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Original Research Articles

# Aging of resting-state functional connectivity in amateur singers, instrumentalists and controls

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Aging is associated with alterations in resting-state functional connectivity (RSFC), which can impact executive functions such as attention and inhibitory control; however, the extent to which lifelong musical practice can influence these age-related changes remains unclear. In this paper, we investigated age-related changes in RSFC and the relationship between RSFC and executive functions among amateur singers, instrumentalists and active controls. We analyzed the resting-state fMRI (rs-fMRI) data using independent component analysis (ICA) collected from 106 healthy adults, including 31 singers, 37 instrumentalists and 38 active controls, aged 20 to 88 years. Attention was measured using the Test of Attention in Listening (TAiL), inhibitory control and cognitive flexibility were measured using the Colour-Word Interference Test (CWIT), and working memory was measured using the Digit Span Task. Our results indicate that while aging is associated with both higher and lower RSFC, age-related reductions in RSFC are more prominent. The musicians exhibited fewer age-related RSFC changes, with distinct patterns of association with cognitive performance for singers and instrument players. Our results indicate that the relationship between RSFC and executive functions is complex and varies across resting state networks, regions, and tasks. We end this paper by proposing a framework for the interpretation of RSFC in neurocognitive aging based on our findings.

## INTRODUCTION

Aging is associated with behavioural and cognitive decline affecting notably attention, inhibition, and working memory,[1-5] with negative impacts on the quality of life of older adults.[6] There is evidence suggesting that older adults with musical experience perform better in specific behavioural and cognitive tasks.[2,7,8] Studies have found that music training in childhood can enhance certain cognitive functions and is accompanied by neuroplastic changes in brain structure and function.[9,10] These cognitive benefits have been shown to persist into later life.[11] Music-induced brain plasticity can also occur later in life,[7,8,12,13] as the adult human brain retains the ability to reorganize itself when acquiring new skills throughout the lifespan.[14] One explanation of this link between music and cognitive improvement is the OPERA hypothesis,[15] which posits that musical training enhances the brain's sensitivity to speech and other auditory tasks by engaging shared neural networks. Specifically, OPERA is an acronym for five key conditions that musical training satisfies to drive plasticity: Overlap, Precision, Emotion, Repetition, and Attention. These conditions suggest that musical training leads to enhanced neural plasticity by requiring the brain to achieve a higher level of precision in auditory processing (e.g., pitch, timing, and timbre) in a manner that overlaps with speech processing. The emotional engagement, repetitive practice, and sustained attention inherent in musical training further amplify this effect, which not only facilitates long-term memory consolidation but also contributes to broader cognitive benefits.

Musical experience has been suggested to reduce the effects of cognitive aging through changes in brain structure and function.[16-18] Singing and instrument playing are both musical activities that involve auditory, cognitive and fine motor function.[19] While both singing and instrument playing could have an impact on cognition, each is associ-

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ated with some unique abilities. For example, singing involves fine motor control of the larynx and structures of the oral cavity, which are also engaged while speaking. Singing also requires the use of language. Instrument playing, in contrast, necessitates fine control over the fingers. Studies comparing instrumentalists and singers are rare.[20] A previous study from our lab found that musical activities, particularly singing, were associated with a slower age-related decline in cortical thickness in specific regions compared to non-musical activities (the same sample is used in the present paper).[17] Singers and instrumentalists exhibited distinct patterns of gray matter plasticity. Singers followed the "Expansion and Renormalization" model, where gray matter volume initially expands and then returns to baseline levels after reaching peak performance. Instrumentalists, on the other hand, aligned with the "Brain Reserve" hypothesis, which suggests an initial expansion in gray matter volume followed by stabilization over time.

In addition to a potential effect on brain structure, engaging in musical activities can have a transformative effect on resting-state fMRI (rs-fMRI) as well.[13,21] Rs-fMRI captures low frequency fluctuations in the blood oxygenation level dependent (BOLD) signal when participants are awake and resting, not engaged in any specific task.[22] These fluctuations are believed to represent the brain's intrinsic activity and are driven by coordinated neural oscillations and vascular response.[23,24] Resting-state functional connectivity (RSFC) measures the functional coupling between brain regions, reflecting spontaneous interactions between regions and network organization.[25] RSFC is sometimes regarded as an index of overall neural health.[26] RSFC been shown to correlate with direct measurements of cortical activity including electroencephalography (EEG), electrocorticography (ECoG) and local field potentials (LFP).[27,28] These correlations suggest that RSFC reflects underlying neural dynamics across different spatial and temporal scales, providing a bridge between fMRI signals and electrophysiological measures of brain activity.

Several resting-state networks (RSN) have been documented including the default mode network (DMN), the auditory network, the speech network, and the dorsal and ventral attention networks.[29] The DMN is the most investigated RSN. It is inhibited during tasks and is active when the brain is at wakeful rest or engaged in self-referential activities such as daydreaming, mind-wandering, recalling the past or planning the future.[30] To analyze RSFC, independent component analysis (ICA), an unsupervised machine learning method, is frequently employed.[31,32] ICA can decompose fMRI data into independent components, each representing a brain network. ICA allows researchers to identify distinct brain networks and can untangle brain activity from noise. This approach is important in rs-fMRI studies, where it helps to uncover intrinsic connectivity networks without prior knowledge of the data.[33]

Age-related changes in RSFC are complex, with variations observed across brain regions and networks.[34-36] RSFC was found to lower with aging,[12,37] as well as lower within networks and higher between two or more networks with aging.[38,39] The relationship between RSFC and cognition is also complex, varying based on specific networks, cognitive tasks, and age.[21] A study involving 237 healthy older adults using the Stroop colour naming task study, which measures attentional selection, inhibitory control and cognitive flexibility,[40] showed that worse Stroop performance was associated with lower RSFC in DMN and salience network (SAL), and no significant relations in dorsal attention network (DAN) and sensory motor network (SMN).[41] However, another study of healthy older adults with a smaller sample (21 participants) found no significant association between RSFC and Stroop task performance.[42] A recent study found that the deactivation of DMN was associated with better performance in a speech-in-noise task.[43] Working memory, defined as the ability to temporarily store and manipulate information,[44] has been linked to resting-state fMRI regional homogeneity (ReHo), which reflects local RSFC by measuring the synchronization of a voxel's activity with its neighbours.[45] High working memory performers exhibited increased ReHo in the executive control network (ECN) and decreased ReHo in the DMN and SAL in healthy adults.[46] An intervention study showed that transcranial direct current stimulation (tDCS) improved working memory performance, which was associated with decreased the RSFC between DMN and the right frontoparietal network (FPN).[47] These findings underscore the complexity of RSFC's relationship with cognitive aging, highlighting how its effects may vary depending on the task, network, and neurological condition.

