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2017

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How to cite

QIAN, Tianyi et al. Detecting Perfusion Pattern based on the Background Low-frequency Fluctuation in Resting-State Functional MRI Data and its Influence on Resting-State Networks: An Iterative Post-processing Approach. In: Brain Connectivity, 2017, vol. 7, n° 10, p. 627–634. doi: 10.1089/brain.2017.0545

This publication URL: <https://archive-ouverte.unige.ch/unige:100223>

Publication DOI: [10.1089/brain.2017.0545](https://doi.org/10.1089/brain.2017.0545)

COVER PAGE**Title**

Detecting Perfusion Pattern based on the Background Low-frequency Fluctuation in Resting-State Functional MRI Data and its Influence on resting state networks: an Iterative post-processing approach.

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Running head

Iterative Approach of RS-fMRI Perfusion

Key words

Resting-state functional connectivity magnetic resonance imaging (R-fMRI), Default mode network, Cerebral vascular territories, Time-shift mapping, Iterative algorithm

ABSTRACT

Introduction

RS-fMRI is based on the assumption that the vascular response and the blood oxygenation level dependent (BOLD) response are homogenous across the entire brain. However, this a priori hypothesis is not consistent with the well-known variability of cerebral vascular territories. In order to explore whether the RS networks are influenced by varied vascular speed in different vascular territories, we assessed the time-shift maps that give an estimate of the local timing of the vascular response and check whether local differences in this timing have an impact on the estimates of RS networks.

Methods

217 elderly (≥ 60 years), healthy participants (73.74 ± 4.41 years, 143 female, 203 right-handed) underwent one MRI examination including an RS-fMRI session. After preprocessing, statistical analyses included time-shift analyses and RS-fMRI analyses using as regressor the delay maps obtained from the time-shift analyses. The functional connectivity map of default mode network of each participant was then calculated by using the seed-to-voxel analysis in the REST toolbox.

Results

Faster cerebrovascular responses were notably present in the primary motor and somatosensory and peri-insular cortex while slower responses were present in various regions including notably the posterior cingulate cortex (PCC). Moreover, significant changes notably in the default mode network (DMN), including medial pre-frontal cortex ($t=11.95$), posterior cingulate cortex ($t=11.52$), right middle temporal lobe ($t=10.72$) and right angular gyrus ($t=10.88$), were observed also taking into account the cerebrovascular delayed maps.

Discussion

As the most prominent example of the RS networks, DMN activation patterns change as a function of the cerebrovascular delay. These data suggest that a group correction for vascular maps in RS-fMRI measurements is essential to correctly depict functional differences and exclude potential confounding effects, notably in the elderly with increasing prevalence of vascular co-morbidity.

INTRODUCTION

Resting state fMRI (RS-fMRI) measures the synchronized spontaneous low-frequency fluctuations of the BOLD signal in a task-free environment. The synchronizations are known to represent the functional connections of different brain areas (Fox and Raichle, 2007). RS-fMRI has been improving for the last 20 years and its applications extend from research to clinics (Barkhof *et al.*, 2014; Fox and Greicius, 2010). The spotlight here is on the intrinsic brain activity in the absence of any sensory or cognitive stimulus (Smitha *et al.*, 2017). Application of this technique has allowed the identification of various Resting State Networks (RSNs) (Lee *et al.*, 2013). Among them, the Default Mode Network (DMN) has been exhaustively studied, and changes in the patterns of its activation have been repeatedly reported in a broad spectrum of neuropsychiatric disorders (Greicius, 2008; Laird *et al.*, 2011; Zhang and Raichle, 2010).

RS-fMRI assumes that the vascular response and the BOLD response are homogenous across the entire brain (Katanoda *et al.*, 2002; Yuan *et al.*, 2013). However, several ASL studies demonstrated that the patterns of vascular response vary substantially in neocortical areas (Emmert *et al.*, 2016; Hartkamp *et al.*, 2013; Wang *et al.*, 2014) as arterial spin labeling (ASL) MRI provides concomitant information on cerebral perfusion and brain anatomy allows for differentiating vascular territories in the brain (Damasio, 1983; Floyd *et al.*, 2003; Tatu *et al.*, 1998; 2012).. Although some previous studies suggested that the “back ground noise” from white matter, grey matter and CSF should be regressed out during the post-processing pipeline of RS-fMRI data (Murphy *et al.*, 2009; Schölvinck *et al.*, 2010; Shmueli *et al.*, 2007), the effects of vascular territories haven’t been fully considered. It is thus possible that the functional connectivity of RS networks (and in particular the DMN) may change according to the differences in vascular speed.

In the present study, we assess the time-shift maps that give an estimate of the local timing of the vascular response using an innovative approach and examine whether local differences in the speed of the vascular response influence the functional connectivity of the DMN.

