



## Onset age of L2 acquisition influences language network in early and late Cantonese-Mandarin bilinguals



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### ABSTRACT

Early second language (L2) experience influences the neural organization of L2 in neuro-plastic terms. Previous studies tried to reveal these plastic effects of age of second language acquisition (AoA-L2) and proficiency-level in L2 (PL-L2) on the neural basis of language processing in bilinguals. Although different activation patterns have been observed during language processing in early and late bilinguals by task-fMRI, few studies reported the effect of AoA-L2 and high PL-L2 on language network at resting state. In this study, we acquired resting-state fMRI (R-fMRI) data from 10 Cantonese (L1)-Mandarin (L2) early bilinguals (acquired L2: 3 years old) and 11 late bilinguals (acquired L2: 6 years old), and analyzed their topological properties of language networks after controlling the language daily exposure and usage as well as PL in L1 and L2. We found that early bilinguals had significantly a higher clustering coefficient, global and local efficiency, but significantly lower characteristic path length compared to late bilinguals. Modular analysis indicated that compared to late bilinguals, early bilinguals showed significantly stronger intra-modular functional connectivity in the semantic and phonetic modules, stronger inter-modular functional connectivity between the semantic and phonetic modules as well as between the phonetic and syntactic modules. Differences in global and local parameters may reflect different patterns of neuro-plasticity respectively for early and late bilinguals. These results suggested that different L2 experience influences topological properties of language network, even if late bilinguals achieve high PL-L2. Our findings may provide a new perspective of neural mechanisms related to early and late bilinguals.

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### 1. Introduction

Neural plasticity in second language (L2) acquisition is influenced by early language experience (Perani & Abutalebi, 2005). However, with age, L2 learning becomes increasingly difficult.

Although achieving native-like proficiency level in L2 (PL-L2) is still possible, this comes at the cost of the engagement of more neural resources supporting L2 processing (Abutalebi & Green, 2007; Wartenburger et al., 2003). Generally, age of second language acquisition (AoA-L2) and PL-L2 are the two factors widely known

*Abbreviations:* AAL, automated anatomical labeling; ANG, angular gyrus; AoA, age of acquisition; AoA-L2, age of second language acquisition; AI, anterior insula; BA, Brodmann's Area; CEFR, Common European Framework of Reference; CUN, cuneus; EBG, early bilingual group; EHI, Edinburgh Handedness Inventory; FC, functional connectivity; FWE, family-wise error; FFG, fusiform gyrus; FO, frontal operculum; FWHM, Full-Width at Half Maximum; IFG, inferior frontal gyrus; IFGoperc, inferior frontal opercularis; IFGtring, inferior frontal triangular part; IPG, inferior parietal gyrus; ITG, inferior temporal gyrus; L1, first language; L2, second language; LBG, late bilingual group; MFG, middle frontal gyrus; MOG, middle occipital gyri; MTG, middle temporal gyrus; ORBinf, inferior frontal orbital; PMC, premotor cortex; PL, proficiency level; R-fMRI, resting-state fMRI; ROIs, regions-of-interest; SFGmed, superior medial frontal gyrus; SMG, Supramarginal gyrus; SPG, superior parietal gyrus; STG, superior temporal gyrus; VWFA, visual word form area.

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to contribute to neural differences in bilinguals during L2 processing (Hernandez & Li, 2007; Perani & Abutalebi, 2005). Although the neural mechanisms of L2 processing have been widely studied (Jing et al., 2012; Mahendra, Plante, Magloire, Milman, & Trouard, 2003; Perani & Abutalebi, 2005; Williams, Darcy, & Newman, 2015) and different brain activation patterns were observed between early and late bilinguals (Archila-Suerte, Zevin, & Hernandez, 2015; Rüschemeyer, Fiebach, Kempe, & Friederici, 2005; Suh et al., 2007), very few studies reported its influences on language network at resting state.

Using a task-fMRI approach, previous studies (see for review Abutalebi 2008) have investigated the effects of AoA-L2 and PL-L2 on language processing in bilinguals. Late bilinguals were found to elicit more activations than early bilinguals during L2 processing. For example, Berken et al. (2015) investigated the effect of AoA-L2 on phonetic processing in early and late French-English bilinguals and a group of English-speaking monolinguals. They found that compared to early bilinguals, late bilinguals showed increased activation in the left inferior frontal gyrus (IFG), left premotor cortex (PMC), and left fusiform gyrus (FFG) when reading aloud in L2 compared with their L1. Saur et al. (2009) performed a word order task which involves syntactic processing in three groups of German-French bilinguals that, included two late bilingual groups and an early bilingual group. Compared to the early bilinguals group, they observed significantly increased activation in the inferior frontal triangular part (IFG<sub>trng</sub>) and inferior frontal opercularis (IFG<sub>operc</sub>), left inferior temporal gyrus (ITG), and the basal ganglia during L2 processing in the two late bilingual groups. These studies revealed the effect of AoA-L2 on phonetic and syntactic processing in bilinguals. In addition, several studies (Hernandez, Hofmann, & Kotz, 2007; Isel, Baumgaertner, Thrän, Meisel, & Büchel, 2010; Wartenburger et al., 2003) suggested that PL-L2 plays a key role in semantic processing in L2. Wartenburger et al. (2003) investigated the effects of AoA-L2 and PL-L2 on semantic processing in Italian-Germany bilinguals, and found that during a semantic judgment task, bilinguals with low PL-L2 displayed increased activation in the left MFG and in right FFG (Brodmann's Area, BA 37), but decreased activation in left IFG and right MFG compared to bilinguals with high PL-L2. This finding suggested that semantic processing is strongly influenced by PL-L2 in late bilinguals.

Actually, there are still some considerations about the effects of AoA-L2 on language processing in bilinguals. First, language processing is associated with a widely distributed brain regions of a language network (Fedorenko & Thompson-Schill, 2014; Friederici & Gierhan, 2013). Although previous studies (Archila-Suerte et al., 2015; Rüschemeyer et al., 2005; Suh et al., 2007) tried to reveal the different patterns of brain activation responsible for different language processing, little is known about the effect of AoA-L2 on language network in bilinguals. Second, different language domains such as phonology, grammar and lexico-semantics require the coordination of specific networks (Blumstein & Amso, 2013) and the literature is lacking specific network studies focusing on each of these domains. Finally, most previous studies (Archila-Suerte et al., 2015; Saur et al., 2009; Wartenburger et al., 2003) employed the task-fMRI to investigate the effects of AoA-L2 on language processing in bilinguals. However, resting-state fMRI (R-fMRI) investigates the low-frequency fluctuations of spontaneous activity which is considered to reflect intrinsic functional organization of the brain (Fox & Raichle, 2007; Raichle, 2010). There is a recent study, which used the R-fMRI approach to investigate the effect of AoA on brain intrinsic functional connectivity (FC) in bilinguals. Berken, Chai, Chen, Gracco, and Klein et al. (2016) selected IFG as a seed to study the effect of AoA-L2 on resting-state FC in French-English bilinguals. They observed stronger FC between the left and right IFG as well as

between the IFG and the brain regions involved the language control in early bilinguals. Meanwhile, they also found FC between left IFG and right IFG was significantly positively correlated with AoA-L2 in late bilinguals. These findings indicate that the AoA-L2 influences brain intrinsic functional patterns in bilinguals. In addition, another study also found resting-state FC in adults is related to the ability of L2 Learning. Chai et al. (2016) investigated English speakers before and after a 12-weeks French course training by using whole-brain resting-state FC between the left anterior insula/frontal operculum (AI/FO) and visual word form area (VWFA), and found that FC between the left AI/FO and left posterior STG as well as FC between the left AI/FO and dorsal anterior cingulate cortex were positively correlated with improvement in L2 lexical retrieval in spontaneous speech, while FC between the VWFA and left STG was positively correlated with improvement in L2 reading speed. Hence, resting-state connectivity may reflect the cumulative effects of language experience on neuronal functional organization of the brain.

