Age-Related Changes in Resting-State Networks of A Large Sample Size of Healthy Elderly

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SUMMARY

Aims: Population aging is burdening the society globally, and the evaluation of functional networks is the key toward understanding cognitive changes in normal aging. However, the effect of age on default mode subnetworks has not been documented well, and age-related changes in many resting-state networks remain debatable. The purpose of this study was to propose more precise results for these issues using a large sample size. **Methods:** We used group-level meta-ICA analysis and dual regression approach for identifying resting-state networks from functional magnetic resonance imaging data of 430 healthy elderly participants. Partial correlation was used to observe age-related correlations within and between resting-state networks. **Results:** In the default mode network, only the ventral subnetwork negatively correlated with age. Age-related decrease in functional connectivity was also noted in the auditory, right frontoparietal, sensorimotor, and visual medial networks. Further, some age-related increases and decreases were observed for between-network correlations. **Conclusion:** The results of this study suggest that only the ventral default mode subnetwork had age-related decline in functional connectivity and several reverse patterns of resting-state networks for network development. Understanding age-related network changes may provide solutions for the impact of population aging and diagnosis of neurodegenerative diseases.

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Introduction

Population aging is a global phenomenon and is currently in an unprecedented and enduring condition, resulting in considerable implications for many dimensions of human life and markedly burdening a wide range of economic, political, and social conditions [1]. Aging leads to a large number of physical, biological, chemical, and psychological changes, steadily declining many cognitive processes across the lifespan. The introduction of functional imaging techniques reveals that age-related cognitive impairment is associated with alterations in brain functional connectivity [2–4], wherein many important findings from the evaluation of

resting-state networks (RSN) [5,6] that had strongly functional connected neural networks, as revealed by resting-state functional magnetic resonance imaging [7]. Exploring age-related changes in RSN is a key toward understanding the cognitive trajectory of aging and further serves as a physiological standard for overcoming clinical challenges occurring while diagnosing neurodegenerative diseases, particularly Alzheimer's disease (AD) [8].

Age-related decline in functional connectivity is observed both within and between several RSNs [5,6], the most consistently identified of which is the default mode network (DMN) [6,9,10]. DMN is a set of brain regions with intrinsic activities originally found as a pattern of decreased activity at task but increased

activity at rest [11,12]. Neuroscientists suspect that some neurodegenerative diseases, such as AD and depression, may be associated with DMN functional impairment [13]. Moreover, recent studies proposed that DMN is separable and the subnetworks are responsible for distinct cognitive functions [14–22]. The different effects of age on these cognitive functions are supported by behavioral science but with some controversy [19,23,24] and evidence from RSN is lacking. Clarifying the vulnerability of these subnetworks to aging can elucidate a functional basis for age-related changes of these cognitive functions.

In addition to DMN, some other RSNs and even between-network connections are influenced by aging; such changes may be the neural basis of cognitive decline in the elderly [5,6]. A previous study revealed that age-related decrease in connectivity exists in salience and visual networks and that the salience network change is associated with impaired frontal and parietal functions. Age-related decrease in connectivity is also present between DMN and visual network, suggesting attenuation of the hub function of DMN [6]. Another study reveals that age-related decrease in connectivity is found in the high-function networks, while the primary function networks are preserved [5]. Compared with young adults, the elderly have a reverse process of reduced modularity of RSNs, signifying increased internetwork connections and decreased intranetwork connections that were probably related to cognitive decline [5]. Furthermore, a previous study has found decreased connectivity in the dorsal attention network, probably underlying the impairment in sustained attention, and increased connectivity in the somatosensory cortex, cerebellum, amygdala, and thalamus, which are relatively less vulnerable networks or regions [25]. Functional connectivity also decreases in the motor network, possibly related to an impaired motor ability [26]. RSNs involving frontal and parietal areas have been reported to decrease in functional connectivity with age [9]. Age-associated modifications within and between many RSNs are critical in the comprehension of cognitive aging; however, previous results have been quite inconsistent [5,6,9,25]. A large sample size can contribute toward more precise results and detect small differences, delineating a closer and more complete picture of the cognitive conditions [27].