Several hypotheses based on task-fMRI have been proposed to explain brain aging and the mechanisms by which the brain preserves its functions, emphasizing both preservation and compensation processes.[48-50] The *preservation* mechanism hypothesis posits that the brain sustains cognitive functions during aging by strengthening existing connections, which allows RSFC levels to remain elevated, akin to those observed in young adults. The *compensatory* mechanism hypothesis posits that the brain sustains cognitive functions during aging by recruiting additional connections to maintain functions. However, the effect of aging on rs-fMRI and the protective role of musical activities on RSFC remain unclear. The objective of this study was to investigate RSFC aging patterns among singers, instrumentalists, and active controls, as well as the relationship between their RSFC and cognitive performance using ICA. We hypothesized that 1) age-related effects on RSFC would vary across different regions and networks, but would mainly be negative, 2) both singers and instrumentalists would exhibit fewer age-related RSFC changes compared to controls, 3) RSFC would be related to cognitive performance, with the nature of the relationship differing by networks and regions, and 4) based on our previous work, we expected singers and instrumentalists to exhibit distinct patterns of association between RSFC and cognitive performance.

# METHODS

## PARTICIPANTS

A non-probabilistic sample of 106 participants was recruited through emails, Facebook messages and posters distributed in the Quebec City area, targeting choirs, music harmonies and various groups, as well as emails and posters distributed at Université Laval and within the *Centre intégré universitaire de santé et de services sociaux de la Capitale-Nationale*. The participants were aged 20–88 years (mean 56.19 ± 19.47, 50 females), and had no history of hearing, speech, language, psychological, neurological, or neurodegenerative disorder. No participants had contraindications to MRI. All participants were native Quebec French speakers, or they spoke Quebec French as their main language since childhood. All participants were right-handed according to the Edinburgh Handedness Inventory.[51] Each passed a general cognitive functioning test, the French version of the Montreal Cognitive Assessment (MoCA),[52] using norms for the Quebec middle-aged and elderly population[53] as well as the 15-item version of the Geriatric Depression Scale (GDS).[54] They also passed a pure-tone threshold test with a calibrated clinical audiometer (AC40, Interacoustic, Denmark) at .5, 1, 2, 3, 4, 6 kHz for each ear. A pure tone average was calculated for each participant (PTA) across all these frequencies. We computed a score based on the 2020 Lancet Commission for dementia prevention, intervention, and care[55] to control for potential group differences in brain health and dementia risk. Livingston and colleagues have identified twelve potentially modifiable risk factors for dementia that could reduce dementia prevalence by 40% if eliminated. Nine of these factors were measured in this study, corresponding to a maximum risk reduction of 31%. These included low education, hearing loss, traumatic brain injury, depression, social isolation, Type 2 diabetes, physical inactivity, alcohol consumption and obesity. The details of the calculation are provided in Joyal et al, 2024.[1] While these scores were not used in the analyses, they were computed to identify potential differences between the groups (see below).

The participants were divided into 31 amateur singers (mean age 61.48±16.88, 20 females), 37 amateur instrumentalists (mean age 53.35±18.28, 11 females), and 38 active controls (mean age 54.63±19.58, 19 females). Amateur musicians (singers and instrumentalists) were individuals who sang or played an instrument for more than five years with a minimum weekly practice of 3 hours. Controls practised at least one non-musical psychomotor activity (e.g., golf, knitting, billiards, yoga, curling, strategy and precision video games, French bocce, bowling, tai chi and sewing, etc.) for a minimum of three hours per week in the past five years. Professional or semi-professional sports players and professional musicians were excluded. None of the participants practised other musical activities, such as dance, gymnastics, or figure skating. Eligibility criteria were verified through screening telephone interviews. Information regarding their history of practice, including number of years of practice, practice ratio, and age of onset was also collected, and a practice experience score was computed, which revealed that the groups were overall similar.[17] The practice ratio was calculated using the age of onset (AO) and the ratio of practice (AAr) of the main activity. It consists of the multiplicative inverse of the subtraction between the age of onset and the product of the age of onset and the ratio of practice (1/(AO − (AO − AAr)). A higher score indicates that a person started practising early and has practised for a large proportion of their life.[1]

Participants' characteristics are detailed in Table 1. The three groups did not differ in age, education, number of spoken languages, MoCA, GDS, handedness, self-reported health, dementia risk factor, and hearing (PTA) (all p > .05). The sex distribution differed significantly across groups. However, as in a previous study using the same sample, sex showed no significant effect on cognitive performance.[1] Nevertheless, sex was included in all analyses as a covariate in this paper.

The study included two visits. In the first visit, the screening tests (MoCA, GDS, audiometric evaluation) and cognitive tasks were administered. The cognitive and hearing tests were administered in a double-walled sound-attenuated room. In the second visit, MRI images were acquired at the Clinic IRM Quebec-Mailloux in Quebec City.

This study is part of the PICCOLO project ("Projet de recherche sur les effets de la Pratique d'un Instrument ou du Chant sur la COgnition, le Langage et l'Organisation cérébrale" in French), a research project on the effects of musical activities on cognition, language, and brain organization. The study was approved by the *Comité d'éthique de la recherche sectoriel en neurosciences et santé mentale, Institut Universitaire en Santé Mentale de Québec* (#2019-1733). All participants provided informed written consent. The study also includes speech and voice tasks that have been published elsewhere.[1,17,56]

## PROCEDURES

### *ASSESSMENT OF EXECUTIVE FUNCTIONS*

The assessment included the Test of Attention in Listening (TAiL),[57] an inhibitory control and cognitive flexibility test (Colour-Word Interference Test, CWIT),[58] a working memory test (Digit Span Test)[59] and a verbal fluency test (not reported here). A detailed report of the cognitive assessment was published separately.[1] The main findings of the group differences are summarized in Table 2.