METHODS

Participants

217 elderly (≥ 60 years), community-dwelling healthy participants (mean age: 73.74 ± 4.41 years, 143 female, 203 right-handed) were enrolled in this study (Geneva and Lausanne). These community-based cases were recruited via advertisements in local newspapers and media. All participants had normal or corrected-to-normal visual acuity. Past hearing problems were identified as a part of the medical interview (including both subjects and their proxies). All cases with such problems were *a priori* excluded. All individuals were evaluated with an extensive neuropsychological battery, including the Mini-Mental State Examination (MMSE), the Hospital Anxiety and Depression Scale, and the Lawton Instrumental Activities of Daily Living (Haller et al., 2017).

All individuals were also evaluated with the Clinical Dementia Rating scale. Only cases with intact cognitive abilities and CDR score of 0 were included in our sample. The education level was defined according to the Swiss scholar system, where level 1 = less than 9 years (primary school), level 2 = between 9 and 12 years (high school), and level 3 = more than 12 years (university).

This study was approved by the Commission cantonale d'éthique de la recherche Genève (cantonal ethics committee Geneva) and has been performed in accordance with the ethical standards prescribed in the 1964 Declaration of Helsinki and its later amendments. Written consent was obtained from all participants in accordance with the local guidelines.

MRI data acquisition

MRI exam included routine protocols for screening obvious lesions and a resting-state fMRI (rs-fMRI) session. The rs-fMRI data acquisition used an echo-planar imaging (EPI) sequence with the parameters as follows: TR=2000 ms, TE=30 ms, flip angle=90°, 33 slices, slice thickness=3.5 mm, distance factor=20%, FOV=210 × 210 mm², matrix= 64×64, measurements=200. A high-resolution T1-weighted magnetization prepared rapid gradient echo (MPRAGE) dataset (TR =2300ms, TE=2.27ms, 176 slices, 1mm isotropic, FOV= 256 × 256 mm², matrix= 256×256) was acquired as anatomical reference. All data were collected on a MAGNETOM Trio Tim 3T MR scanner (Siemens Healthcare, Erlangen, Germany) using a 32-channel head-coil.

Pre-processing of fMRI data

The fMRI data was firstly pre-processed with a standard pipeline for resting-state data analysis by using GREYNA toolbox (<https://www.nitrc.org/projects/gretna>) in MATLAB. The pipeline included slice timing correction, rigid head motion correction, normalization from individual space to standard MNI space by using EPI templates and re-sampling to 3mm isotropic, smoothing (FWHM=6 mm), de-trend and band-pass filter data with residual signals within 0.1-0.01Hz to remove the low-frequency signal drift.

Time-shift analysis

After the pre-processing, the time-shift map of each participant was calculated using an iterative algorithm with the following steps: 1) Averaging the time series of the whole brain to create the first time series template (time points from 6TR to 194TR). 2) For each voxel, the time course was shifted from -6TR to +6TR and correlated with the time series template at each TR. Each voxel was then labeled as the number of TR which had the maximum correlation coefficient value, and this value (ranged from -6 to +6) was defined as time-shift value of the voxel. 3) Shifting the time series of all voxels based on their relative time-shift value determined by step2. For example, if the -2TR has the largest

correlation coefficient with the template, then the time point at -2TR was set to time point 0, the rest of the time series were shifted with the same number of TRs in the timeline. 4) Averaging the re-aligned time series of the whole brain to create a new global time series template. 5) Repeating steps 2,3 and 4 until the number of voxels, that had changed their time-shift value between two iterations, is less than 100. 6) Smoothing the final time-shift value of all voxels using Gaussian kernel (FWHM =6 mm). 7) Subtracting the time-shift value of each voxel by the mean value of all voxel. The result of this step was defined as time-shift map (TSM).

The TSM of all participants were then averaged to create a TSM of the elder group. The distributions of time-shift values in different vascular territories were analyzed according to a custom-made atlas (based on previous knowledge of vascular territories and watershed areas WSA as depicted in Radiopaedia.org, courtesy of Dr Frank Gaillard and the IMAIOS online atlas <https://www.imaio.com/en/e-Anatomy/Head-and-Neck/Brain-MRI-3D>).

The time-shift map of each participant was then converted to z-score for group analysis. Voxel-based analysis of the normalized time-shift map in group level was performed by using two-sample t-test in the Resting-State fMRI Data Analysis Toolkit (REST), version 1.8 (Song *et al.*, 2011) to test the age effect to TSM. The differences between the younger group (<70 years, 70-) and elder group (>80 years, 80+) was investigated with gender and education as covariates. The clusters in the t-maps of group analysis were selected under a combined threshold of $p < 0.001$ and a cluster size of 351 mm^3 . This yielded a corrected threshold of $p < 0.05$, determined by Monte Carlo simulation using the AlphaSim program with parameters: FWHM = 6 mm, within the brain mask (afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf). The variance of the time-shift value of each voxel among all participants was also calculated to test which areas had large individual differences.