In this study, we aimed to examine the effect of AoA-L2 on the language network when considering high proficiency-level in both languages. Using graph theory, we analyzed topological properties and inter-modular functional connection of the language networks in the early and late Cantonese (L1)-Mandarin (L2) bilinguals, and determined their differences. Graph theory has been widely used to characterize the topological properties of brain functional networks (Newman, 2012; Sporns, 2013). Accordingly, we used four parameters, clustering coefficient ( $C_w$ ), characteristic path length ( $L_w$ ), global efficiency ( $E_{glob}$ ) and local efficiency ( $E_{loc}$ ) to describe the topological properties of the language networks. The clustering coefficient describes how close the relationship is between a node and its neighboring nodes, and the characteristic path length displays a number of intermediate steps for information transfer in a network. The global and local efficiency reveal the efficiency of information transfer between different nodes in a whole or partial network. Actually, brain functional networks can be divided into a set of sparsely inter-connected (but densely intra-connected) functional modules (Crossley et al., 2013; Meunier, Lambiotte, & Bullmore, 2010) for revealing intra- and inter-modular connectivity properties. In the calculations, we used Newman's algorithm (Newman, 2006) to divide the language network into different functional modules. In brief, topological analysis focuses on the information communication between nodes in the language network, while modular analysis investigates the intra- and inter-modular properties of those nodes in a special module, such as functional connectivity with each other. Considering previous findings (Berken et al., 2015; Zou et al., 2012) that more extended activation and individual variation in later bilinguals may indicate the additional neural resources for processing L2 needed to achieve native-like performance, we hypothesized that there would be detectable differences in local and global topological properties of the language networks between the early and late bilinguals.

## 2. Methods

### 2.1. Subjects

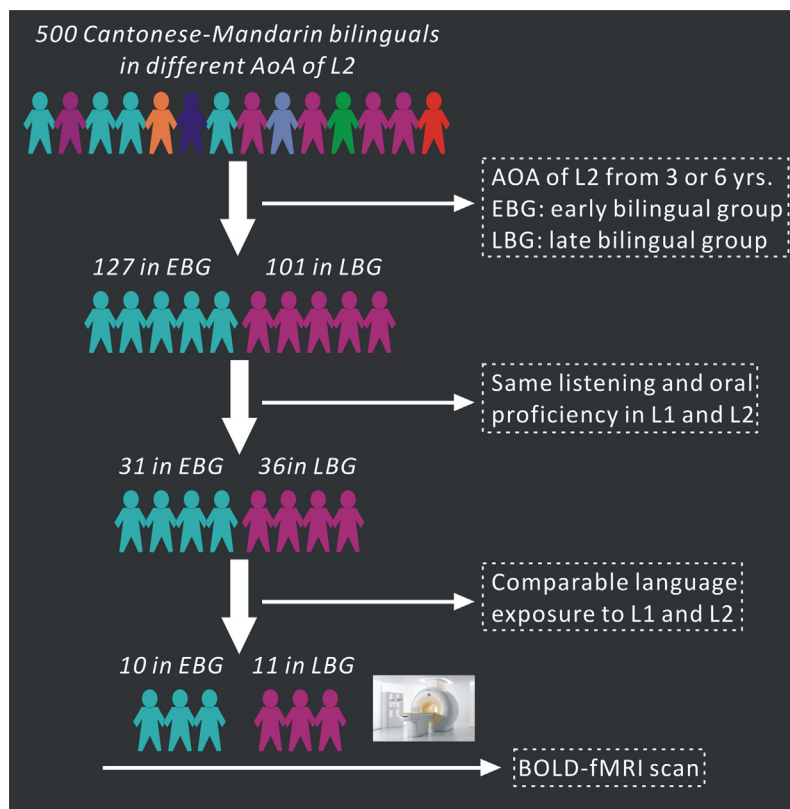
Cantonese is the dominant language spoken by over 80% of the population in Guangzhou, the capital of Guangdong Province, China, and is the official language in Hong Kong. A large segment of the population except of students on campus speaks Cantonese in their daily life. There is a dramatic difference between Cantonese and Mandarin (i. e., the official language of the People's Republic of China). According to Li's statistics (Li, 1990), of all characters in Basic Vocabulary Table of Modern Chinese Characters, only 21.5% have the same pronunciations between the two languages. And

of all the Cantonese words in A Dictionary of the Cantonese (Guangzhou) Dialect (Rao, Ouyang, & Zhou, 1996), only 23.1% have equivalents in Mandarin. The same rate is amazingly as low as 1.78% when the colloquial expressions of the two are compared in A Handbook of Translation of Cantonese and Mandarin Colloquial Expressions (Zeng, 1982). Therefore, Cantonese and Mandarin are generally regarded as two distinct languages in bilingual studies (Cai, Pickering, Yan, & Branigan, 2011; Tu et al., 2015). In this study, 500 volunteer undergraduates who are Cantonese-Mandarin bilinguals were recruited to make a sample pool. They are all native Cantonese speakers, learning Mandarin as their L2. Fig. 1 shows the procedure of selecting subjects.

Main selection criteria involved the age of second language acquisition (AoA-L2), listening and oral proficiency level in L2 (PL-L2), and language exposure to L1 and L2. First, each of these 500 subjects attended an interview and finished a questionnaire to confirm the age of language acquisition. From this sample pool, we obtained 228 bilinguals who acquired Cantonese as their L1, 127 corresponding to the early bilingual group (EBG) whose AoA-L2 was between 3 and 4 years old, and 101 to the late bilingual group (LBG) whose AoA-L2 between 6 and 7 years old. Most of early bilinguals lived with their parents and relatives in childhood. Some of their relatives speak Cantonese or Mandarin. Hence, they acquired Cantonese and Mandarin more early. Most of late bilinguals lived in a Cantonese language environment until they went to primary school. Second, we used the standard scale of Common European Framework of Reference (CEFR) for Languages (Verhelst, Van Avermaet, Takala, Figueras, & North, 2009) to assess the oral and listening PLs of these 228 subjects. The assessment of listening PLs included self-report (10 questions) through the CEFR for Languages and the other one is to estimate response accuracy to 5 probe questions on stories they listened to in L1 and L2. We

randomly arranged 10 questions and 5 stories for all 288 subjects. Our criteria for high listening PLs were not less than 6 questions in self-report part and not less than 4 questions in listening stories part. For oral PL, three language State-level experts were invited to grade the subject's ability of using L1 or L2 according to the standard of CEFR from A1 (break-through) to C2 (mastery). Each of language experts blinded to the status of the subjects and the interview order was randomized. The criteria of grades are as follows: competence-level users were assessed in A1 or A2, independent users were assessed in B1 or B2, and proficient users were assessed in C1 or C2. Early and late bilinguals who reach the B2 grade could be considered as a high oral PLs candidate. After the CEFR test, we selected out a total of 67 subjects who had similar high listening and oral PLs. The approximate language exposure in across different stages of their life time (0–1, 1–2, 2–3, 3–6, 6–12, 12–15, 15–18, and above 18 years old until present) was the final standard for selecting subjects. We used a seven-point scale to evaluate their language exposure in different ages ('1' represents only exposed to L1, '7' represents only exposed to L2, and '4' represents the same language exposure to L1 and L2). In this step, language exposure is an approximate evaluation for usage of L1 and L2 in their past daily, because early and late bilinguals didn't equally use L1 and L2 during their language acquisition, no matter in their home or school language environments. At last, we obtained two groups with similar high listening and oral PLs, 10 subjects in EBG whose AoA-L2 was between 3 and 4 years old and 11 subjects in LBG whose AoA-L2 between 6 and 7 years old, for the further fMRI scan. Table 1 lists more detail information about all of the 21 subjects in this study. For detailed criteria and procedure of selecting subjects, please see our previous paper, Tu et al. (2015).

All subjects were right-handed according to the Edinburgh Handedness Inventory (EHI) (Oldfield, 1971) and had no



**Fig. 1.** The procedure for selecting subjects in this study. We selected the subjects pooling from 500 Cantonese-Mandarin bilinguals. The criteria of selection included the age of acquisition of L2 (AoA-L2), listening and oral proficiency levels, and the language exposure to L1 and L2 across life stages. In the end, we obtained 10 subjects in the early bilinguals group (EBG) and 11 in the late bilinguals group (LBG) for the further analysis. Note: The range of AoA-L2 was 3–4 years old for EBG and 6–7 years old for LBG.