### *TEST OF ATTENTION IN LISTENING (TAIL)*

The TAiL includes three tasks that evaluate the speed of auditory information processing as well as two dimensions of auditory selective attention: involuntary orienting (IO) and conflict resolution (CR). During the three tasks, the participants hear pairs of pure tones (between 476 Hz and 6178 Hz) sequentially through circumaural headphones. In the first task—cued reaction time (RT) task—participants were asked to press a key as fast as they can once they heard the second sound. The average reaction time (RT) was measured. Only RTs between 100 ms and 2 s were included.

**Table 1. Participants' characteristics**

| Characteristics | Control (N = 38, 19 F) | | | | Singers (N = 31, 20 F) | | | | Instrumentalists (N = 37, 11 F) | | | | ANOVA/ Fisher's exact test | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Min | Max | Mean | SD | Min | Max | Mean | SD | Min | Max | F | p |
| Age | 54.6 | 19.6 | 20 | 87 | 61.5 | 16.9 | 23 | 88 | 53.4 | 18.3 | 22 | 88 | 0.1 | 0.78 |
| Education[a] | 14.7 | 2.2 | 11 | 18 | 14.9 | 2.5 | 10 | 18 | 15.4 | 2.3 | 11 | 21 | 1.7 | 0.2 |
| MoCA[b] | 27.5 | 1.6 | 25 | 30 | 27.9 | 1.8 | 24 | 30 | 27.9 | 1.8 | 22 | 30 | 1 | 0.32 |
| GDS[c] | 1 | 1.6 | 0 | 7 | 1 | 1.6 | 0 | 7 | 0.8 | 1 | 0 | 3 | 0.3 | 0.57 |
| Self-reported health[d] | 5.1 | 0.8 | 3 | 7 | 5.4 | 1.1 | 3 | 7 | 5.2 | 1 | 3 | 7 | 0.1 | 0.81 |
| Dementia risk[e] | 9.3 | 6.5 | 0 | 28.4 | 10.3 | 7.6 | 0 | 29.8 | 8.2 | 4.9 | 0 | 16.2 | 0.6 | 0.43 |
| Number of spoken languages[f] | 2.2 | 0.6 | 1 | 4 | 2.3 | 0.6 | 1 | 3 | 2.3 | 0.5 | 1 | 3 | p = 0.156 | |
| Right PTA[g] | 18.9 | 15 | -4.2 | 59.2 | 20 | 14.2 | -0.8 | 54.2 | 17 | 9.6 | -2.5 | 33.3 | 0.4 | 0.54 |
| Left PTA[g] | 19.3 | 15.4 | -3.3 | 70.8 | 19.7 | 14.6 | 0 | 53.3 | 18.2 | 12.1 | -0.8 | 51.7 | 0.1 | 0.74 |
| Better PTA[h] | 16.4 | 13.2 | -4.2 | 50 | 18 | 13.9 | -0.8 | 53.3 | 15.1 | 9.8 | -2.5 | 33.3 | 0.2 | 0.64 |
| Inter-aural Difference[i] | 5.1 | 6.6 | 0 | 34.2 | 3.8 | 2.9 | 0 | 10 | 5.1 | 3.5 | 0.8 | 18.3 | 0 | 0.93 |
| Practice (years)[j] | 25.1 | 15.7 | 8 | 80 | 27.2 | 16.5 | 5.1 | 72 | 32.1 | 18 | 5.5 | 65 | 3.3 | 0.07 |
| Practice (ratio)[k] | 0.5 | 0.2 | 0.1 | 0.9 | 0.4 | 0.2 | 0.1 | 0.8 | 0.6 | 0.2 | 0.08 | 0.9 | 4.3 | 0.04 |
| Age of Onset[l] | 24.3 | 19 | 5 | 62 | 27.2 | 19.4 | 3 | 69 | 14.2 | 10.9 | 5 | 63 | 6.5 | 0.01 |
| Practice (exp)[m] | 0.3 | 0.45 | 0.02 | 2.49 | 0.17 | 0.19 | 0.02 | 0.71 | 0.36 | 0.32 | 0.02 | 1.17 | 0.5 | 0.48 |

***Notes***. SD = standard deviation of the mean, N = number of participants per group.
[a]Education = Number of years of education based on the highest degree obtained in Quebec.
[b]MoCA = Montreal Cognitive Assessment. Higher scores indicate better cognitive functions.
[c]GDS = The Geriatric Depression Scale.
[d]Self-reported health = self-reported general health status on a scale of 0 to 7 (0 being the lowest health level)
[e]Dementia risk factor = To control for the risk of dementia among our sample, we developed a dementia risk factor (DRF)[1] based on the 2020 *Lancet* Commission for dementia prevention, intervention, and care.[55] Livingston and colleagues have identified twelve potentially modifiable risk factors for dementia that could reduce dementia prevalence by 40% if eliminated. Nine of these factors were measured in our sample, corresponding to a maximum risk reduction of 31%. These included low education, hearing loss, traumatic brain injury, depression, social isolation, Type 2 diabetes, physical inactivity, alcohol consumption and obesity.
[f]Number of languages spoken: Fisher's exact test was used due to some of the expected cell frequencies being small, which violates the assumptions needed for the chi-square test. The Fisher test revealed no significant differences in the distribution of the number of spoken languages across groups (p = 0.156).
[g]PTA = pure tone average thresholds measured in decibels at .5, 1, 2, 3, 4, 6 kHz for each ear.
[h]Better ear PTA = pure tone average thresholds (PTA) at .5, 1, and 2 kHz for the better ear, measured in decibels (dB).
[i]Inter-aural Difference = |PTA (Right Ear)−PTA (Left Ear)|.
[j]Years of practice = total years of active practice of signing, playing a musical instrument or practising a cognitive-motor activity.
[k]Ratio of practice = Ratio between Years of practice and Age (AAr).
[l]Age of onset = age at which singers, instrumentalists or control participants began to practise their activity.
[m]The *practice experience score* combines the age of onset (AO) and the ratio of practice (AAr) of the main activity. It consists of the multiplicative inverse of the subtraction between the age of onset and the product of the age of onset and the ratio of practice (1/(AO − (AO − AAr)). A higher score indicates that a person started practising early and has practised for a large proportion of his life.[1]

In the second task—attend frequency (frequency)—participants were asked whether the two tones have the same pitch. In the third task—attend location (location)—participants were asked whether the two tones came from the same ear. Average RT and accuracy in the form of error rate (ER) were recorded for both frequency and location tasks. In the frequency and location tasks, participants oriented their attention to the task-relevant dimension (frequency for frequency task, location for location task). However, they performed better in congruent trials (when the two dimensions were either both the same or different) than in incongruent trials (when the two tones were the same in one dimension and different in the other, e.g., different pitch from the same ears).