Functional connectivity analysis

After the pre-processing steps of fMRI data, the residual signal in the frequency band of 0.1-0.01Hz was further processed by regressing out the background noise with two different methods. The first method used the regressor created by averaging the time-series of all voxels directly. The second method applied the global time-series template obtained in the TSM analysis as regressor and shifted the regressor according to the time-shift value for each voxel using an in-house developed MATLAB code. The functional connectivity map of the DMN of each participant was then calculated by using the seed-to-voxel analysis in REST toolbox. The seed was located in posterior cingulate cortex (PCC, MNI coordinate: X=1 Y=-61 Z=38) with 6 mm radius, and the voxels with correlation coefficient value smaller than 0.2 were masked as 0. Fisher's Z-transform and paired t-test were used to compute the differences in functional connectivity between the datasets processed by two different noise regress strategies.

RESULTS

The averaged time-shift map of the rs-fMRI data over 217 normal participants is shown in Figure 1. The blue-purple represents negative time-shift meaning that blood flow arrives in this area earlier than the averaged time-shift value among all voxels of the brain, while yellow-red represent positive time-shift value and late arrival time. The motor, somatosensory and peri-insular cortex have the shortest while the frontal, parietal and temporal lobes including regions of the DMN notably the posterior cingulate cortex (PCC) and lateral ventricle shows the longest time-shift values.

A vascular territories mask was applied to assess the distribution of the time-shift value in different vascular territories. The box plot in Figure 2 illustrates that the MCA territory has the earliest blood arrival time (median value = 140ms, 25th percentiles= 67ms and 75th percentiles= 211ms) followed by the ACA (median value = 56ms, 25th percentiles= -35ms and 75th percentiles= 152ms) and finally PCA territories (median value = 8ms, 25th percentiles= -108ms and 75th percentiles= 99ms).

Figure 3 and Table 1 show the group differences between 70- and 80+. As compared to the 70- group, the 80+ group has longer time-shift in the frontal horn of the lateral ventricle and the body of the lateral ventricle, with shorter time-shift values in left parahippocampal gyrus.

As shown in Figure 4, the time-shift values have large variance in ventricles, brain stem, temporal lobe, and inferior frontal lobes. When comparing the variance in different vascular territories (figure 5), the PCA (median value = 0.81, 25th percentiles= 0.66 and 75th percentiles= 1.05) territory displays a more pronounced variance compared to ACA (median value = 0.69, 25th percentiles= 0.54 and 75th percentiles= 0.79) and MCA (median value = 0.66, 25th percentiles= 0.54 and 75th percentiles= 0.79).

In respect to the functional connectivity maps of DMN in a single participant (figure 6), an enhanced correlation coefficient between the seed (in PCC) and the medial prefrontal cortex (mPFC) could be observed in maps obtained from the data processed by the time-shift regression method compared to the global average regression. The results of the paired t-test between the two co-variance regression strategies shown in figure 7 indicate that the time-shift regression method could enhance the connectivity within the DMN including PCC, medial pre-frontal cortex (mPFC), middle temporal lobe, and angular gyrus.

DISCUSSION

To our knowledge, this is the first study that evaluates the impact of the local timing of the vascular response on RS network functional connectivity patterns. Using innovative time-shift maps, our results show first that vascular speeds vary across the different cortical areas in the absence of external stimuli. Secondly, we demonstrate that take into account the 'reactivity maps' previously extracted, changes in RS-fMRI network activation in terms of delayed responses are present mainly in the periphery of the DMN.

The present findings show regional differences in terms of vascular speed notably in the pre-central gyrus and motor cortex. These regions have therefore different timing of blood flow arrival than the rest of the brain. These results are in line with previous observations using breath-holding, showing changes in vascular reactivity between different parts of the brain (Geranmayeh *et al.*, 2015; Iranmahboob *et al.*, 2016; Lipp *et al.*, 2015; Tancredi and Hoge, 2013). The motor cortex and pre-central gyrus include representations of the respiratory muscles (Colebatch *et al.*, 1991; Li and Rymer, 2011). This can partly explain the differences in cerebrovascular response of these brain areas.

After creating the 'delayed maps' from the vascular response, we proceeded to take these maps into the RS analyses, using the DMN as the most prominent example. Our findings reveal significant differences in RS DMN functional connectivity patterns (in particular in its periphery) when taking into account the 'delayed maps' of vascular response. In particular, the time-shift regression method could enhance the connectivity within the DMN including PCC, medial pre-frontal cortex (mPFC), middle temporal lobe, and angular gyrus. These results are in line with previous works investigating changes in the cerebrovascular reactivity (Golestani *et al.*, 2016; Haight *et al.*, 2015), showing that the BOLD signal is potentially mediated by concomitant alterations in the cerebrovascular response. Our observations support the idea that the presence of varied in cerebro-vascular response may be the major vascular modulator of RS-fMRI (Golestani *et al.*, 2016). The present results imply that not taking into account any vascular information is a good approximation, yet it is not totally accurate. In line with previous studies (Golestani *et al.*, 2016), we suggest a group correction for vascular maps in RS-fMRI analyses, to depict cerebrovascular differences and exclude potential confounding effects.

