the SN (Fig. 3 (B, H)), five components of the FPN (Fig. 3 (A, D ~ G)) and one cerebellum region (Fig. 3 (C)). Each of the networks has been reported to show structural/functional degenerations in former studies on healthy aging. Specifically, the fronto-parietal areas are known to be involved in a variety of high-level cognitive control processes, such as working memory, task switching, problem solving, decision making, and visual-spatial processing, which are often impaired in the elderly. Indeed, impaired cognitive functions in the elderly have been much associated with structural/functional degeneration of the FPN (Alichniewicz et al., 2012; Drag et al., 2016; Gold et al., 2010; Luft et al., 1999; Madden et al., 2010). The SN plays a crucial role in rapid detection of goal-relevant events and efficient utilization of appropriate cognitive resources, which are impaired in the elderly (Fabiani and Friedman, 1995; Walhovd and Fjell, 2001). Many studies have reported grey matter volume and FC decreases in the SN in the elderly (He et al., 2014; Onoda et al., 2012; Siman-Tov et al., 2016), and the functional/structural degenerations of the SN has been linked to cognitive decline (He et al., 2014; Onoda et al., 2012). Moreover, reduced FCs within the SN have been reported to be an important feature for distinguishing the elderly from the young (Meier et al., 2012). Taken together, the present findings provide a novel perspective with regard to functional degeneration of the SN and the FPN in the elderly. That is, in addition to static FCs, dynamic FCs associated with the two networks also changed in the elderly.

Methodological issues

One limitation of this study is the relatively small sample size. In fact, there were only 18 subjects aged 55–85 years (Fig. S1). Insufficient sampling of the elderly may limit the generalizability of the results, as possible heterogeneities in the dynamic FCs in the elderly (which may be closely related to heterogeneities in cognitive and psychological performance in them) may not be fully characterized. Further studies including sufficient samples of old age are expected to capture such heterogeneities. The Enhanced NKI Sample (http://fcon_1000.projects.nitrc.org/indi/enhanced/), which included nearly 1000 subjects aged 6–85 years, may be applicable for the purpose.

Like many other fMRI studies of aging and psychiatric diseases, we excluded subjects with high motion in this study, and the practice may not withstand close scrutiny. In general, the elderly and patients with psychiatric diseases tend to move more (e.g., due to physical discomfort). Once the subjects with high motion were excluded, the remaining elderly/patients may not be representative, and bias will consequently occur. Although we did not observe a significant age-vs-head-motion correlation ($R = 0.10$, $p = .38$) based on the NKI-RS dataset (78 samples, including those with high motion) (Fig. S11), we cannot rule out the possibility that the elderly included in the NKI-RS dataset happened to have good control of head motion. Therefore, conclusions drawn in the current study (as well as many other fMRI studies on aging and psychiatric diseases) may be applicable only for the samples that have good control of head motion.

Determining the k-value and setting the initial cluster centroids are two critical and challenging problems for k-means clustering analyses. Indeed, there is not yet a widely accepted method for determining the k-value for k-means clustering, and the same is true for initializing the cluster centroids. In this study, we performed k-means clustering with eight k-values (3–10), and performed ten rounds of clustering based on each k-value. The results indicate that the three FC states demonstrated in Fig. 1 (A, B, C) were highly repeatable, in their spatial patterns (Figs. S3–S10, Tables 1, 2, S2), and in their age-vs-dwell-time correlations (Tables 1, 2, S2). Therefore, we suggest that the present results are robust to inappropriate settings of k-values and initial centroids.

The choice of distance measure is an additional critical problem for k-means clustering. We used the cityblock distance as the distance measure in this study, and results regarding FC states were based on the cityblock distance. According to Allen et al. (2014), extremely similar results could be obtained based on different distance measures including cityblock, correlation, Euclidean, and cosine. However, when we performed the same analyses using correlation, cosine, and squared Euclidean as distance measures, it was found that only the results based on the squared Euclidean distance were highly similar to those based on the cityblock distance (Fig. S12, Table S3). This may be result from the fact that the cityblock and squared Euclidean distances measure the "absolute distances" between two variables, while correlation and cosine measure the "similarity" between two variables. Consequently, two variables that are near to each other based on the cityblock/squared Euclidean distance (e.g., [1.0, 1.1, 1.0, 1.1, 1.0] and [1.1, 1.0, 1.1, 1.0, 1.1]) may be quite distant from each other based on correlation/cosine. Similarly, two variables that are distant from each other based on the cityblock/squared Euclidean distance (e.g., [1.0, 1.1, 1.0, 1.1, 1.0] and [5.0, 5.1, 5.0, 5.1, 5.0]) may be near to each other based on correlation/cosine. In the current study, FC matrices with relatively weak interactions throughout the brain may be deemed to be near to each other and clustered into one FC state (the loose interaction state) based on the cityblock and squared Euclidean distances, while they may not be clustered into one FC state based on correlation/cosine, as the correlation/cosine between them are not necessarily high.