The degree to which age affects the relevant cognitive functions of default mode subnetworks seems to be different. Therefore, we hypothesize that the effect of age on the functional connectivity of these subnetworks would be different, and functional evaluation can serve as complementary evidence on age-related cognitive changes in behavioral science. Further, the importance of within- and between-network changes on other RSNs in cognitive aging is highlighted. Here, we used a large sample size to acquire more precise results and better understand cognitive changes in aging.

Methods

Participants

The participants were recruited from the I-Lan Longitudinal Aging Study, a community-based aging cohort study in I-Lan, Taiwan, aiming to investigate the association between normal aging, cognitive decline, and brain imaging markers [28]. In brief, 1008

community-dwelling adults aged 50 years or older from the town of Yuanshan were randomized through household registrations of the I-Lan County Government and were invited to participate in the study via mail or telephone. This study randomly sampled half ($n = 504$) of the cohort subjects with the following inclusion criteria: (1) without a plan to move out in the near future and (2) age equal or older than 50 years old. Any participant with the following conditions was excluded: (1) unable to communicate and complete an interview, (2) unable to complete a simple motor task within a reasonable period of time because of functional disability, (3) suffering from any major illness with limited life expectancy, (4) presence of ferromagnetic foreign bodies or implants that were electrically, magnetically, or mechanically activated anywhere in the body, and (5) presence of major neuropsychiatric diseases. All participants provided written informed consent before participating in this study. The entire experiment was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of National Yang-Ming University, Taipei, Taiwan.

MR Image Acquisition

Magnetic resonance imaging scans were acquired on a Siemens 3T whole-body MRI scanner (Siemens Magnetom Tim Trio, Erlangen, Germany) using a twelve-channel head coil at the National Yang-Ming University in Taiwan. All acquired images were aligned to the anterior and posterior commissure. Whole-brain resting-state blood oxygen level-dependent (BOLD) functional MRI (rs-fMRI) images were collected using a T2*-weighted gradient-echo-planar imaging (EPI) sequence with the following imaging parameters: repetition time (TR)/echo time (TE) = 2500/27 ms, flip angle = 77°, matrix size = 64×64 , field of view = 220×220 mm, voxel size = $3.4 \times 3.4 \times 3.4$ mm, 43 interleaved axial slices without intersection gap, and 200 continuous image volumes. Further, anatomical three-dimensional T1-weighted images were collected using magnetization-prepared rapid gradient-echo sequence with the following imaging parameters: TR/TE/TI = 3500/3.5/1100 ms; flip angle = 7°; NEX = 1; field of view = 256×256 mm²; matrix size = 256×256 ; voxel size = $1.0 \times 1.0 \times 1.0$ mm³. An additional axial T2-weighted fluid-attenuated inversion recovery multishot turbo spin echo sequence (2D-T2-FLAIR BLADE; TR/TE/TI = 9000/143/2500 ms; flip angle = 130°; 63 slices; NEX = 1; echo train length = 35; matrix size = 320×320 ; field of view = 220 mm; slice thickness = 2.0 mm; voxel size = $0.69 \times 0.69 \times 2.0$ mm) was performed. All structural MRI scans were visually reviewed by an experienced neuroradiologist to confirm that participants were free from any morphologic abnormality. The total scanning time for each participant was 40 min.

Resting-State Functional Magnetic Resonance Imaging

The technique of rs-fMRI has been widely used to calculate intrinsic functional connectivity of the brain by measuring the BOLD signal fluctuations at the resting state [29]. RSNs have been proven to be a powerful tool for studying large-scale *in vivo* brain function [30–32]. The connectivity pattern of the human brain

during rest was most commonly investigated by seed-based correlation or data-driven methods. For seed-based techniques, there were some biases in the seed selection, and the resulting RSN maps were markedly affected even by a slightly different seed location [33,34]. These disadvantages can be avoided using techniques driven by exploratory data, such as independent component analysis (ICA), which can identify at least 10 large-scale RSNs in previous studies [35,36]. Therefore, we use ICA to approach RSNs in our study.