Functional connectivity analyses, regardless whether seed-based or ICA-based, intrinsically assume that the vascular BOLD response to neuronal activation is equal across the brain. We however know from vascular anatomy, that this is not the case. The known vascular variations in the blood supply of the brain have been ignored in most fMRI studies, yet may cause a systematic and local effect in the brain - while other physiological parameters such as e.g respiration in general affects the entire brain globally. Without the cerebro-vascular response, the BOLD signal after standard pre-processing steps may still contain

the perfusion related pattern, but in different phases across different brain areas. This type of “noise” will reduce the correlation coefficient when running functional connectivity analysis. By regressing out the global averaged signal, this perfusion pattern could be removed from the areas that are in the same phase of the global pattern, but still exist in the voxels containing perfusion patterns in different phases.

DMN is a widely distributed network that has areas located in at least two brain-vascular territories, ACA and MCA. It is further known that the DMN recovers in an antero-posterior temporal gradient when switching from task to rest (Van et al., 2012).

The correlation coefficient value between PCC and mPFC will be affected by the brain-vascular response time in ACA and MCA. The shifted regressor can provide better modeling of the BOLD fluctuation due to brain-vascular response. Removing this part of noise can further improve the accuracy of real functional connectivity without “perfusion relationship”. Several brain-vascular territories based regressors’ combination could also be considered to remove this component, paralleling the idea of modeling of the physiological patterns proposed in a previous study (Catie Chang, et al., 2009). Our results could further demonstrate that when taking into account this local variations in time shift, this may indeed and as expected increase the estimated correlation.

Besides their neuroradiological relevance, our observations may also have clinical significance. Small but notable differences in the cerebro-vascular response may be particularly important in a clinical population. In fact, elderly individuals (80+) may have stronger variation in cerebrovascular response as compared to younger controls (70-) due to increasing prevalence of vascular co-morbidity. The observed variations of this parameter between cases younger than 70 years and older than 80 years of age further suggest that this hemodynamic parameter may display significant fluctuations in elderly individuals due to vascular morbidities. Consequently, vascular-related confounding effects on RS-fMRI may be stronger in very old individuals. Therefore, when performing RS analyses in young volunteers, correcting for vascular maps might not generate prominent differences. However, in an older cohort with vascular comorbidity the situation may be very different.

The temporal delay in BOLD signals has first been investigated in acute stroke by Yating et al., (Lv *et al.*, 2013). Some other groups also tried to calculate the time delay of the lesion area in ischemic stroke and Moyamoya patients (Christen *et al.*, 2015; Khalil *et al.*, 2017; Siegel *et al.*, 2016). These studies used the averaged time-series of BOLD signal within a whole-brain mask or manually picked ROI as the template of fluctuation pattern to measure the time delay. Due to the changed time-shift in different vascular territories, simply averaging the time-series of all voxels together will mix the patterns in different phases. The manually selected ROI might have subjective bias and might also lead to unclear patterns due to the insufficient SNR. One of our previous study (Qian et al., 2015) showed that the abnormal perfusion areas detected with iterative algorithms were more consistent with dynamic susceptibility contrast MR than the method without iteration. In such cases, the iterative algorithm proposed in this study could extract the time series pattern automatically and more accurately. Although the results in normal subjects were closely match to the known anatomic vascular territories, this algorithm still based on the hypothesis that the first template obtained by average the time-series of all brain voxels still keeps the main pattern for calculating time-shift map. However, this hypothesis may not always correct, (e.g. using the prior knowledge of whole brain perfusion pattern may improve the starting template in the iterative algorithm). In this study, we didn't acquire any CT/MR perfusion data with contrast agent injected. So the accuracy and reliability of this method in normal subjects still need to be evaluated in future study.

To conclude, the present study aims to specifically assess the time-shift maps that give an estimate of the local timing of the vascular response and to check whether local differences in the speed of the vascular response influence the estimated RS networks. Our results show that taking into account the cerebrovascular delayed maps slightly modify the DMN as the most prominent example of the RSNs. Therefore, we suggest that a group correction for vascular maps in RS-fMRI measurements is essential to correctly depict functional differences and to exclude potential confounding effects.

LIMITATIONS

Two main limitations should be considered when interpreting our data. First, the present analysis is confined to the DMN and does not address the impact of the cerebro-vascular reactivity on other RSNs activation patterns. Second, the temporal resolution of the BOLD signal was only 2000ms, so we can only detect the large time-delay. Future works should extend this investigation and study possible influences of cerebro-vascular reactivity on other RSNs. Moreover, a simultaneous-multi-slice EPI sequence to acquire BOLD signal in higher spatial and temporal resolution should be applied to detect the time-delay more accurately.