The IO and CR scores were calculated based on RT and ER for both frequency and location tasks.[57] The IO score reflects the impact of incongruence in the unattended dimension. A higher IO score means greater distractibility. The CR score reflects the difference between incongruent and congruent trials. A higher CR score means greater costs for resolving conflict. Details of the calculation for IO and CR scores are provided in Supplementary Material 1.

The detailed analysis of the cognitive data collected as part of the PICCOLO Project revealed that scores in the attend frequency task showed more group differences.[1] In the present study, we therefore focused on the relationship between performance in the attend frequency task (IORT, IOER, CRRT and CRER) and RSFC.

*COLOUR-WORD INTERFERENCE TEST (CWIT)*

Delis-Kaplan Executive Function System (D-KEFS) Colour-Word Interference Test (CWIT)[58] is a commonly used version of the Stroop test.[60] It measures inhibitory control and cognitive flexibility. The CWIT has four conditions: colour naming (C1), word reading (C2), inhibition (C3), and inhibition/switching (C4). In each condition, the participants are given a white paper with 50 stimuli in French. In C1, participants are asked to name the colour of the squares (red, green, and blue). In C2, the participants are asked to read the colour words ("red," "green," and "blue") printed in black ink. In C3, the participants are asked to name the colour of words (same as C2, "red," "green," and "blue") printed in an unmatched colour (e.g., the word "red" printed in blue ink). In C4, the participants are asked to name half of the word colours and to read the other half (printed in a box). C1 and C2 measure the fundamental skills of colour naming and word reading. C3 and C4 measure the executive function: inhibition and cognitive flexibility. Participants practised ten stimuli for each condition before starting. The RT and ER were calculated for all conditions. Based on previous analyses of the dataset,[1] which showed that only RT is associated with age and group, we decided to focus on the RT of C3 and C4.

*WORKING MEMORY*

Working memory (WM) was measured using the digit span subtest from the French version of the Wechsler Adult Intelligence Scale (WAIS-III).[59] The test includes two conditions: forward and backward. Participants are asked to repeat a series of numbers (length from 2 to 8) either in the same order (forward) or a reverse order (backward). The number of correct responses was measured for each condition, along with the total score. Based on our previous analyses,[1] which showed that the backward condition is harder than the forward condition. The singers were better than the controls. We decided to focus on the accuracy score in the backward condition.

MRI DATA ACQUISITION

The data were acquired on a whole-body Philips 3.0 Tesla Achieva TX. The participant's head was immobilized with a set of cushions and pads during the procedure. Structural MR images were acquired with 3D T1-weighted MPRAGE sequence (TR = 8.2 ms, TE = 3.7 ms, FoV= 250 $mm^2$, 256×256 matrix, 180 slices/volume, slice thickness = 1 mm,[3] no gap). Resting-state fMRI data were acquired using the following parameters: TR = 2399 ms, TE= 13 ms; FOV = 240 x 240 mm; 80 x 80 matrix; 45 interleaved 3 $mm^3$ axial slices, no gap, SENSE = 2.1, 250 volumes. The resting-state scan duration was approximately 10 minutes. During the resting-state fMRI scan, participants were asked to lie still, to keep their eyes open and to avoid falling asleep. The functional sequence started with five dummy scans to ensure the magnetization reached a stable state. Two additional diffusion-weighted spin-echo EPI images were acquired with opposite phase-encode blip polarities (A-P, P-A) to sample distortions in opposite directions within the same field. They were used to estimate and correct the distortion caused by the susceptibility distribution of the head and rapid switching of eddy currents.

FMRI DATA ANALYSES

The rs-fMRI data were pre-processed using AFNI V23.1.07 and FSL. The data were first preprocessed using afni_proc.py[61] with the following steps: EPI distortion correction, motion correction, motion censor, slice-time correction, T1 alignment, non-linear MNI transformation (using the TT_N27.nii template) and nuisance-removal regression. A frequency filter was not applied because it can induce a loss in degree of freedom.[62] The time series were then smoothed using a 6 mm FWHM filter. The results of the preprocessing were visually inspected by the first author. Next, the rs-fMRI data of each participant was analyzed with single ICA using FSL MELODIC.[63,64] The motion correction reporting and standard space registration information were extracted using FSL FEAT.[65] Then the independent components (IC) representing noise were identified using ICA-AROMA.[66] After removing the noise ICs, the rs-fMRI signal was rebuilt using fsl_regfilt. From the cleaned rs-fMRI data, 20 and 70 ICs were extracted separately from all the participants using MELODIC group ICA.[32,67] While ICA primarily identifies spatial patterns of ICs, these patterns are widely recognized as reflecting key aspects of RSFC by identifying regions with co-activation or functional synchronization. In this paper, we use RSFC to describe the rs-fMRI signal's relative contribution to IC.

**Table 2. Summary of cognitive findings emphasizing group differences. The full results are presented in Joyal et al. 2024[1]**

| Task | Measurements | Results |
|---|---|---|
| TAiL | Processing speed | No significant group differences were found. |
| | Auditory involuntary orientation (IORT, IOER) | Older singers were disadvantaged compared to older controls. |
| | Auditory conflict resolution (CRRT, CRER) | Instrumentalists had better accuracy than controls and singers.<br>Age effect was lower in instrumentalists compared to singers and controls; no difference between singers and controls.<br>There was no significant results for CRRT. |
| Inhibitory control (CWIT) | C3 and C4 | Singers and instrumentalists had lower RT compared to controls, with no difference on accuracy. |
| Working memory | Digit span backward test | Singers showed an advantage compared to controls and instrumentalists in working memory performance. |

Each IC of the group ICA results represents one resting-state network (RSN) shared by all the participants. With more components, the resulting networks are smaller and constitute subnetworks of the solutions with fewer components. The correlation between the 20-IC and 70-IC solutions was calculated using fslcc to evaluate how the results from these two levels of analysis are related.