Image Preprocessing

Resting-state fMRI data were preprocessed using FSL v5.0.6 (Functional Magnetic Resonance Imaging of the Brain Software Library; <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) [37]. The preprocessing steps applied to these datasets included (1) correcting for subject movement during the imaging session using a three-dimensional rigid-body motion correction (realigned to the first EPI acquisition) using MCFLIRT (Motion Correction using FMRIB's Linear Image Registration Tool), (2) correcting for temporal shifts in fMRI data acquisition (slice timing correction), (3) removing nonbrain tissue using Brain Extraction Tool (BET), (4) smoothing with a 5-mm full-width at half-maximum Gaussian kernel, and (5) high-pass temporal filtering set at 0.01 Hz to remove low-frequency drifts. Any participant showing a maximum rotation of 1.5° or displacement of 1.5 mm in any direction was excluded from further analysis. Before large-scale intrinsic functional network identification using group ICA, the preprocessed time series data were registered into stereotactic space [MNI152 template; Montreal Neurological Institute (MNI), Montreal QC] using a two-stage spatial registration framework [38,39]. Because of the large sample size in this study, we resampled the MNI-space time series data into 4-mm resolution for further group ICA analysis.

Group-Level Large-Scale Intrinsic Functional Network Identification using Meta-ICA Approach

Preprocessed rs-fMRI dataset was used to generate robust unbiased data-driven large-scale intrinsic functional network templates using a metalevel ICA approach [40] using the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) package in FSL [35].

According to the optimized resampling number for meta-ICA approach in a previous simulation study [40], the preprocessed rs-fMRI datasets of the 430 subjects were randomized into twenty-five subsets with fifty age and sex-matched subjects for each subset. Because of limited computation resource capacity and for reducing over-fitting problem, we did not include subjects into the temporal concatenation ICA analysis all at once. We identified 40 components displaying maximal spatial independence effects and the characteristics of our own dataset for each MELODIC analysis. The 40 components subsequently were concatenated into a single image file and served as the input for single metalevel ICA to generate the 40 most consistent group-level component templates. This group-level meta-ICA approach decomposes the concatenated 4D dataset (40 components per group-level ICA analysis $\times$ 25 subgroup-level ICA analysis = 1000 image

volumes) into spatial maps of structured component signals in the data.

Individual Large-Scale Intrinsic Functional Network Identification using Dual Regression Approach

Following group-level meta-ICA, subject-specific measures of functional connectivity strength of all 40 independent components were calculated using a dual regression approach [33,41]. The dual regression procedure was separately applied to each individual preprocessed rs-fMRI dataset using a multiple regression framework. For each independent component, each participant's voxel-wised functional connectivity strength map (regression coefficients) was calculated as follows: (1) the 40 nonthreshold spatial component maps identified by group-level meta-ICA were spatially regressed against the preprocessed rs-fMRI dataset of each individual's rs-fMRI scan. This spatial regression produced separate 40-column matrices describing mean temporal dynamics at the individual subject level of each equivalent group-level meta-ICA component; (2) these 40-column matrices were then used to temporally regress against the individual preprocessed rs-fMRI dataset, producing a set of 40 individualized spatial functional connectivity strength maps, which corresponded to group-level meta-ICA analysis for each participant. These resultant spatial functional connectivity strength maps indicated synchronization between BOLD temporal dynamics at each voxel and mean scan-specific BOLD time series of the individualized component.

Identification of Individual Default Mode Subnetwork and Other RSNs using Template Matching Technique

Through spatial cross-correlation with RSN templates from previously published network templates [42], the top 10 best-fits were manually compared with three default mode subnetworks and other RSNs based on their neuroanatomical configurations [19,42].