Acknowledgements

The authors would like to acknowledge Dr. Ignacio Vallines, Dr. Aurelien Stalder, & Dr. Yi Sun for discussing this research project and giving useful suggestions during the preparation of the manuscript.

Disclosure

Tianyi Qian is an employee of Siemens Healthcare.

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Acronyms

ACA: Anterior Cerebral Artery

ASL: Arterial Spin Labeling

BOLD: Blood Oxygenation Level Dependent

DMN: Default Mode Network

EPI: Echo-Planar Imaging

MCA: Middle Cerebral Artery

mPFC: Medial Pre-Frontal Cortex

PCC: Posterior Cingulate Cortex

RS-fMRI: Resting State Functional MRI

RSNs: Resting State Networks

TSM: Time-Shift Map

Table 1. Regions showing decreased and increased time-shift value identified by age effect.

Brain Regions	MNI coordinate			Cluster size	t score
	X	Y	Z		
70- < 80+					
	-24	-45	-12	16	3.54
70- > 80+					
	39	-45	0	25	-3.38
	12	24	9	15	-3.90
	30	-39	18	46	-3.51

Table 2. Regions showing increased functional connectivity with time-shift compared to global average regression.

Brain Regions	MNI coordinate			Cluster size	t score
	X	Y	Z		
Medial pre-frontal cortex	3	60	12	1402	11.95
Posterior cingulate cortex	-6	-45	42	756	11.52
Middle Temporal lobe (Right)	60	-15	-18	109	10.72
Angular gyrus (Right)	42	-57	36	107	10.88

FIGURE CAPTIONS

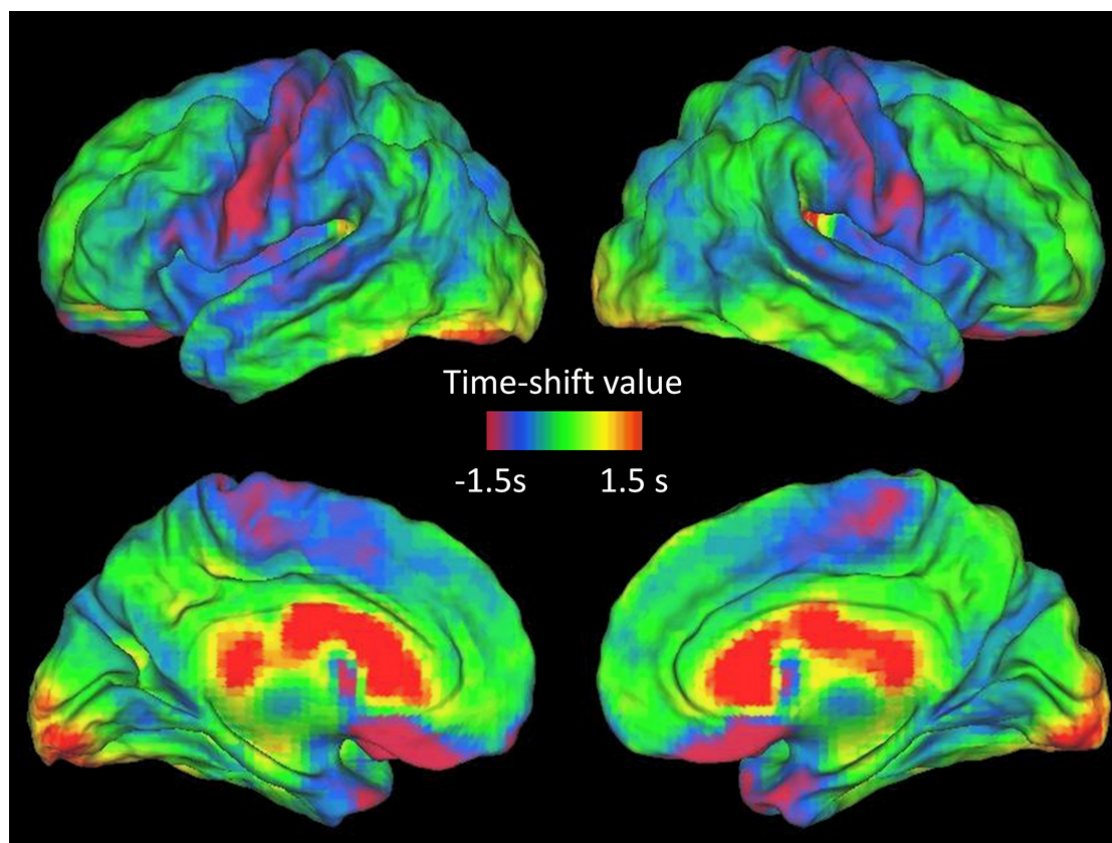


Figure 1, Averaged time-shift maps obtained from resting-state-fMRI data of 217 healthy participants. The blue-purple represents the blood flow that arrives in this area earlier than average while yellow-red represents a late arrival.