We used FSL's dual regression to map the cleaned rs-fMRI data of each participant to the group-level component space.[31,68] Dual regression includes two steps. First, the group ICA components were regressed into the subject's 4D space-time dataset as spatial regressors in a multiple regression for each subject. Next, we regressed these results of subject-specific time series, one per group-level spatial map, into the same 4D dataset as temporal regressors in a multiple regression. With this step, a set of subject-specific spatial maps, one per network, was estimated from the first step. Each participant has 70 sub-bricks, each representing the RSFC for a different network. Embedded in FSL's dual regression, the randomize permutation-testing tool[69] was used to control for multiple comparisons and ensure statistical significance. We examined the effects of age and group (singers, instrumentalists, and controls) on RSFC, as well as the 2-way (age-by-group, group-by-behaviour) and the three-way (age-by-group-by-behaviour) interactions, with sex as a covariate. All the significant regions were masked using the corresponding IC. Clusters smaller than five voxels were excluded.

### RESTING-STATE CONNECTIVITY AND BEHAVIOUR DATA ANALYSES

The cognitive data for the analyses included: IORT, CRRT, CRER in TAiL, RT of C3 and C4 in CWIT, and backward accuracy score in the WM test. To illustrate the relationship between RSFC and behaviour in each group, we decomposed the group-by-behaviour and age-by-group-by-behaviour interactions using general linear regression (GLM). For the group-by-behaviour interactions, we calculated the average RSFC in each significant region and performed regression analyses of RSFC and behavioural performance for each group using RStudio (version 2024.04). For the age-by-group-by-behaviour three-way interactions, the participants were divided into three age terciles: young, middle-aged, and older. The average RSFC extracted from the significant clusters of the ICA dual regression results were regressed with cognitive performance for each group within each age subgroup. A multiple comparison correction was performed using the False Discovery Rate (FDR) approach using RStudio; results with an adjusted p-value $\leq 0.05$ were considered statistically significant.

The full data analysis process is summarized in Figure 1.

## RESULTS

### GROUP ICA RESULTS

The 20-IC and 70-IC group ICA methods decompose the rs-fMRI data into 20 and 70 resting-state networks (RSNs) respectively. The first 5 ICs of each analysis are shown in Figure 2. The full IC maps of 20-IC and 70-IC are provided in the Supplementary Material (Figure S2.1 and S2.2). A comparison of spatial correspondence between the 20 and 70 solutions (provided in Supplementary Material Table S2) showed that the smaller 70-IC results can be considered subnetworks of the 20-IC results. The 70-IC solution showed less overlap among ICs, facilitating the analyses of specific networks. Larger ICs may obscure the small significant regions in the dual regression outcome.[32] Therefore, we focus here on the 70-IC results.

### AGE ASSOCIATIONS

Positive and negative age associations with RSFC were found. To facilitate the identification of the RSNs presented here, Figure 3b displays the corresponding ICs derived from the 70-IC group ICA results shown in Figure 3a. The main effect of sex is reported in Supplementary Material 3.

Positive age associations were found on IC #36, 41 and 57/70 (Figure 3a). This includes the globus pallidus in the IC#36; the left supramarginal gyrus and the right middle frontal gyrus in IC #41, which overlaps with the speech and language networks; and the right occipital gyrus in IC#57, which overlaps with the dorsal attention network.

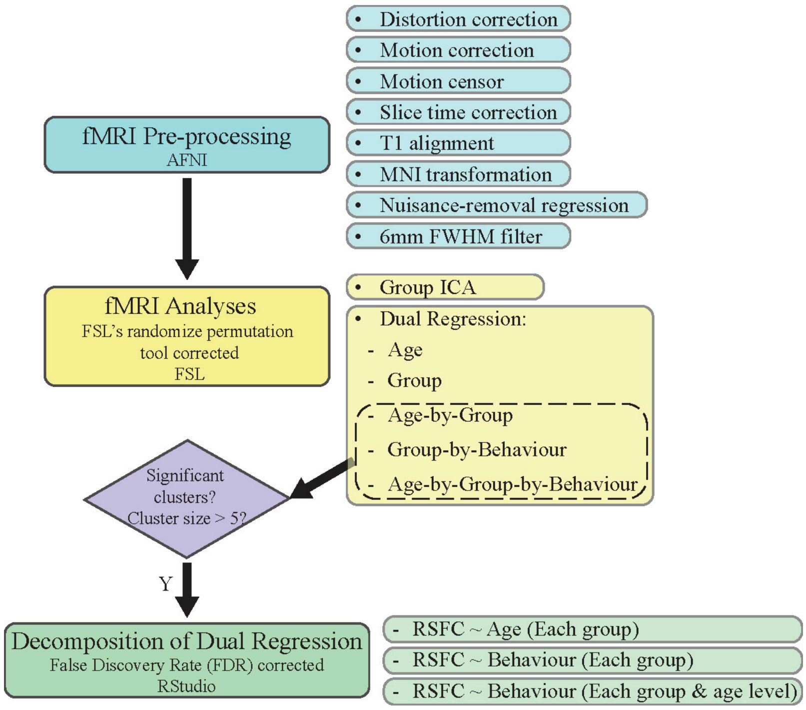


**Figure 1. Summary of the data processing steps.**

Negative age associations were found on IC #11, 12, 15 and 34 (Figure 4a). This includes the right precuneus of the default mode network (IC#11); the bilateral postcentral gyrus, transverse temporal gyrus, insula, and planum temporale in the auditory network (IC#12); the bilateral occipital gyrus (IC#15) and the left occipital gyrus in the visual network (IC#34).

GROUP DIFFERENCES

Group differences were limited. As shown in Figure 5a, singers exhibited higher RSFC than controls in the right middle frontal gyrus and left superior parietal lobule in the somatomotor dorsal network (IC#16). Instrumentalists exhibited higher RSFC than controls in the right inferior parietal lobule in IC48 (Figure 5b). We found no significant differences between singers and instrumentalists.

AGE BY GROUP INTERACTIONS

Age and group interactions were found in a few regions. The decomposition of these interactions using regressions revealed a few different patterns.

There were a few cases of singers and instrumentalists differing from controls but not from one another. Lower RSFC with aging in singers and higher RSFC in controls (Figure 6a) was found in the right precentral and postcentral gyri of the dorsal attention network (IC#4) and in the left middle frontal gyrus of the FPN (IC#13). Higher RSFC with aging in singers and lower RSFC in controls (Figure 6a) was found in the right superior parietal lobule of the right FPN (IC#39). Finally, higher RSFC with aging in instrumentalists and lower RSFC with aging in controls was found in the bilateral superior frontal gyrus of the FPN (IC#32) (Figure 6b).