Retrieve Connectivity Strength for Each Default Mode Subnetwork and Other RSNs in Each Participants

To examine the mean network connectivity of each RSN for each participant, the mean value of connectivity strength across voxels within the RSN template images was calculated for each subject-specific connectivity map. These specific RSN template images were generated by normalizing group-level meta-ICA results by the maximum value and by setting the threshold at 0.4 based on a previous metalevel ICA simulation study [40]. These resultant RSN template images were used to retrieve individualized final dual regression results for each participant. These subject-based mean RSN connectivity scores were the average connectivity strength across all voxels within the corresponding RSN template masks.

Statistical Analysis

Statistical analyses of demographic data and clinical evaluations were performed using the Statistical Package for Social Sciences

(SPSS) program, version 17 (SPSS Inc., Chicago, IL, USA). Partial correlation was used to observe the association between (1) age and all RSNs after controlling sex and (2) between-network connectivity and age after controlling other RSNs and sex. The threshold for statistical significance was a P value of <0.05 .

Results

Demographics

The final sample comprised 430 subjects (224 men and 206 women) from the original 504 participants. We excluded participants because of severe distortion or artifacts in imaging data ($n = 26$) or because of relatively poor Mini-Mental State Examination scores (corrected by age and educational level) [43] ($n = 48$). The mean age of the participants was 63.78 ± 8.4 years old, ranging from 51 to 85 years old.

Default Mode Subnetworks and Other RSNs

We performed a 40-component meta-ICA using rs-fMRI data from 430 healthy participants. Based on the visual inspection of spatial maps, we identified 12 components as RSNs and 28 components as ventricular, vascular, susceptibility, or motion-related artifacts. The spatial maps of the three default mode subnetworks were anterior DMN (aDMN), ventral DMN (vDMN), and posterior DMN (pDMN) (Figure 1), similar to those previously identified [19]. The spatial maps of the remaining nine RSNs included the auditory, cerebellum, executive control, left and right frontoparietal, sensorimotor, visual medial, occipital, and lateral networks (Figure 1).

Age Effect on RSNs

Among the three default mode subnetworks, only vDMN had a marked negative correlation with age after controlling sex ($r = -0.163$, $P < 0.01$) (Figure 2). Furthermore, a marked negative correlation was also noted in the auditory ($r = -0.144$, $P < 0.01$), right frontoparietal ($r = -0.199$, $P < 0.01$), sensorimotor ($r = -0.113$, $P = 0.02$), and visual medial ($r = -0.188$, $P < 0.01$) networks. Among these five networks, the sensorimotor network does not survive corrections for multiple comparisons using the Bonferroni procedure in the analysis of totally 12 RSNs ($P < 0.05/12 = 0.004$) and should therefore be interpreted with caution.

Age-related Pair Relationship of RSNs

Age-related connectivity changes between networks were also evaluated after controlling other RSNs and sex, and we discovered that some pairs revealed increased connectivity ($0.1 < r < 0.2$), some decreased ($0.1 < |r| < 0.2$), and some had no significant findings (Figure 3). Age-related increases in functional connectivity were found between aDMN and auditory ($r = 0.19$, $P < 0.01$), aDMN and visual medial ($r = 0.12$, $P = 0.01$), vDMN and pDMN ($r = 0.12$, $P = 0.02$), vDMN and right frontoparietal ($r = 0.14$, $P < 0.01$), pDMN and right frontoparietal ($r = 0.11$, $P = 0.02$), auditory and left frontoparietal ($r = 0.14$, $P < 0.01$), cerebellum and right frontoparietal ($r = 0.13$, $P < 0.01$), cerebellum and visual occipital ($r = 0.19$, $P < 0.01$), and visual medial and visual lateral ($r = 0.14$, $P < 0.01$) networks. Age-related decreases in functional connectivity were noted between aDMN and vDMN

Figure 1 Resting-state networks. From left to right, the functional connectivity of each component is displayed in coronal, sagittal, and axial views. Anterior default mode subnetwork (aDMN) includes the anterior cingulate, paracingulate gyrus, insular cortex, frontal, and temporal pole. Ventral default mode subnetwork (vDMN) includes the precuneus and posterior cingulate, hippocampus, parahippocampal gyrus, and occipital cortex. Posterior default mode subnetwork (pDMN) includes the posterior cingulate and precuneus, lateral parietal and middle temporal gyrus. The nine other resting-state networks identified in our study include auditory, cerebellum, executive control and sensorimotor networks, left and right frontoparietal networks; the three visual networks were as follows: visual medial, occipital, and lateral networks.