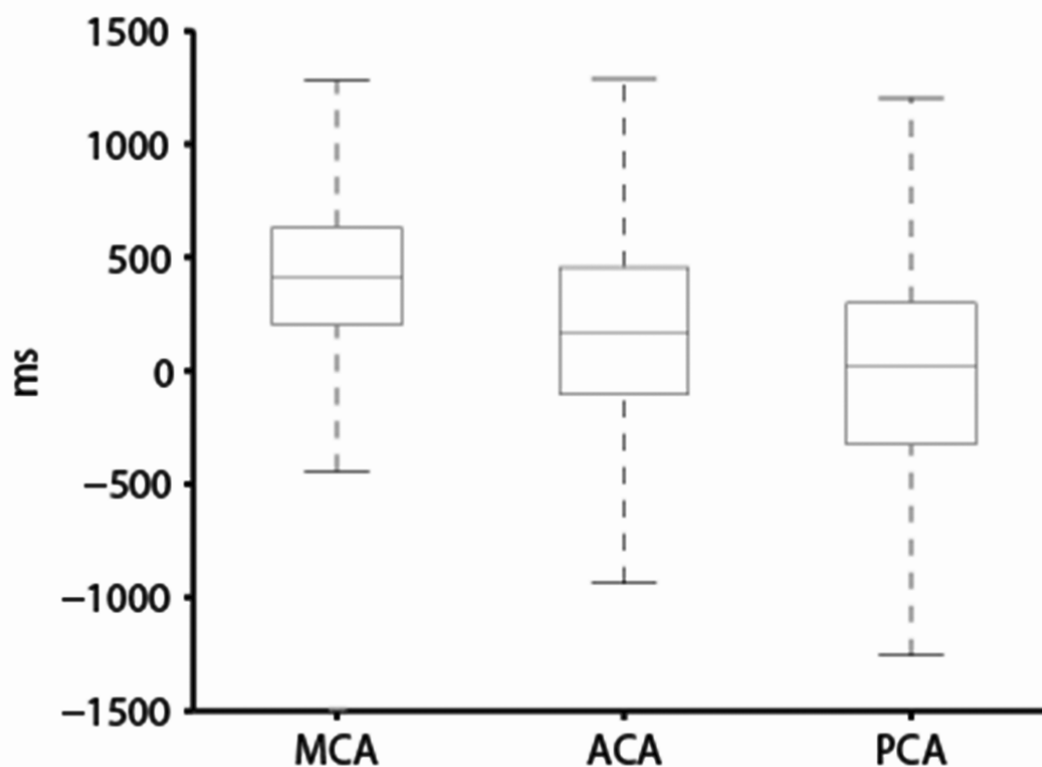


Figure 2, The statistic results of the time-shift value of voxels in different vascular territories. MCA: middle cerebral artery, ACA: anterior cerebral artery, PCA: posterior cerebral artery.

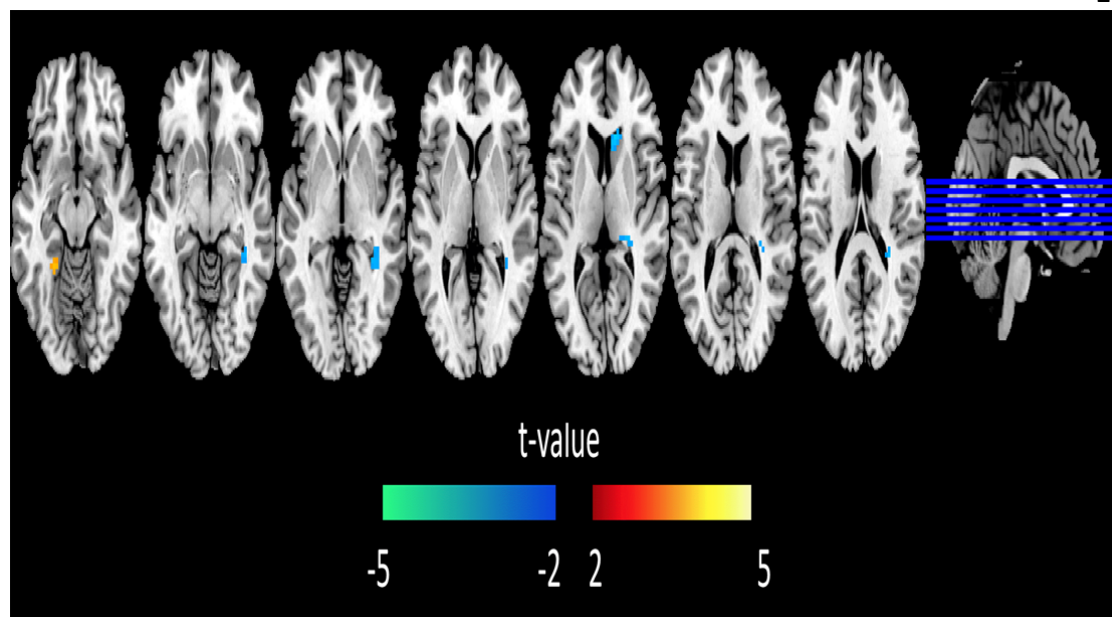


Figure 3, Group differences between 70- and 80+ cases, based on a 2-sample t-test with gender and education as covariates. The threshold was set to a corrected threshold of $p < 0.05$, determined by AlphaSim (threshold = $p < 0.001$). The color scale represents t-values. Green-Blue color represents longer time-shift in 80+ as compared to 70-.