**Table 1**

The demographic characteristics (sex and age), language proficiency level (PL), and language exposure to the first language (L1, Cantonese) and the second language (L2, Mandarin) for all subjects in this study. The listening PLs of L1 and L2 were assessed using the self-report method (10 questions) according to the Common European Framework of Reference (CEFR) for Languages and answering accuracy to the just listened stories in L1 and L2 (5 probe questions). The oral PLs of L1 and L2 were assessed in two interviews, respectively, in which three language experts graded each subject's usage of L1 or L2 according to a standard scale of CEFR. The grade ranges from A1 (break-through) to C2 (mastery). A1 and A2 refer to competence level of the basic users, B1 and B2 to the independent users, and C1 and C2 to the proficient users. The language exposure in different life stages indicates the language exposure to L1 and/or L2 from birth to the time of this study. It was assessed by a seven-point scale, ranging from 1 (only using L1) to 7 (only using L2). Abbreviations: Ex, language expert for L1 or L2. Ex1, Ex2, Ex3, Ex4, Ex5 and Ex6 represent six language expert raters, respectively. Self, self-report of listening PL. Acc, response accuracy. Sex, '0' represents the female and '1' represents the male.

Group	Subject	Sex	Age	Listening PLs				Oral PLs						Language exposure across life stages							
				L1		L2		L1			L2			0–1 years	1–2 years	2–3 years	3–6 years	6–12 years	12–15 years	15–18 years	18 years–
				Self	Acc	Self	Acc	Ex1	Ex2	Ex3	Ex4	Ex5	Ex6								
Early bilingual group (EBG)	S1	0	22	7	5	8	5	C2	C2	C2	C1	C1	C2	1	1	2	2	3	3	3	4
	S2	1	20	10	5	6	5	C2	C1	C2	C2	B2	C1	1	1	2	3	5	4	4	3
	S3	1	21	10	4	10	5	C2	C2	C2	C2	C2	C2	2	2	2	2	3	3	3	4
	S4	1	21	10	5	7	5	C2	C2	C2	B2	B2	C1	1	1	1	2	3	3	2	4
	S5	0	20	6	5	7	5	C1	C1	C2	C1	C1	C1	1	1	2	3	4	5	5	5
	S6	1	19	10	5	9	4	C2	C1	C2	C1	C1	C1	1	1	3	4	4	4	4	4
	S7	0	20	8	5	9	5	C2	C2	C2	C1	C2	C2	1	1	2	4	5	4	5	2
	S8	0	21	10	5	10	5	C2	C2	C2	C1	C1	C2	1	2	3	3	3	4	4	6
	S9	1	27	6	3	6	5	C2	C2	C2	B2	C1	C1	1	1	2	2	4	4	6	6
	S10	0	20	9	5	9	4	C2	C2	C2	C1	C1	C1	1	1	2	2	3	3	4	4
Late bilingual group (LBG)	S11	1	22	9	5	8	5	C2	C2	C1	C1	C2	C1	1	1	1	1	6	6	3	4
	S12	0	26	9	5	5	5	C2	C2	C2	C1	C1	C2	1	1	1	1	4	4	5	5
	S13	0	20	8	5	7	5	C2	C2	C2	C1	C2	C1	1	1	1	1	3	4	5	4
	S14	0	26	9	5	6	5	C2	C2	C2	C1	C1	C2	1	1	1	1	2	3	3	4
	S15	1	20	7	5	6	4	C2	C2	C2	C1	C1	C1	1	1	1	1	2	2	3	5
	S16	1	23	8	4	8	5	C2	C2	C2	C2	C2	C1	1	1	1	1	4	4	4	5
	S17	1	24	9	4	9	5	C2	C2	C2	C2	C1	C1	1	1	1	1	3	4	4	4
	S18	0	20	7	4	7	5	C2	C2	C2	C2	C1	C1	1	1	1	1	4	4	4	5
	S19	0	19	7	5	7	4	C2	C2	C2	C2	C1	C1	1	1	1	1	2	3	3	5
	S20	0	24	10	5	10	4	C2	C2	C1	B2	C1	C1	1	1	1	1	3	4	4	5
	S21	0	22	8	4	7	5	C2	C2	C2	C1	B2	C1	1	1	1	1	3	4	4	4

experience that their left-handedness was adjusted to right-handedness by their parents when they were toddlers. None of subjects had a current or history of neurological or psychiatric disorders or brain injury. Written informed consent was obtained from each subject prior to the study. The protocols were approved by the Review Board of South China Normal University.

## 2.2. Image acquisition

All MRI data were acquired on a 1.5 T Philips Achieva Nova Dual MR scanner at Department of Radiology, Huangpu Clinical Medical Centre, Sun Yat-sen University First Affiliated Hospital. In order to reduce head movements which could impact the quality of images, we used two pieces of foam pad to fix the head of every subject. The R-fMRI data were obtained using a single-shot gradient-echo echo-planar imaging (GE-EPI) sequence. Specific parameters of the sequence were as follows: repetition time (TR) = 3000 ms, echo time (TE) = 40 ms, flip angle (FA) = 90°, field of view (FoV) = 240 mm × 240 mm, data matrix = 64 × 64, slice thickness = 4 mm without gap, 36 slices along the PC-AC line covering the whole brain, and 180 time points acquired in 9 min. During the R-fMRI scanning, each subject was requested to lie quietly and close their eyes but not think about anything. In addition, we also acquired high-resolution brain structural images for each subject using a T1-weighted 3D rapid interference phase gradient echo flip recovery pulse sequence (TR/TE = 9.6 ms/3.8 ms, FA = 8°, FoV = 256 × 256 mm, data matrix = 288 × 288, slice thickness = 1 mm, voxel size = 1 mm<sup>3</sup>, and 176 sagittal slices covering the whole-brain).

## 2.3. Data preprocessing

The functional images were processed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) and DPARSF-A (<http://rfmri.org/DPARSF>). For each subject, the first 10 volumes were discarded for adapting the scanning environment. We then performed slice timing for the remaining 170 volumes to account for the acquisition time delay between different slices and realigned to the first volume for head-motion correction. After that, the images were normalized to the EPI template in the MNI space using the affine transformation and resampled to 3 × 3 × 3 mm<sup>3</sup> with a kernel of Full-Width at Half Maximum (FWHM) of 8 mm. Last, we performed signal linear detrending and band-pass filtering (0.01–0.08 Hz) and regressed out the nuisance covariates (head motion profiles derived from the Friston 24-parameter model, white matter signal, and cerebrospinal fluid signal) within each voxel in whole brain. Because regressing out the global signal in R-fMRI analysis is controversial (Fox, Zhang, Snyder, & Raichle, 2009; Murphy, Birn, Handwerker, Jones, & Bandettini, 2009), we hence have not regressed out the global signal in this study.

## 2.4. Definition of language network

We first selected two seed regions-of-interest (ROIs), Broca's and Wernicke's areas and used a standard seed-voxel correlation approach to define the language network. In specific, we selected two ROIs with 3mm-radius spheres as seeds according to automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) and a previous study about resting-state FC of language network (Tomasi & Volkow, 2012). One ROI was around the left pars triangularis (MNI coordinate: –53 20 15) to represent Broca's area, and the other ROI was around the left supramarginal gyrus (MNI coordinate: –51 –51 30) to represent Wernicke's area. Average time courses of these two selected 3mm-radius sphere ROIs, representing Broca's and Wernicke's areas, were abstracted for each subject. For a given ROI, we took it as a seed and estimated its

FC, Pearson's correlation coefficient  $r$  between the ROI and each voxel in whole brain. Thus, we obtained the FC maps for Broca's area and for Wernicke's area separately. The Fisher's  $r$ -to- $z$  transform was used to convert these connectivity maps to  $z$ -value maps for statistical analysis. One sample  $t$ -test was applied to detect the group-level FC patterns across all 21 subjects in this study. We determined the clusters according to the following criteria: significant threshold  $p < 0.05$  (after family-wise error, FWE correction,  $p_{corrected} < 1.41e^6$ ), and the number of voxels in each clusters no less than 50 voxels. After performing one sample  $t$ -test, we applied FWE correction, which considered smoothness of the 3D images with a Gaussian kernel of FWHM of 8 mm, to control type-I error. Finally, we got the  $p_{corrected}$  threshold of  $1.41e^6$ . In this way, we determined the clusters which connect with the Broca's and Wernicke's areas respectively. For each of these clusters in the FC map, we used the voxels with statistical peak values of one sample  $t$ -test to draw spheres (3mm radius). By taking these spheres as nodes and the inter-nodal FC as the edge weight, we defined the language network for this study.