Figure 2 Correlation between age and functional connectivity of each default mode subnetwork. Mean connectivity score of each default mode subnetwork for each participant is the average connectivity strength across all voxels within the corresponding default mode subnetwork mask. The resulting connectivity score for each participant is plotted against age. **(A)** Anterior default mode subnetwork: the regression line shows minimal, but not significant, increase in functional connectivity with age ($r = 0.067$, $P = 0.16$). **(B)** Ventral default mode subnetwork: the regression line shows a mild but marked decrease in functional connectivity with age ($r = -0.163$, $P < 0.01$). **(C)** Posterior default mode subnetwork: the regression line shows minimal, but not significant, increase in functional connectivity with age ($r = 0.008$, $P = 0.88$). (* $P < 0.05$).

($r = -0.14$, $P < 0.01$), aDMN and pDMN ($r = -0.17$, $P < 0.01$), aDMN and visual lateral ($r = -0.13$, $P < 0.01$), pDMN and auditory ($r = -0.18$, $P < 0.01$), left frontoparietal and right frontoparietal ($r = -0.12$, $P < 0.01$), left frontoparietal and visual lateral ($r = -0.14$, $P < 0.01$), and visual medial and visual occipital ($r = -0.12$, $P < 0.01$) networks. The rest between-network connectivity was not markedly correlated with age. Among these 16 significant between-network connections, only four of them

survive corrections for multiple comparisons using the Bonferroni procedure in the analysis of totally 66 between-network connections, ($P < 0.05/66 = 0.0007$), including two increased connectivities (aDMN v.s. auditory network, and cerebellum v.s. visual occipital networks) and two decreased connectivities (aDMN v.s. pDMN, and pDMN v.s. auditory network). Therefore, the interpretation of the age-related change of the rest between-network connectivities should be with caution.

Figure 3 Age-related associations of resting-state networks. Age-related between-network connectivity changes were evaluated after controlling other RSNs and sex. Red and blue cells represent significant positive and negative correlation with age, respectively. Age-related increases in functional connectivity were found between aDMN and auditory, aDMN and visual medial, vDMN and pDMN, vDMN and right frontoparietal, pDMN and right frontoparietal, auditory and left frontoparietal, cerebellum and right frontoparietal, cerebellum and visual occipital, and visual medial and visual lateral networks. Age-related decreases in functional connectivity were noted between aDMN and vDMN, aDMN and pDMN, aDMN and visual lateral, pDMN and auditory, left frontoparietal and right frontoparietal, left frontoparietal and visual lateral, and visual medial and visual occipital networks. The rest between-network connectivity was not markedly correlated with age. The absolute r values of all red and blue cells are between 0.1 and 0.2.

Discussion

Using a large sample size, age-related decreased functional connectivity was found in vDMN, auditory, right frontoparietal, sensorimotor, and visual medial networks; there were nine increased between-network connections and seven decreased connections. The increased vulnerability of vDMN compared with the other two subnetworks is consistent with some previous behavioral studies [19,23,24]. In healthy elderly, both high and primary function networks deteriorate, demonstrating age-related network changes in the late stage. The presence of several increased between-network connections was probably a compensatory reaction or the dedifferentiation of networks, a reverse process to network development [19,44–46].