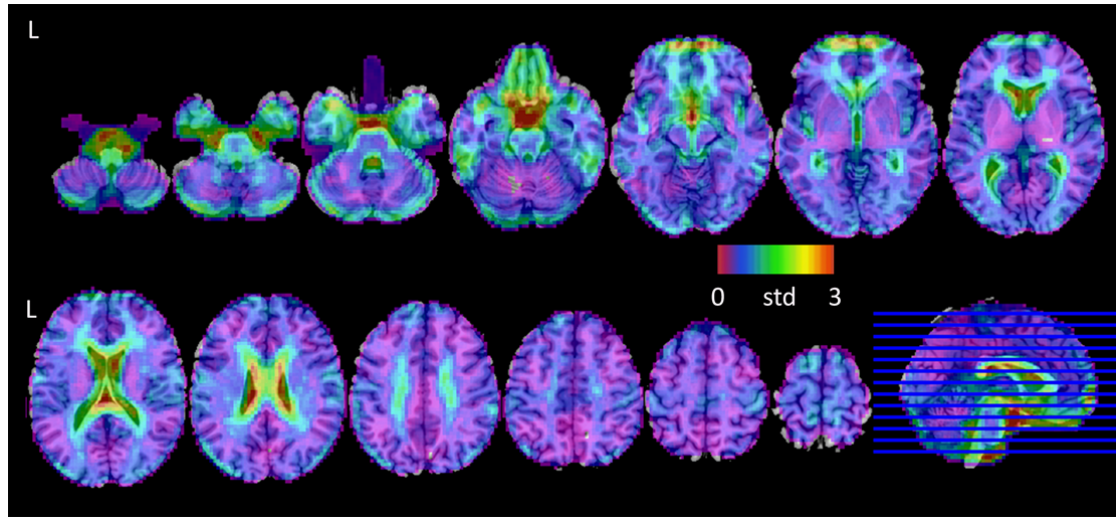


Figure 4, Cross-participants variability of time-shift map.

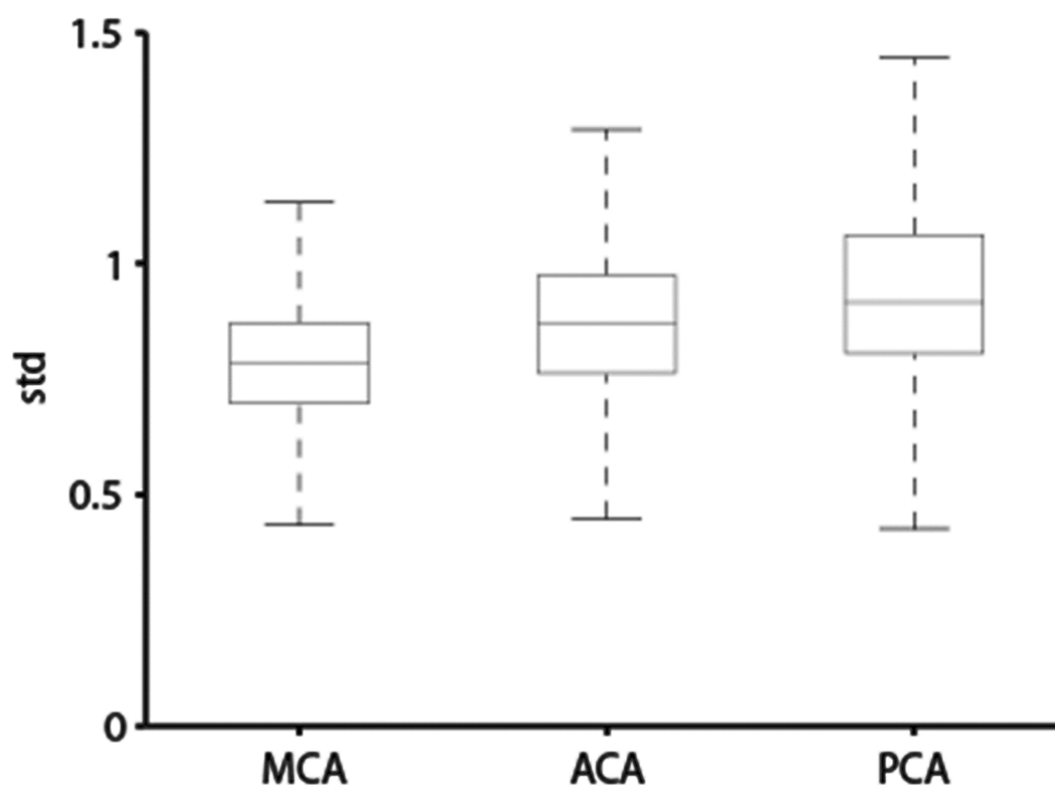


Figure 5, Cross-participants variability of time-shift values in different vascular territories. MCA: middle cerebral artery, ACA: anterior cerebral artery, PCA: posterior cerebral artery.