## 2.5. Topological properties of language network

The topological parameters for the language network were estimated with GREYNA software (<http://www.nitrc.org/projects/gretna/>). To measure the inter-nodal FC, we extracted average time courses for all of these nodes, calculated the Pearson's correlation coefficients for any two nodes, and generated a symmetric connectivity matrix for each subject. To reduce confounding effects of noisy correlations on subsequent network analysis, we determined inter-nodal FCs when they satisfy to a threshold of significance-level in this study. In particular, we assumed an inter-nodal FC existed only if its corresponding  $p$ -value met a statistical threshold of  $p < 0.05$  (after FWE correction,  $p_{corrected} < 2.9e^4$ ) compared to all others in the entire connectivity matrix, otherwise, we assumed no inter-nodal FC (Cruse et al., 2012). Last, we obtained the brain functional network for each subject and estimated the topological properties of the language network, including clustering coefficient ( $C_w$ ), characteristic path length ( $L_w$ ), global efficiency ( $E_{glob}$ ) and local efficiency ( $E_{loc}$ ). The definitions and descriptions of these topological parameters are listed in Table 2 or can be found in Rubinov and Sporns (2010).

## 2.6. Modularity of language network

In this study, we divided the language network into different functional modules according to Newman's algorithm (Newman, 2006). The module is defined as sets of network nodes that are densely linked with each other and less so with other nodes in the network. That is, the delineated nodes in the same module showed more and denser association with each other than with the nodes of the other modules. The modularity index,  $Q$ , can be expressed as:

$$Q(p) = \sum_{s=1}^{NM} \left[ \frac{w_s}{W} - \left( \frac{W_s}{2W} \right)^2 \right], \quad (1)$$

where  $Q$  quantifies the goodness of a network being optimally divided into different modules. The modular analysis will stop when it can't increase the  $Q$  value while divided into any modules from the network (the related information of module is described in Table 2). In the calculations, we first extracted the average time courses for all of nodes in the language network and calculated the Pearson's correlation coefficients for any two nodes (FWE correction,  $p_{corrected} < 2.9e^4$ ), then averaged these weighted inter-nodal FC matrix across all 21 subjects in this study. Next, we estimated  $Q$  value for all subjects in this study according to Eq. (1) and divided

**Table 2**

Mathematical definition of the topological parameters of a given network  $G(N, M)$  with  $N$  nodes and  $M$  edges. The detailed descriptions of these network parameters can also be found in [Rubinov and Sporns \(2010\)](#). The modularity index was given by [Newman \(2006\)](#).

Network Parameters / Module index	Definitions	Descriptions
Clustering coefficient	$C_w = \frac{1}{N S_i (k_i - 1)} \sum_{j, h \in G} \frac{(w_{ij} + w_{ih})}{2} a_{ij} a_{ih} a_{jh}$	Here $w_{ij}$ is the weight between node $i$ and $j$ in a network, and $S_i$ is the strength of node $i$ . $C_w$ is the mean of the weighted clustering coefficients over all nodes in a network.
Characteristic path length	$L_w = \frac{1}{1/(N(N-1)) \sum_{i=1}^N \sum_{j \neq i}^N 1/L_{ij}}$	$L_{ij}$ is the characteristic path length between nodes $i$ and $j$ . It measures a harmonic mean length between pairs and quantifies the ability for information propagation in parallel.
Global efficiency	$E_{glob} = 1/L_w$	$E_{glob}$ is the inverse of the harmonic mean of the characteristic path length between each pair of nodes within the network. $E_{glob}$ measures the global efficiency of parallel information transfer in the network.
Local efficiency	$E_{loc}(G) = \frac{1}{N} \sum_{i \in G} E_{glob}(G_i)$	$E_{loc}$ is defined as the average of the local efficiencies across all nodes.
Modularity	$Q(p) = \sum_{s=1}^{N_M} \left[ \frac{w_s}{W} - \left( \frac{W_s}{2W} \right)^2 \right]$	$N_M$ : the number of modules; $W$ : the total weight of the network; $w_s$ : the sum of the connective weights between all nodes in modules; $W_s$ : the sum of the nodal strength in modules. Therefore, $w_s/W$ represents the fraction of the total connective weight for the edges in a module divided by the total connective weight for the edges in the whole network; $W_s/2W$ represents the fraction of the total nodal strength for the nodes in module $s$ divided by the total nodal strength for the nodes in the whole network.

**Table 3**

Demographic statistics. Excepting of the L1 and L2 oral PLs, all of the data are presented as mean  $\pm$  SD. The values of L1 and L2 oral PLs were presented as a grade (Frequency) from six language experts. The grade ranges from A1 (break-through) to C2 (mastery). A1 and A2 refer to competence level of the basic users, B1 and B2 to the independent users, and C1 and C2 to the proficient users.

	Early bilinguals (n = 10)	Late bilinguals (n = 11)	p-value
Gender (male/female)	5/5	4/7	0.53 <sup>a</sup>
Age (years old)	21.1 $\pm$ 2.23	22.36 $\pm$ 2.46	0.10 <sup>b</sup>
Age of L2 acquisition (years old)	3.30 $\pm$ 0.48 (range: 3–4)	6.45 $\pm$ 0.52 (range: 6–7)	<0.001 <sup>b</sup>
L1 listening PLs (Self)	8.6 $\pm$ 1.71	8.27 $\pm$ 1.00	0.25 <sup>b</sup>
L1 listening PLs (Acc)	4.7 $\pm$ 0.67	4.64 $\pm$ 0.50	0.27 <sup>b</sup>
L2 listening PLs (Self)	8.1 $\pm$ 1.52	7.27 $\pm$ 1.42	0.08 <sup>b</sup>
L2 listening PLs (Acc)	4.8 $\pm$ 0.42	4.73 $\pm$ 0.47	0.35 <sup>b</sup>
Oral PLs (L1)	C2 (26) C1 (4) B1 (0)	C2 (28) C1 (2) B (0)	0.26 <sup>c</sup> , 0.10 <sup>b</sup>
Oral PLs (L2)	C2 (8) C1 (18) B1 (4)	C2 (9) C1 (22) B1 (2)	0.17 <sup>c</sup> , 0.32 <sup>b</sup>
Language exposure in 2–3 years old	2.10 $\pm$ 0.57	1.00 $\pm$ 0.00	<0.001 <sup>b</sup>
Language exposure in 3–6 years old	2.70 $\pm$ 0.82	1.00 $\pm$ 0.00	<0.001 <sup>b</sup>
Language exposure in 6–12 years old	3.70 $\pm$ 0.82	3.27 $\pm$ 1.19	0.12 <sup>b</sup>
Language exposure in 13–15 years old	3.70 $\pm$ 0.67	3.82 $\pm$ 0.98	0.29 <sup>b</sup>
Language exposure in 16–18 years old	4.00 $\pm$ 1.15	3.81 $\pm$ 0.75	0.25 <sup>b</sup>
Language exposure in 19 years old	4.20 $\pm$ 1.23	4.55 $\pm$ 0.52	0.14 <sup>b</sup>

<sup>a</sup> The p-value was obtained using a  $\chi^2$ -test.

<sup>b</sup> The p-value was obtained using a non-parametric permutation test.

<sup>c</sup> The p-value was obtained using a Kendall coefficient of concordance test.

the language network into different modules. We also estimated the differences in intra- and inter-modular FC between the EBG and LBG.

### 2.7. Statistical analysis

A nonparametric permutation test ([Nichols & Holmes, 2002](#)) was used to determine the between-group differences in topological parameters as well as in intra- and inter-modular FC. Briefly, for a given parameter, we randomly reallocated all the parameter values for all each subjects into two new groups and recalculated the differences in the mean values of the parameter between the two re-generated groups. This permutation was repeated 10,000 times to obtain the empirical distribution of the difference. In the calculations, we selected significant levels at  $p < 0.05$  to determine significant between-group differences at 95% of the empirical distribution in a two-tailed test. Once significant difference was observed for the given parameter, we then estimated the effect size (Cohen  $d$ ) and statistical power according to Cohen's definition and to determine the statistical power

([Cohen, 2013](#)). The levels of small, medium and large effect size corresponding to 0.2, 0.5, and 0.8, respectively.