While the overall DMN functional connectivity decreases with age [6,9,10], our study further revealed that only vDMN showed this decline, suggesting that normal aging process may first influence only the specific vDMN. vDMN was associated with the function of constructing a mental scene based on memory [19]. This hippocampus-dependent function deteriorates with age, partially resulting in memory impairment [23]. In contrast, the functions of self-referential processing, supported by aDMN, and familiarity and autobiographical memory, supported by pDMN, have been reported to be relatively intact in older adults, although it remains

controversial [19,23,24]. These behavioral results are consistent with our functional network findings. Moreover, the hippocampus, a part of vDMN, played an important role in episodic long-term memory and navigation; therefore, the reduction of vDMN functional connectivity may also partly underlie age-related decline of these two functions [24,47,48]. A recent study showed stable condition of the DMN across lifespan but decreased functional connectivity in the PCU–dPCC network, which is composed of dorsal posterior cingulate cortex, dorsal precuneus, and ventral precuneus [49]. The ventral precuneus in PCU–dPCC network is overlapped with DMN and is highly similar to the vDMN in our study. Thus, the specific functional overlapping of vDMN with PCU–dPCC network probably also explains why only vDMN decreased functional connectivity with age but not the rest of DMN. Furthermore, the majority of previous rs-fMRI studies on AD revealed more functional reduction of DMN than normal subjects [50,51]. A previous study compared AD patients with controls based on default mode subnetworks and revealed a more increased functional connectivity of aDMN and vDMN and more decreased functional connectivity of pDMN in patients with AD at baseline. Consistent findings for aDMN and pDMN in AD were mentioned before; however, there is a difficulty in explaining the increased functional connectivity of vDMN in AD because AD pathologically affects the medial temporal lobe first, which is a part of vDMN [19]. This decreased functional connectivity in the medial temporal lobe has been largely supported [24]. Our results suggest a possible explanation that perhaps, the increased vulnerability of vDMN to normal aging results in less difference or even reverses the finding between AD and controls, but further evaluation is required.

Furthermore, in other RSNs, we found that the auditory, right frontoparietal, sensorimotor, and visual medial networks were negatively correlated with age. Perception considerably interacts with cognition and deficits in the auditory and visual acuity; the sensory and motor ability were common in the elderly [24]. Decreased functional connectivity in these three basic function networks was noted in both our study and previous task-dependent fMRI studies [52,53]. Therefore, these deficits are probably because of not only structural or physical degeneration but also functional processing in the brain or perhaps, decreased functional connectivity is just the result of less external information input. The right frontoparietal network is involved in several functions, such as memory, language, attention, and visual processing, all of which are impaired in the elderly [24,54]. However, the left counterpart was not correlated with age. Previous task studies revealed that some cognitive functions, including some common age-related impaired functions, such as inhibition and working memory, are more likely related to the right frontoparietal network [24,55]. Perhaps this lateralization explains the different vulnerability between the bilateral frontoparietal networks. The within-network results of some previous studies were partially different from ours, probably because of variations in population, network template, or imaging analysis methods or software. A notable finding was that visual network revealed age-related functional connectivity decline [6]; however, we discovered that only the visual medial network possessed this decline. Visual medial network was associated with simple visual stimuli (e.g., a flickering checkerboard), whereas visual occipital and

lateral networks were related to higher-order visual stimuli (e.g., orthography) and complex (emotional) stimuli, respectively [56]. Previous studies have favored that complex or high-order visual processing is more affected by aging [57], but our results supported that the visual medial network, involved in processing simple visual stimuli, declined more with age. A possible explanation for this discrepancy is that our data may have revealed the late stage of visual network change, whereas the other two networks with higher-order functions have already decreased to a stable status earlier in life. To resolve this problem, further behavioral studies and RSN evaluation on a wider age group are required.