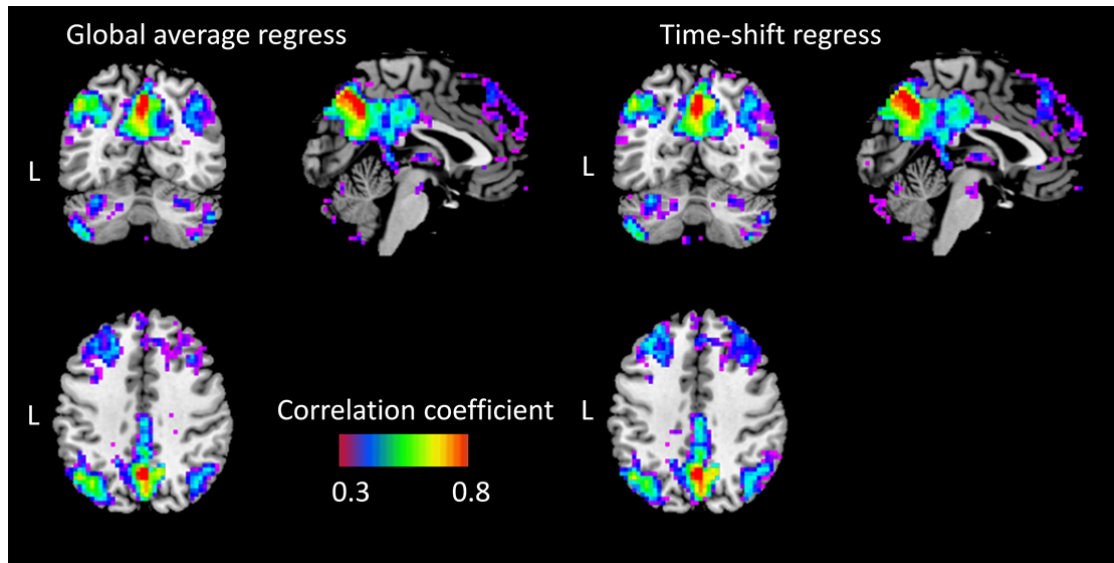


Figure 6, Functional connectivity map of the DMN obtained from two different types of covariance regression strategies.

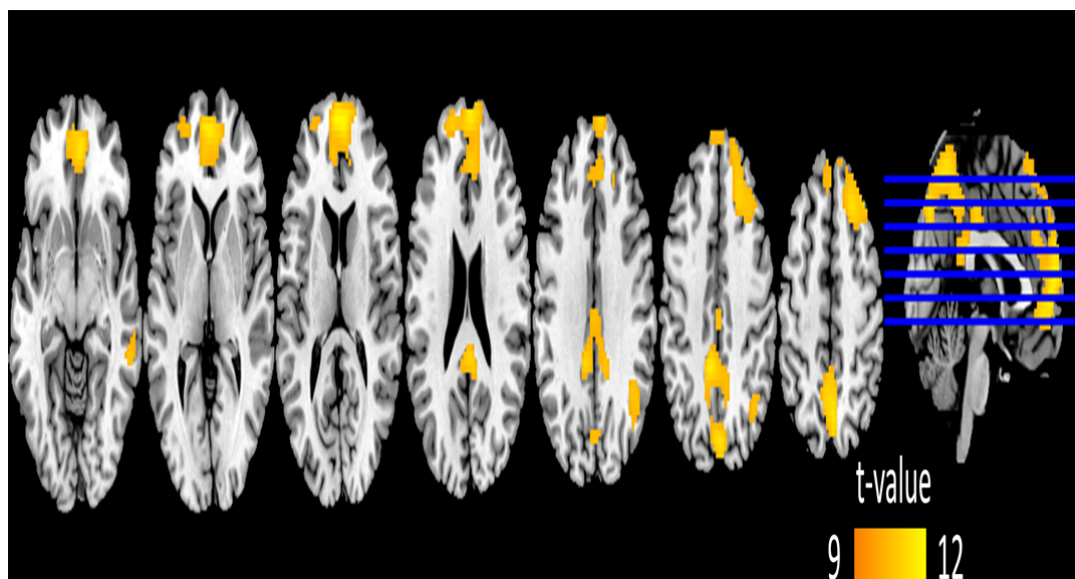


Figure 7, Time-shift regression versus global average regression differences of the functional connectivity maps among all participants. The orange-yellow represent higher correlation coefficient values with time-shift regression compared to global average regression.