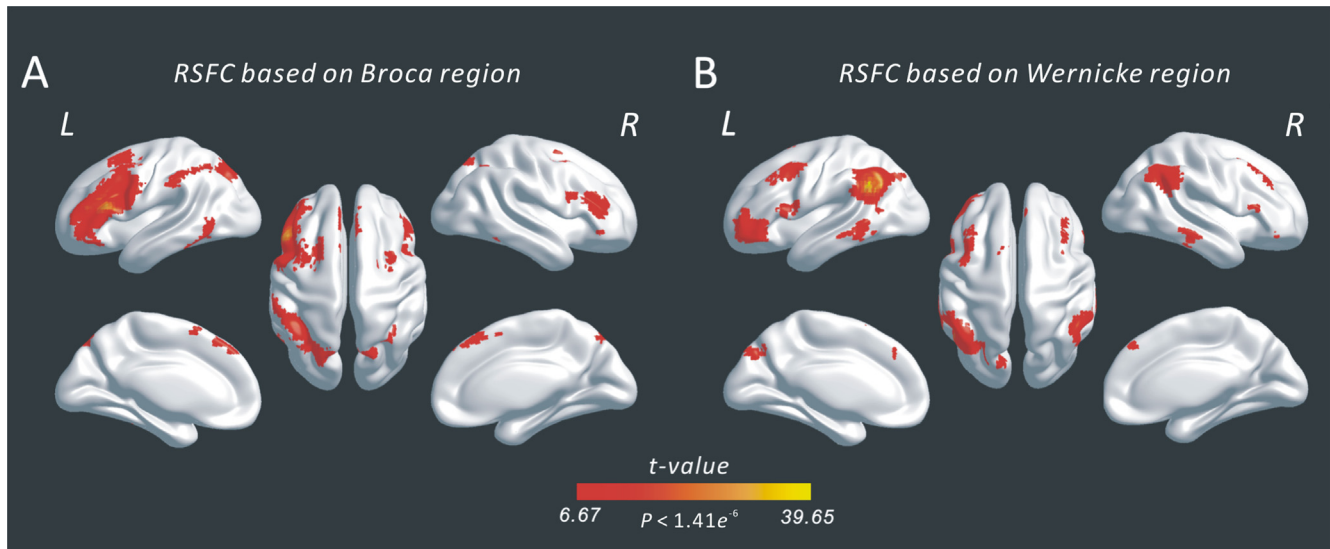
## 3. Results

### 3.1. Behavioral tests

[Table 3](#) lists the statistic information for 21 subjects in this study. No significant difference was found in the age and gender between the EBG and LBG group. Permutation test ( $p < 0.05$ ) revealed no significant differences in the scores of L1 and L2 listening PLs, L1 and L2 Oral PLs, and language exposure test in separate grades after 6 years old. We also employed Kendall coefficient of concordance to investigate the assessment of oral PLs in L1 or L2 among three language experts. No significant differences were found among three language experts for oral assessment in L1 or L2.

### 3.2. Language network

[Fig. 2](#) shows the determined connectivity patterns of Broca's and Wernicke's areas ( $p_{corrected} < 1.41e^6$ , FWE corrected,



**Fig. 2.** Resting state functional connectivity (RSFC) maps reflecting the average spatial distribution of temporal correlations with time-varying signals in Broca's (A) and Wernicke's (B) area across 21 subjects. The color bar represents  $t$ -value ( $p_{corrected} < 1.41e^{-6}$ , FWE corrected). L(R), left (right) hemisphere.

**Table 4**  
Clusters locations and peak coordinates corresponding to the Broca's and Wernicke's regions. The statistical significance was set at  $p < 1.41e^{-6}$  (FWE corrected). BA: Brodmann's Area. We used symbols of 'a', 'b', and 'c' to present the same region in the language network but in different coordinates (not overlapped).

Seed region	Cluster location	Cluster size (# voxels)	BA	$t$ -value in peak voxel	Peak coordinate in MNI space		
Broca's area	ITG.L	397	37	14.19	-54	-60	-15
	ITG.R	132	37	10.23	57	-63	-15
	IFGtriang.L	1680	46	39.65	-51	30	18
	ORBinf.R <sup>a</sup>	87	47	10.13	54	39	-6
	IFGtriang.R	436	45	13.46	51	33	15
	IPL.L	553	40	11.09	-42	-48	36
	SPG.R	174	7	10.77	15	-69	51
	SFGmed.L <sup>b</sup>	123	8	9.22	-3	36	48
Wernicke's area	MFG.R <sup>c</sup>	86	6	8.89	33	3	54
	MTG.R	108	21	9.26	69	-27	-6
	ANG.L	1268	39	31.00	-51	-51	33
	ORBinf.R <sup>a</sup>	90	47	12.01	54	36	-12
	MFG.L	826	9	13.79	-42	12	45
	IFGoperc.R	81	45	9.90	60	18	9
	SMG.R	544	48	12.89	48	-42	33
	MFG.R <sup>c</sup>	239	9	10.48	42	33	36
	SFGmed.L <sup>b</sup>	55	9	8.03	-3	45	51
	CUN.L	72	/	9.45	-9	-75	36
Cerebellum.R	747	/	12.00	33	-69	-42	

and cluster size  $> 50$  voxels) across all subjects in this study. We detected 9 clusters which were uniformly significantly positively correlated with Broca's area, including the bilateral inferior temporal gyrus (ITG), bilateral inferior frontal triangular part (IFGtriang), left inferior parietal gyrus (IPL.L), left superior medial frontal gyrus (SFGmed.L), right inferior frontal orbitalis (ORBinf.R), right superior parietal gyrus (SPG.R), and right middle frontal gyrus (MFG.R). Similarly, we also detected 10 clusters which were uniformly significantly positively correlated with Wernicke's area, including bilateral middle frontal gyrus (MFG), right middle temporal gyrus (MTG.R), right inferior frontal opercularis and orbitalis (IFGoperc.R, ORBinf.R), supramarginal gyrus (SMG.R), right cerebellum crus, left angular gyrus (ANG.L), left superior medial frontal gyrus (SFGmed.L), and left cuneus (CUN.L). In this study, no cluster was found to show significantly negative correlation with Broca's and Wernicke's areas after performing FWE correction. It is worth noting that the time courses in ORBinf.R, SFGmed.L, and MFG.R were significantly positively correlated not only to the time course of Broca's area, but also to the time course of Wernicke's area. The

detailed information of these clusters is listed in Table 4. Last, we determined the voxels with statistical peak values of one sample  $t$ -test in 19 clusters to draw spheres (3 mm radius) as the nodes and to construct the language network, which is shown in Fig. 3.

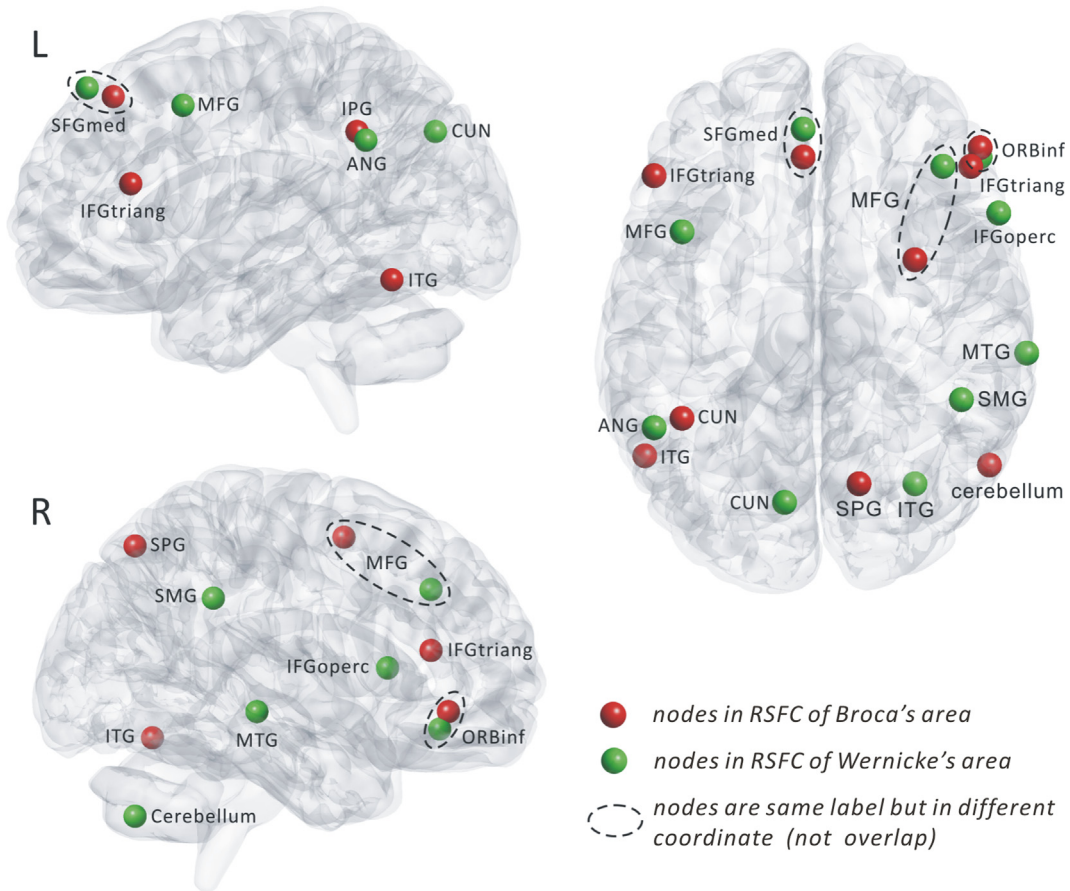
### 3.3. Topological properties

Fig. 4 shows the global parameters of language network for both the EBG and LBG. Statistical analysis showed that the EBG had significantly higher clustering coefficient ( $p = 0.026$ ), global efficiency ( $p = 0.010$ ), and local efficiency ( $p = 0.018$ ), compared to the LBG. While the EBG showed significantly lower characteristic path length ( $p = 0.009$ ) compared to the LBG.