A previous study has revealed that age-related decreased connectivity predominantly affects high-function networks, such as DMN and cingulo-opercular and frontoparietal control networks, whereas primary function networks, such as somatomotor and visual networks, are preserved [5]. Nonetheless, in our study, functional connectivity decreased in both high-function networks, including vDMN and right frontoparietal network, and primary function networks, including auditory, sensorimotor, and visual medial networks. This difference could be because of the different age groups; the previous study compared younger and older adults, whereas our study merely included relatively older adults. Therefore, the aging process may have initially affected high-function networks then influenced both high and primary function networks later. This pattern is reverse to the development in children, whose basic function networks mature earlier [58]. This phenomenon may be explained by a developmental theory, last-in-first-out theory, or retrogenesis model. This theory which suggested that brain regions developing later were more vulnerable has been applied to gray and white matter changes in aging and neurodegenerative disease [59,60]. The theory is probably also applicable in RSN degeneration. Further, in AD, there was increased functional connectivity in executive network or more complex results that include increased, decreased, and mixed conditions in RSNs [50,61]. Although increased connectivity was not found in our RSNs, it has been reported to occur in the medial and lateral prefrontal regions in healthy elderly [51]. However, the condition of increased connectivity in RSNs is more common in AD, probably resulting from more compensation to overcome more deterioration of RSNs. This compensatory change has been noted in task experiments and within DMN and is commonly used to explain overactivation in elderly [19,44,59].

A previous study revealed that as age increased, there were decreased correlations between DMN and visual network, salience and temporal networks, and salience and visual networks [6]. Much more complex age-related between-network changes were identified in our study, including both decreased and increased conditions. These bidirectional changes have been previously noted [5]. One explanation is that decreased correlations may be the result of breakdown of between-network connectivity in the aging process, and increased correlations may be compensatory reactions for preserving global brain function. Another explanation can refer to de-differentiation in older adults, suggesting the nonselective recruitment of brain regions, probably because of a decreased ability of selecting proper brain regions for performing tasks [59]. De-differentiation is an opposing pattern to the maturation of networks, during which, networks increased internal

connectivity and were segregated to achieve functional specialization, thereby underlying cognitive functions [45,46]. From this viewpoint, increased between-network connectivity can represent decreasing segregation, a process against network development and probably serving as one cause of cognitive impairment. Decreased segregation with age has been found in a recent study and may be considered as an age-invariant marker of individual difference in cognitive function [62]. This process involves both the primary function sensory-motor system and the high-function association system, but the decreased segregation in the association system is more prominent after an approximate age of 50, which fits our age group. Our results are compatible with this study, showing decreased segregation with age within primary function system and high-function system, and we further demonstrate that decreased segregation also exists between primary and high-function system. For the development of DMN, the anterior–posterior connection, more specifically, the medial prefrontal cortex and posterior cingulate cortex, developed last; this link was suspected to be associated with the formation of self- and social-cognitive functions during adolescence [45,63]. Our results displayed weaker age-related connectivity between the anterior portion of DMN (aDMN) and posterior portions of DMN (vDMN and pDMN), whereas the connection between the posterior two subnetworks was stronger. Our results support that the breakdown of DMN may start from the lastly developed anterior–posterior connection and that the posterior components may be connected more tightly in a compensatory manner, supporting the last-in-first-out theory.

There are several limitations to our study. We only evaluated cross-sectional conditions of the participants; thus, the results reveal age difference, not aging change, which should rely on follow-up data. Longitudinal evaluation was also helpful for differentiating whether vDMN is the only one or the first one with age-related decline. Moreover, we focused on functional changes without including any structural analysis, which could provide a broader view of age-related brain changes by considering structural differences and their associations with functional results. Besides, the significant findings of the sensorimotor network and many between-network connectivities do not pass multiple comparison corrections; therefore, whether these results are significant needs further analysis of other data for comparison.

Conclusion

Based on a large sample size, we propose more reliable results for improving the understanding of functional basis of cognitive changes in aging. We found that vDMN is the specific default mode subnetwork with age-related decline compatible with behavioral science. We also discover several reverse patterns to network development and supporting evidence for last-in-first-out theory in the elderly, including (1) first breakdown of anterior–posterior connection in DMN, (2) decreased connectivity in both high and primary function networks in elderly participants, and (3) emergence of less segregation of networks. A better comprehension of these age-related network changes can help find solutions to the impact of population aging and can provide a diagnostic pearl for neurodegenerative diseases.

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Conflict of Interest

The authors declare no conflict of interests.

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