In a few regions, the singers and instrumentalists exhibited significantly different patterns. Higher RSFC with aging in instrumentalists and lower RSFC in singers was found in the right precentral and postcentral gyrus, and a small region in the left inferior parietal lobule of the dorsal attention network (IC#4); the bilateral superior frontal gyrus, left middle frontal gyrus of the FPN (IC#13 and 17) and left thalamus (Figure 6c). The slopes and p-values of the age-by-group analysis are provided in the supplementary material S3.

After analyzing the age association and group effect, along with age-by-group interactions, we examined how RSFC is associated with cognitive performance across groups. Figure 7 shows the summary of this analysis. Figure 7a displays the age association, group effect, age-by-group interactions and the behaviour analyses marked with the task name. Figure 7b summarizes the group-by-behaviour results. The complete group-by-behaviour results, together with the slopes and p-values, are provided in Supplementary Material S4. Figure 7c summarizes the age-by-group-by-behaviour three-way interaction results. The full age-by-group-by-behaviour results together with the slopes and p-values are provided in Supplementary Material S5. Only ICs with significant results are shown in this figure. The full matrices with all ICs are provided in Supplementary Material S6.

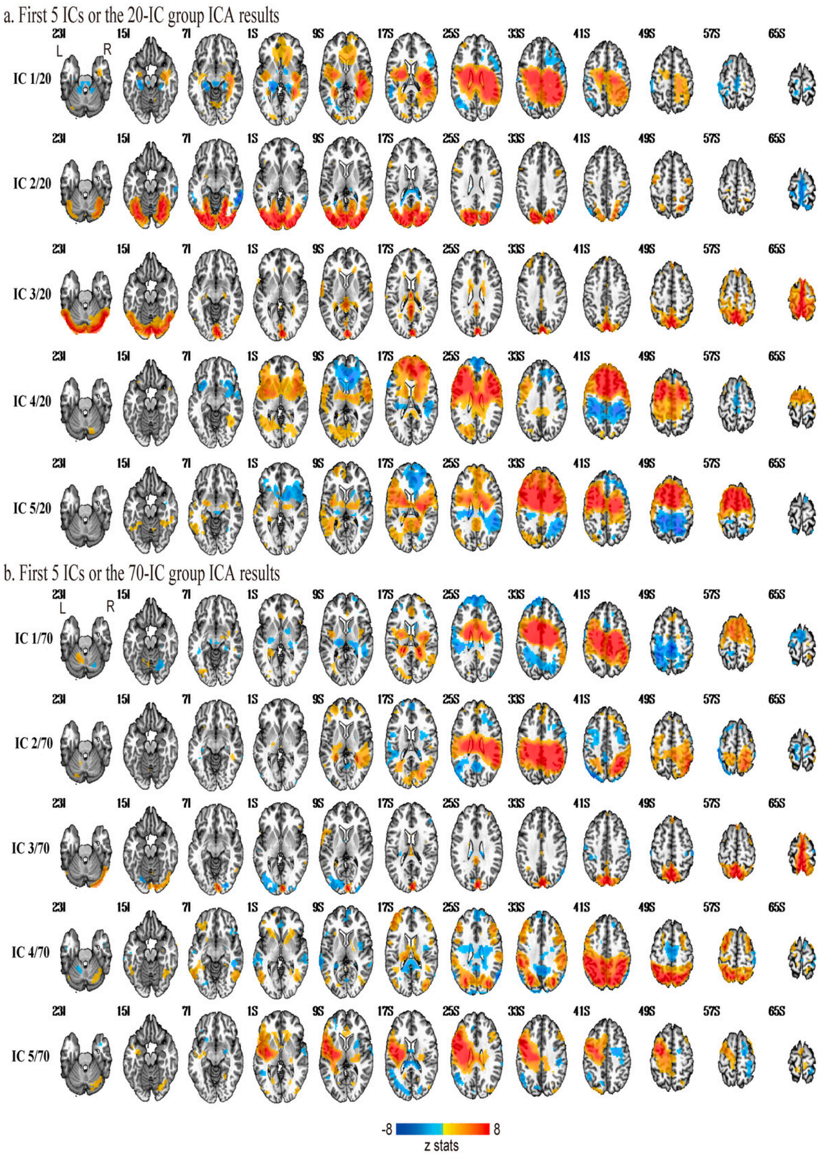


**Figure 2. a) The first 5 ICs of the 20-IC group ICA analyses. b) The first 5 ICs of the 70-IC group ICA analyses. Red indicates positive correlation; blue indicates negative correlation. The numbers on the left of the brain slices represent the IC order of the group ICA results.**

TAIL

*GROUP BY COGNITIVE PERFORMANCE INTERACTIONS*

There was no relationship between RSFC and the TAiL IO score, but we found significant relationships with the CR scores (CRRT and CRER).

For CRRT, Figure 8 shows that instrumentalists differ from controls in the right cingulate gyrus; and from singers in right insula in the frontal parietal network (IC#30). In these regions, the average RSFC of controls and singers showed no significant relation with the CRRT. In contrast, for the instrumentalists, lower RSFC was significantly related to lower CRRT, which indicates a better performance. A similar pattern was observed in other networks (IC#25, #17, #43, and #57). The complete group-by-CRRT results are presented in Supplementary Material S4.