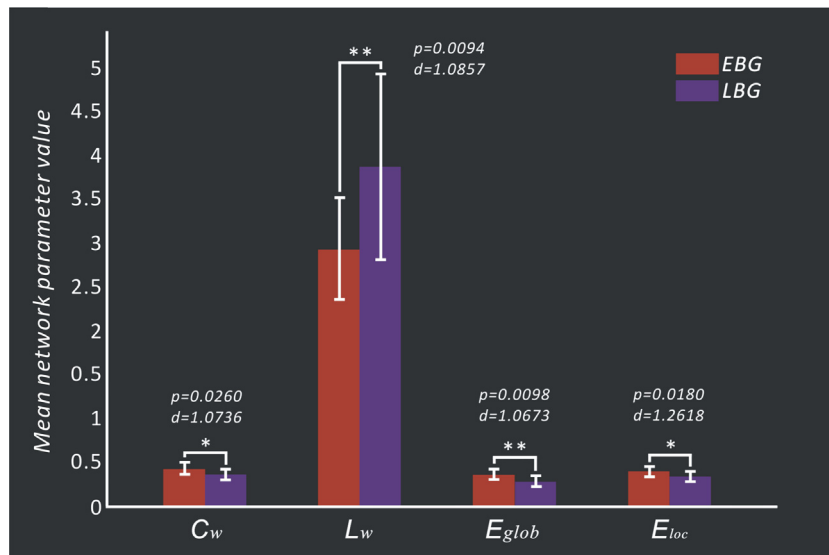
### 3.4. Modularity of language network

Fig. 5 shows the identified three modules in the language network ( $Q = 0.0758$ ,  $p_{corrected} < 2.9e^{-4}$ , FWE corrected). The first module,

### Label and location of 19 nodes in language network

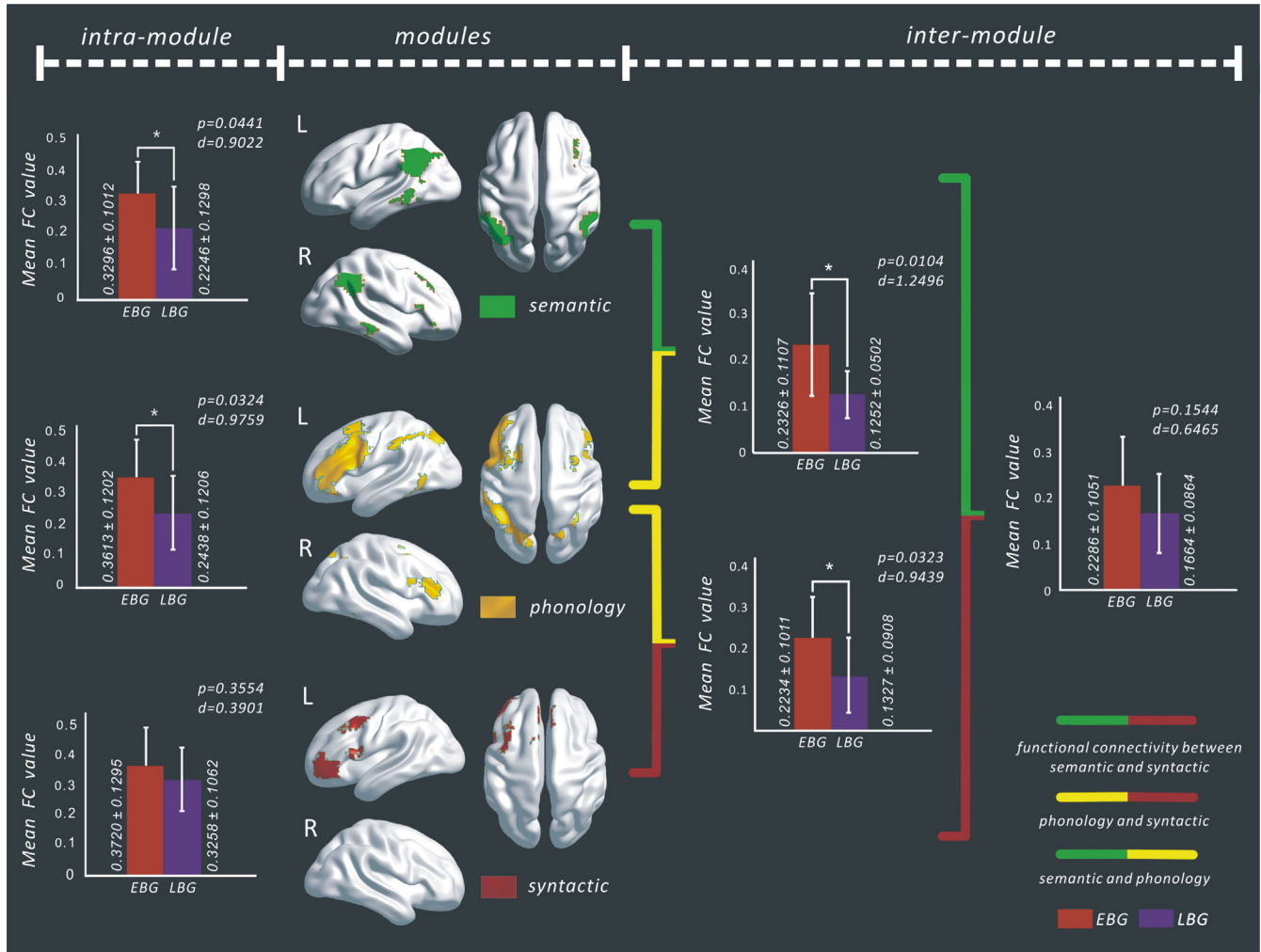


**Fig. 3.** Location of brain regions belonging to the language network derived from the RSFC of Broca's (red spherule) and Wernicke's (green spherule) regions. Totally, we detected 19 regions in the group of EB and LB. The nodes with same label and with much closed MNI coordinates were grouped together. Abbreviation: ITG, inferior temporal gyrus; IFGtriang, inferior frontal triangular part; ORBinf, inferior frontal orbital; IPL, inferior parietal gyrus; SPG, superior parietal gyrus; SFGmed, superior medial frontal gyrus; MFG, middle frontal gyrus; MTG, middle temporal gyrus; ANG, angular gyrus; IFGoperc, inferior frontal opercularis; SMG, supramarginal gyrus; CUN, cuneus; Cerebellum, cerebellum crus; L(R), left (right) hemisphere.



**Fig. 4.** The Bar plot show global parameters of language networks for both the early bilingual group (EBG) and late bilingual group (LBG). Given a parameter, the bar corresponds to the mean value and the error bar to the standard deviation of a group. Abbreviations:  $C_w$ , Clustering coefficient;  $L_w$ , characteristic path length;  $E_{glob}$ , global efficiency;  $E_{loc}$ , local efficiency. Note: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .





**Fig. 5.** Intra- and inter-modular RSFC of language network for the early bilingual group (EBG) and late bilingual group (LBG) in this study. (a) Intra-modular RSFC. (b) Three modules derived from the language network: semantic, phonology, and syntactic modules. (c) Inter-modular RSFC. Note: \*\*,  $p < 0.05$ ; \*\*\*,  $p < 0.01$ .