Controversy remains in the debate of whether to remove the global signal from the RS-fMRI time-series (Liu, 2016; Liu et al., 2017). In this study, all results were based on data with the global signal regressed out. When we performed the same analyses based on data without regressing out the global signal, similar results were obtained (Figs. S13–S15, Table S4). Specifically, when performing FC state analyses, we observed three FC states with similar patterns to those based on data with the global signal regressed out. The dwell time of the loose interaction state (State 1)/the SMN-CCN interaction state (State 3) was also significantly positively/negatively correlated with age (Fig. S13). Furthermore, the results regarding the three FC states were repeatable across different k-values (Table S4). With regard to analyzing the temporal fluctuations in FC time-series, significant age-vs-amplitude correlations were observed at both the global ($R = -0.44$, $p = .00040$) (Fig. S14) and regional level ($p \leq .05$, FDR corrected) (Fig. S15). Moreover, all eight regions exhibiting significant age-vs-amplitude correlations based on RS-fMRI data with the global signal regressed out (Fig. 3) were included in the 21 regions exhibiting significant age-vs-amplitude correlations (Fig. S15). These results indicate that the practice of regressing out the global signal from RS-fMRI data had limited influence on the present results and conclusions drawn.

We also evaluated whether the results are dependent on ROI definition by performing the same analyses (as have been performed on the set of 160 ROIs) based on two other sets of ROIs. One set was defined according to the study by Power et al. (2011), which included 264 ROIs and will be referred to as "Power [264]". Another set was defined according to the study by Monge et al. (2017), which included 397 ROIs and will be referred to as "Monge [397]". Based on each of the two sets of ROIs, one loose interaction state and one SMN-CCN interaction state were obtained by k-means clustering, and the loose interaction state occurred more frequently than other FC states (Fig. S16). The dwell time of the loose interaction state (State 1)/the SMN-CCN interaction state (State 3) was significantly positively/negatively correlated with age (Fig. S16). In addition, the results were repeatable across k-values (Table S5). Consistent with the results based on the set of 160 ROIs, the age-vs-amplitude correlation based on each set of ROIs was stronger than the age-vs-variability correlation (Fig. S17). As there were more comparisons here (264 for Power [264], and 397 for Monge [397]), no significant regional age-vs-amplitude correlation was observed at a threshold of $p \leq .05$ (FDR corrected), though the age-vs-amplitude correlation of 5/6 ROIs based on Power [264]/Monge [397] were comparable to those shown in Fig. 3 ($R \leq -0.4$). Therefore, the present results are

independent of ROI definition.

Conclusion

In this study, we investigated the changes in dynamic FCs with aging based on RS-fMRI. We observed that the elderly spent more time in an FC state characterized by weak interactions throughout the brain and less time in an FC state characterized by strong interactions within the SMN and the CCN. These results may reflect an overall weakening of connections in the elderly, which support less efficient information transfer in them. The results indicate that amplitude may be another effective metric for assessing FC fluctuations in addition to the widely-used variability metric. Moreover, the observed declines in the amplitude of FC fluctuations in the elderly may support the assumption that it should be the weakening of absolute interactions between brain regions, rather than toggling between positive and negative correlations, that causes the repeatedly reported widespread (static) FC decreases with aging. Despite these findings, it should be noted that the measures used in this study were relatively simple and straightforward, and further studies utilizing more sophisticated measures of the dynamic FCs, such as the flexibility of brain regions (Shine et al., 2016) and time-resolved network efficiency (Zalesky et al., 2014), are expected to provide a better understanding of the changes in dynamic FCs with aging.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2018.01.040.

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