For CRER, we found that group-by-CRER interactions showed significant differences between controls and singers in the left cingulate gyrus (CG) and right precentral gyrus in IC#1 (Figure 9a). Controls were significantly different from instrumentalists in the bilateral cerebellum, bilateral occipital gyrus and striate area in IC#25; the left precentral gyrus in IC#29 (Figure 9b). Instrumentalists were significantly different from singers in the left cingulate gyrus (CG) and right precentral gyrus in IC #2; bilateral superior parietal lobule (SPL), right postcentral gyrus (PoG), and right inferior parietal lobule (IPL) in IC #14; the bilateral superior frontal gyrus (SFG) in IC #17 (Figure 9c). The regression results are presented in Figures 9e, 9f, 9g. In most regions and ICs, for instrumentalists, lower RSFC was associated with better auditory attention performance. For singers, in most regions, higher RSFC was associated with better performance except in IC #29. For controls, in most regions, RSFC was not related to CRER. However, in IC #1,

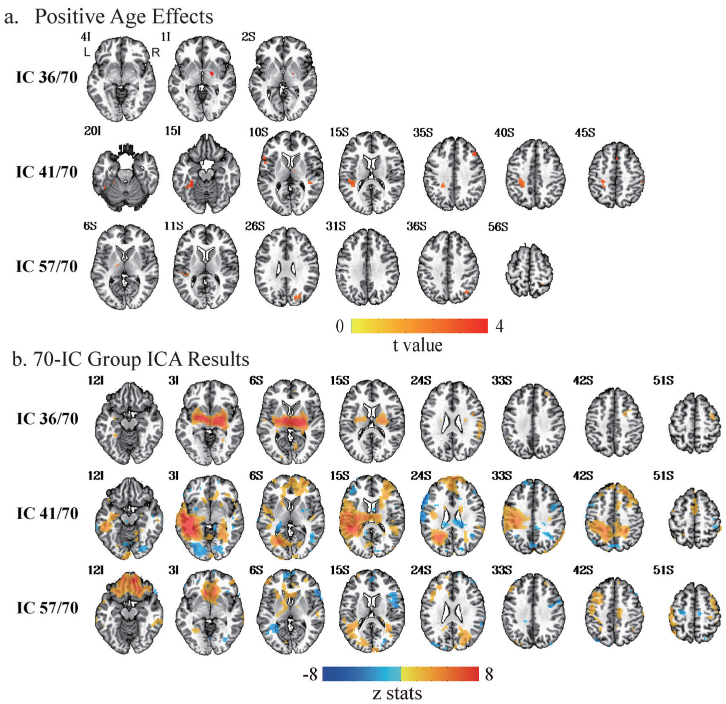


**Figure 3. Age associations on RSFC. a) The regions showing positive age associations on RSFC are shown on axial slices of the TT_N27 template in the MNI space. b) The networks where age effects were identified in a. The numbers on the top left of the brain slices represent the z-coordinate (in mm), S=superior, I = inferior, L = left, R = right. The same convention is used in the following figures.**

lower RSFC was associated with better performance while in IC #29, higher RSFC is associated with better performance. The complete group-by-CRER results are presented in Supplementary Material S4.

*AGE BY GROUP BY COGNITIVE PERFORMANCE INTERACTIONS*

For CRRT, as shown in Figure 10a, instrumentalists exhibited significantly different RSFC from the singers in the right thalamus in IC #36 in the bilateral superior frontopolar gyrus and middle frontal gyrus. Decomposition of these interactions using linear regression of RSFC (y-variable) and CRRT (x-variable) revealed that, among the middle-aged instrumentalists, lower RSFC was associated with better conflict resolution. The details of the statistical results are provided in Supplementary Material 5.

For CRER (figure 11), significant differences between instrumentalists and controls were found in the bilateral cerebellum and right entorhinal cortex in IC #25; and in the bilateral superior frontal gyrus (SFG) and left middle frontal gyrus (MFG) in IC#29. A significant difference between instrumentalists and singers was found in the left SFG in IC#41. The regression showed that better CRER was significantly related to lower RSFC for the older instrumentalists. In contrast, for the middle-aged controls, better CRER was significantly related to higher RSFC. The full results are provided in Supplementary Material S4.

CWIT

*GROUP BY COGNITIVE PERFORMANCE INTERACTIONS*

For C3 of CWIT, we found that instrumentalists were significantly different from controls in the bilateral superior frontal gyrus and middle frontal gyrus in IC#32, and in the right occipital gyrus in IC#49. The regression results showed that higher RSFC was associated with better C3 performance for controls (Figure 12).

For C4 of CWIT, we found that instrumentalists were significantly different from controls in the left striate cortex, right occipital gyrus, and left middle frontal gyrus (MFG) in IC#57. The instrumentalists were significantly different from singers in the right occipital gyrus in IC#57. The regression results showed that higher RSFC was associated with better C4 performance for singers while lower RSFC was associated with better C4 performance for instrumentalists (Figure 13).

*AGE BY GROUP BY COGNITIVE PERFORMANCE INTERACTIONS*

For the age-by-group-by-C3 interaction, significant differences between instrumentalists and controls were found in the bilateral superior frontal gyrus SFG in IC #17 (Figure 14a). The instrumentalists and singers showed significant differences in bilateral SFC in IC#32 (Figure 14b). The decomposition of these interactions using linear regression of RSFC (y-variable) and C3 RT (x-variable) showed no significant slopes after FDR correction. However, the trends of the