**Table 5**  
MNI coordinates of each brain region in the language network. These regions were classified into three functional modules, semantic, phonological, and syntactic modules. We use symbols of 'a', 'b', and 'c' to present the same pair regions in the language network but in different coordinates (not overlapped).

Functional module	Brain region	Abbreviation	Peak coordinate in MNI space		
Semantic	Inferior frontal orbital <sup>a</sup>	ORBinf.R	54	39	-6
	Middle temporal gyrus	MTG.R	69	-27	-6
	Angular gyrus	ANG.L	-51	-51	33
	Inferior frontal orbital <sup>a</sup>	ORBinf.R	54	36	-12
	Inferior frontal opercularis	IFGoperc.R	60	18	9
	Supramarginal gyrus	SMG.R	48	-42	33
	Middle frontal gyrus <sup>b</sup>	MFG.R	42	33	36
Phonological	Inferior temporal gyrus	ITG.L	-54	-60	-15
	Inferior temporal gyrus	ITG.R	57	-63	-15
	Inferior frontal, triangular part	IFGtriang.L	-51	30	18
	Inferior frontal, triangular part	IFGtriang.R	51	33	15
	Inferior parietal gyrus	IPG.L	-42	-48	36
	Superior parietal gyrus	SPG.R	15	-69	51
	Middle frontal gyrus <sup>b</sup>	MFG.R	33	3	54
Syntactic	Cuneus	CUN.L	-9	-75	36
	Superior medial frontal gyrus <sup>c</sup>	SFGmed.L	-3	36	48
	Cerebellum crus	Cerebellum.R	33	-69	-42
	Middle frontal gyrus	MFG.L	-42	12	45
Superior medial frontal gyrus <sup>c</sup>	SFGmed.L	-3	45	51	

mod-1, was composed of six regions mainly strongly coupled to Wernicke's area, including IFGoperc.R, ORBinf.R, MTG.R, MFG.R, SMG.R, and ANG.L. The second module, mod-2, involved the regions mainly strongly coupled to Broca's area, including bilateral ITG,

IFGtriang, IPG.L, SPG.R, MFG.R and CUN.L. The third module, mod-3, included SFGmed.L, right cerebellum crus, and MFG.L. Table 5 lists these three modules and the corresponding regions of the language network.

Fig. 5 also shows the intra- and inter-modular FC for both the EBG and LBG. Statistical analysis revealed significantly strong intra-modular FC within mod-1 ( $p = 0.0441$ ) and mod-2 ( $p = 0.0324$ ) in the EBG compared to the LBG. We also found the inter-modular FCs between mod-1 and mod-2 ( $p = 0.0104$ ) as well as between mod-2 and mod-3 ( $p = 0.0323$ ) were significantly stronger in the EBG compared to the LBG.

#### 4. Discussion

In this study, we investigated the effect of AoA-L2 on language network in bilinguals. Through analyzing the resting-state FC of Broca's and Wernicke's areas for both the EBG and LBG, we constructed a brain language network, which included 19 nodal regions. Network analysis showed that the EBG had significantly higher clustering coefficient, local and global efficiency, but lower characteristic path length, compared to the LBG. At the modular level, we detected three functional modules from the language network. The EBG showed not only significantly higher intra-modular FC within mod-1 and mod-2, but also significantly higher inter-modular FC between mod-1 and mod-2 as well as between mod-2 and mod-3 compared to the LBG.

The definition of early and late bilinguals varies in different studies (Hyltenstam, 1992; Johnson & Newport, 1989; Pinker, 1994). In fact, it is hard to set a "boundary", a specific age or critical period, to classify bilinguals into early and late bilinguals. For example, the specific age for early bilinguals has been set as 0 years old (Tu et al., 2015; Wartenburger et al., 2003), 3 years old (Isel et al., 2010; Saur et al., 2009), 5 or 6 years old (Archila-Suerte, Zevin, Bunta, & Hernandez, 2012; Chee, Tan, & Thiel, 1999; Mechelli et al., 2004). While the specific age for late bilinguals was set at 6 years old (Archila-Suerte et al., 2012; Isel et al., 2010) or 12–15 years old (Chee et al., 1999; Jeong et al., 2007). In our study, we chose the early bilinguals whose AoA-L2 was 3–4 years old, while the late bilinguals whose AoA-L2 was 6–7 years old. Considering previous studies (Perani et al., 2003; Waldron & Hernandez, 2013), we took the AoA-L2 in 6 years old as the "boundary" age to subdivide the bilinguals into the early or late bilinguals throughout the paper.

##### 4.1. Language network

Based on the resting-state FC of Broca's and Wernicke's areas, we defined a language network (Fig. 3) which includes 19 regions (Table 4), such as the inferior frontal network (IFGtriang, IFGoperc and ORBinf), MTG, SFGmed, ITG and temporoparietal regions (SMG, SPG, IPG and ANG). This result is in line with previous studies (Archila-Suerte et al., 2015; Saur et al., 2009; Wartenburger et al., 2003) which suggested these regions involved in different functions in language processing in bilinguals. For example, Archila-Suerte et al. (2015) found that during phonetic processing, late English-speaking bilinguals showed increased activation in the bilateral STG, rolandic operculum, IPG, but decreased activation in the right MFG, relative to early bilinguals. Saur et al. (2009) investigated the syntactic processing in German-French bilinguals, and found that late bilinguals showed stronger activation in the IFGtriang, IFGoperc, left ITG, and the basal ganglia during L2 processing compared to early bilinguals.

Also, several regions in the language network were responsive for the phonetic and semantic processing of Chinese characters (Booth et al., 2006; Cao et al., 2013; Tan, Laird, Li, & Fox, 2005). A meta-analysis compared 19 fMRI studies in Chinese and English phonetic processing of written word forms (Tan et al., 2005), and found that the left dorsal lateral frontal region, left inferior parietal regions, bilateral FFG and middle occipital gyri (MOG), and left

ventral prefrontal regions are four major regions involving in the phonetic processing of Chinese characters. Booth et al. (2006) investigated the phonetic and semantic processing in native Chinese by employing the parallel rhyming and meaning association judgment tasks. They found that subjects showed more activation in the IFG/MFG (BA 9/44) and the IPG (BA 40) during the rhyming task, while more activation in the IFG/MFG (BA 47) and the STG/MTG (BA 22/21) during the meaning task. These findings suggested that the left MTG was involved in representing semantic information and the left IPG was involved in mapping between orthographic and phonetic representations.

In this study, we also found several language functional regions are located in the right hemisphere, such as right IFGtriang, IFGoperc, ORBinf (Fig. 3), although language is widely believed lateralization to the left hemisphere. This result indicates that language processing involves bilateral frontal, temporal, and parietal cortices (Bozic, Tyler, Ives, Randall, & Marslen-Wilson, 2010; Huth, de Heer, Griffiths, Theunissen, & Gallant, 2016). Two meta-analyses based on language fMRI-studies (Vigneau et al., 2011; Jie Yang, 2014) found that the right hemisphere works in an inter-hemispheric manner during language processing. Although the right hemisphere may not play a key role in phonetic representations, it participates in lexico-semantic processing (Vigneau et al., 2011; Jie Yang, 2014). In this study, we detected that the IFGtriang, IFGoperc, ORBinf, MTG, ITG, SMG, FMG, SPG, and cerebellum in language network are located in the right hemisphere. Hull and Vaid (2007) suggested that bilinguals, who acquired L1 and L2 before 6 years old, showed balanced activation in bilateral hemispheres for processing both languages. Therefore, given that most of the subjects in LBG acquired L2 at about 6 years old, our result indicated that the language network involves bilateral brain regions concurs with Hull and Vaid (2007).

##### 4.2. Topological properties of language network

Language processing is supported by the language network for transferring information. Based on graph theory analysis, we found that in the language network, the EBG showed significantly higher clustering coefficient, global and local efficiency, but significantly lower characteristic path length compared to the LBG (Fig. 4). Different topological properties may reflect the influence of different L2 experience on local and global transfer efficiency of information in bilinguals.

###### 4.2.1. Local properties

The clustering coefficient (Table 2) described how close is the relationship between a node and its neighboring nodes (Rubinov & Sporns, 2010). We observed significantly higher clustering coefficient in the EBG compared to the LBG, which may reflect that early L2 experience would strengthen the connectivity profile for each language's functional region. This result is consistent with previous studies (Klein, Mok, Chen, & Watkins, 2014; Wei et al., 2015) that suggested the effect of AoA-L2 on brain neural plasticity in bilinguals. For example, a brain structural plasticity study suggested that late bilinguals displayed increased density of grey matter in the left inferior parietal cortex, while the PL-L2 and the AoA-L2 commonly modulated the degree of structural reorganization in this region (Mechelli et al., 2004). And another study found late bilinguals were associated with significantly thicker cortical thickness in the left IFG and thinner cortical thickness in the right IFG (Klein et al., 2014). As for the local efficiency (Table 2), it measures the capacity of a node for regional specialization. We found the late bilinguals had lower local efficiency compared to the early bilinguals. This may be probably resulted from an increase of language processing demands in late bilinguals (Consonni et al., 2013; Hernandez et al., 2007) or late bilinguals need to employ additional

neural resources to process L2 (Hernandez et al., 2007; Jeong et al., 2007). Nevertheless, we detected significant difference in local efficiency between early and late bilinguals who all had high PL-L2. Although late bilinguals could achieve high PL-L2, our finding suggested that the neural plasticity in language learning is still influenced by different AoA-L2.

#### 4.2.2. Global properties

EBG displayed higher global parameters in the language network, which may be related to an increase in their neural efficiency as influenced by early language experience (Fig. 4 and Table 2). The characteristic path length represents a number of intermediate steps for information transfer in language network. Bilinguals with different L2 experience may induce different global parameters in language network, even if they achieve high PL in both languages later. A recent study (Sheppard, Wang, & Wong, 2012) suggested that the differences in global and local efficiency can be detected after a short-term training in language learning. Different global and local efficiency were associated with language learning abilities. Sheppard et al. (2012) analyze the large-scale brain functional networks before and after sound-to-word learning by 17 younger adult subjects. They found that compared to less successful learners, successful learners performed with higher global efficiency and a more cost-efficient network organization. In addition, short-term training could not only influence the global and local efficiency in a new language learning, but also alter the effective connectivity among language functional regions (Jing Yang, Gates, Molenaar, & Li, 2015). In this study, we focused on how AoA-L2 influences the language network in early and late bilinguals who have comparably high PL in both L1 and L2. Different from short-term training in language learning, the subjects in this study had high PL in both languages. We found that the early bilinguals showed not only higher global efficiency, but also higher local efficiency in language network than the late bilinguals. This result may suggest that short-term language training and long-term language experience result in different forms of brain plasticity.

#### 4.3. Modularity

In this study, we detected three modules from the language network (Fig. 5 and Table 5). Different modules in language network were associated with different language functions, such as semantic, phonetic and syntactic processing (Fig. 5 and Table 5). The mod-1 (semantic module), grouped regions strongly coupled to Wernicke's area, is associated with semantic processing (Binder, Desai, Graves, & Conant, 2009; Price, 2010). The mod-2 (phonetic module), grouped regions strongly coupled to Broca's area, is responsible for phonetic processing (Gauthier, Duyme, Zanca, & Capron, 2009; Wong, Perrachione, & Parrish, 2007). And the mod-3 (syntactic module), including four regions, is associated with syntactic processing (De Smet, Paquier, Verhoeven, & Mariën, 2013; Grodzinsky & Friederici, 2006).

We found that the intra-modular FC in the EBG was significantly higher in two modules, semantic and phonetic modules, compared to LBG (Fig. 5). These results were consistent with several previous studies (Archila-Suerte et al., 2015; Fiebach, Friederici, Müller, von Cramon, & Hernandez, 2003; Hernandez & Fiebach, 2006), which detected that AoA-L2 influences semantic and phonetic processing in bilinguals. Particularly, in phonetic learning, a previous study (Archila-Suerte et al., 2012) showed early L2 learners usually display more native-like patterns of L2 perception than late learners, while late learners who achieve high PL-L2 also improve the perception of L2 sounds. In our study, we observed the AoA-L2 group difference in intra-modular FC for the phonetic module even though the subjects have comparable PL

in L1 and L2. Hence, we suggest that although late bilinguals have achieved high PL-L2 and improve their L2 pronunciation in their long-term language learning environment, the phonetic processing may still be influenced by their AoA-L2. In addition, several studies (Hernandez, Bates, & Avila, 1996; Hernandez & Reyes, 2002) found that AoA-L2 influence the syntactic more than semantic processing in late bilinguals. However, we observed that the semantic module displayed significant differences in intra-modular FC between early and late bilinguals. This different result may be related to subjects' variability in different studies. Bilingual subjects in previous studies (Hernandez & Reyes, 2002; Hernandez et al., 1996) mainly used alphabetic languages, but we recruited Cantonese-Mandarin bilinguals who use Chinese, a logographic language. Several differences have been found between alphabetic and logographic language in syntax, phonology, semantic and orthography (Zhu, Nie, Chang, Gao, & Niu, 2014).

We also analyzed the Inter-modular FC between different language modules. Compared to the LBG, we found that the EBG showed significantly higher resting-state FC between semantic and phonetic modules as well as between phonetic and syntactic modules (Fig. 5). Connectivity between different language modules also reflects the local property of language network. A recent study (Jing Yang et al., 2015) found that efficient resting-state FC among different language function regions for lexical access, phonetic working memory, auditory and phonetic processing could predict successful in language learning. Hence, differences between inter-modular FC suggest that different AoA-L2 may influence bilinguals to learn a further language.

#### 5. Limitations

There are several limiting factors in this study. First, the sample size is small which may bias our findings (10 subjects in EBG, 11 subjects in LBG). Actually, we recruited 500 volunteer undergraduates of Cantonese-Mandarin and recorded the age of L2 acquisition, listening and oral PL, and language exposure to L1 and L2. We found only 21 subjects met the criteria to take part in the MRI scan. In order to test the power of our findings, we estimated the effect size by conducting a nonparametric permutation test. The results (Figs. 4 and 5) revealed that the effect size were quite high for each network parameter and functional connectivity in both intra- and inter-module. We also calculated functional connectivity between thousands of brain voxels, but effect sizes are only reported for a small subset of significant network parameters between groups. It will make the effect size larger than their true values. Hence, for the high effect size in this study, we should still prudently explain the results and don't get into the 'winner curse'. Meanwhile, although effect size describes the statistical power of results, it is worth noting that increasing sample sizes will often reduce the effect size observed between groups. Second, our R-fMRI datasets were obtained from a 1.5 T MRI scanner, which is less sensitive to detect brain spontaneous activity and topology of resting-state brain network. Thus, the findings observed in the current study should be further tested using a high field MR scanner. Third, the overlapped modular patterns were not considered in this study as we adopted the average FC matrix of 21 subjects to define functional modules by using Newman's algorithm (Newman, 2006). We only used Pearson's correlation to construct the language network. Actually, we tried to use partial correlations to construct the language network, but only few connections survived after FWE correction. Thus, based on partial correlations, we can hardly apply graph theory to analyze topological properties of the language network. Fourth, we didn't regress out the global signal in R-fMRI analysis on account of controversies in fMRI study (Fox et al., 2009; Murphy et al., 2009). The global signal removal in R-fMRI

preprocessing induced more anticorrelated FC in brain network analysis. Finally, we just considered the static language network in this study although the language network is changing in different developmental stages (Skeide & Friederici, 2016). In the future, we should include the dynamic properties of language network to expand the present findings.

## 6. Conclusion

In this study, we mapped the language network and investigated its topological properties in early and late bilinguals of high PL. We found that the AoA-L2 can induce not only significant differences in topological parameters in the language network, but also differences in the intra- and inter-modular functional connectivity, between early and late bilinguals. The findings may provide us a new perspective to understand the effect of AoA-L2 on bilinguals at a language network level.

## 7. Competing interest's statement

The authors declare no competing financial interests.

### Statement of significance

The language network was constructed for Cantonese-Mandarin bilinguals. We found that AoA-L2 can induce significant differences in topological properties, intra- and inter-modular functional connectivity of the language network. The early bilinguals had higher  $C_w$ ,  $E_{loc}$  and  $E_{glob}$ , but lower  $L_w$  in language network compared to the late bilinguals.

### Statement of significance to the neurobiology of language

In this study, we mapped the language network and investigated its topological properties in early and late bilinguals of high PL. We found that the AoA-L2 can induce not only significant differences in topological properties in the language network, but also differences in the intra- and inter-modular functional connectivity, between early and late bilinguals. The finding may provide us a new perspective to understand the effect of AoA-L2 on bilinguals at language network level.

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