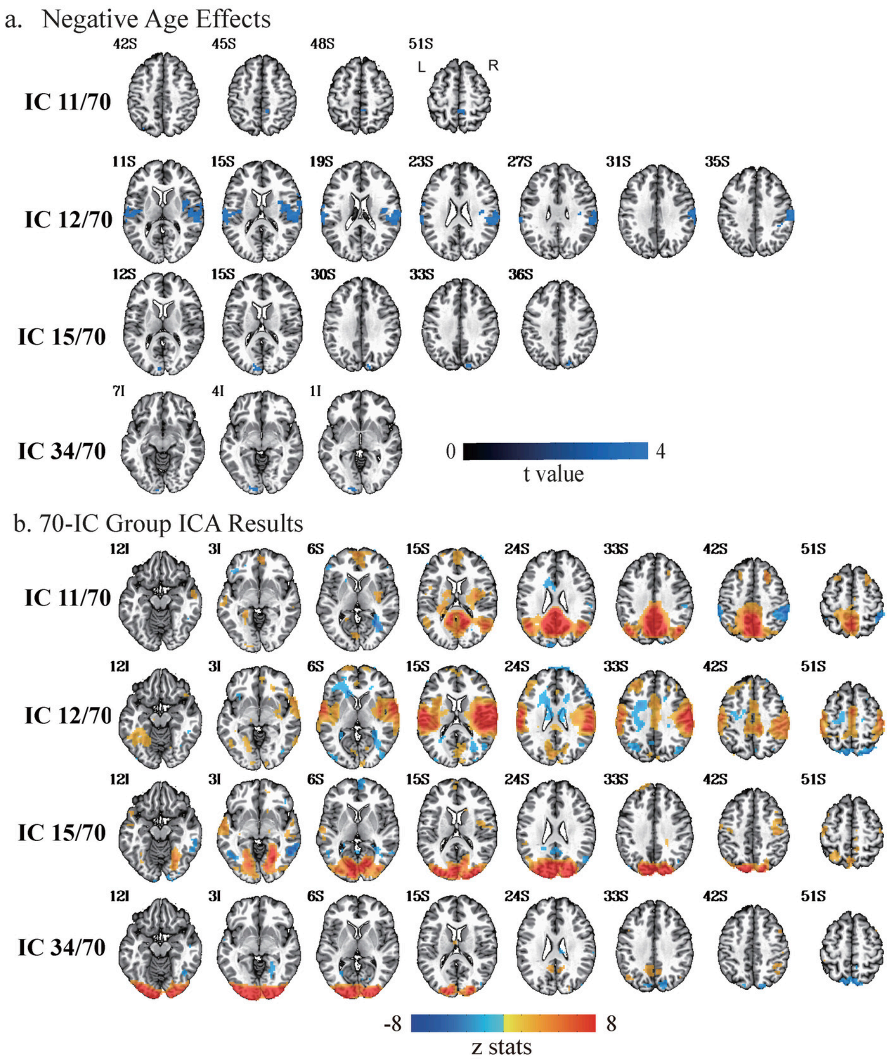


**Figure 4. Age associations on RSFC. a) The regions showing a negative age association are shown on axial slices of the TT_N27 template in the MNI space. b) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space.**

regression lines showed that in IC#17, higher RSFC was related to a shorter C3 reaction time in all ages, and instrumentalists had different trends than controls in young and middle-aged groups (Figure 14d). In IC#32, the young musicians showed different patterns in the relation between RSFC and C3 RT (Figure 14e). The details of the statistical results are provided in Supplementary Material 5.

For the age-by-group-by-C4 interaction, significant differences between instrumentalists and controls and between instrumentalists and controls were found in the bilateral SFG and right middle frontal gyrus (MFG) in IC #17 (Figure 15a and b). In IC#32, significant differences between instrumentalists and controls and between instrumentalists and controls were found in bilateral SFG, bilateral precentral gyrus (PrG), and right inferior frontal gyrus (triangular gyrus) (IFGTr) (Figure 15a and b). The Decomposition of these interactions using linear regression of RSFC (y-variable) and C4 RT (x-variable) shows the trends of the relation between RSFC and C4 RT in each age subgroup. In both IC#17 and #32, older instrumentalists showed a higher RSFC associated with shorter reaction times, a pattern not observed in the control group (Figure 15d and e). The full results are in Supplementary Material 5.

WORKING MEMORY

The dual regression revealed significant age-by-group-by-WM interactions in the bilateral superior parietal lobule (SPL) and right middle frontal gyrus (MFG) in IC#39 (Figure 16a) and in the right SPL and right white matter near the postcentral gyrus (PoG) in IC #1 (Figure 16b). The decomposition of these interactions using linear regression of RSFC (y-variable) and working memory (x-variable) showed no significant trends after FDR correction. Figures 16d and 16e show the pattern of the relation between RSFC and WM performance in each age subgroup. In IC#39, young singers and controls exhibited opposite patterns between RSFC and WM: higher RSFC was linked to better performance in singers, whereas the opposite was true for controls. Among older participants, old controls exhibited a similar pattern as the young controls, while old singers dif-

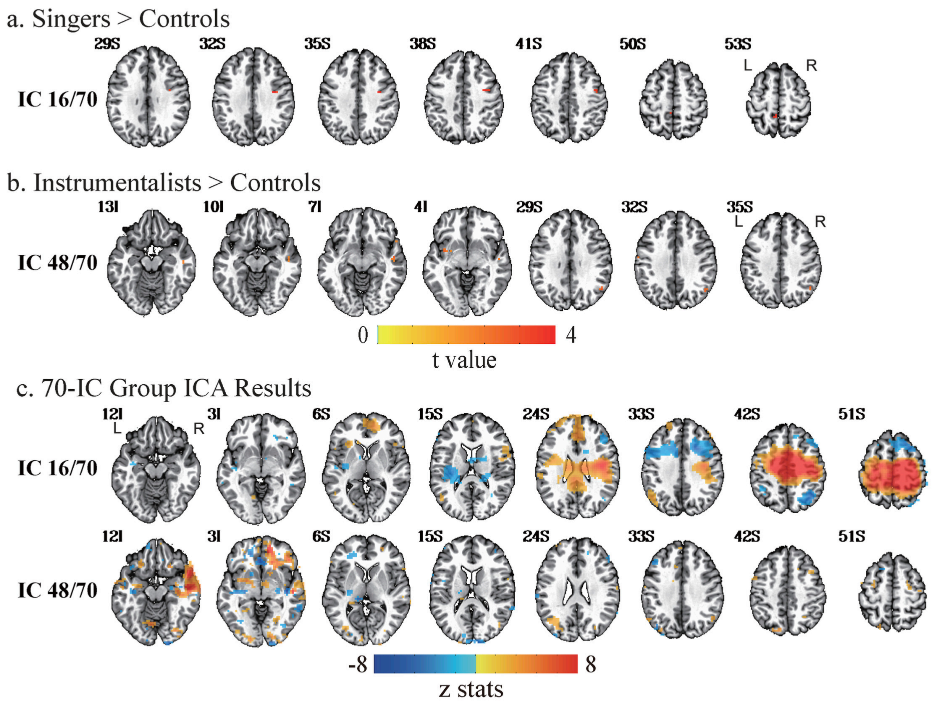


**Figure 5. The RSFC group difference results. a) Regions showing singers have higher RSFC than controls in the red regions in IC #16 on axial slices of the TT_N27 template in the MNI space. b) Regions showing higher RSFC in instrumentalists than controls in IC #48 on axial slices of the TT_N27 template in the MNI space. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space.**

fered from young singers (Figure 16d). In IC#1, the middle-aged controls displayed a different pattern compared to middle-aged musicians: higher RSFC was linked to better WM in controls while lower RSFC was linked to better WM in musicians (Figure 16e). The full results are presented in Supplementary Material 5.

## DISCUSSION

This study aimed to investigate RSFC aging in singers, instrumentalists, and active controls, as well as the relationship between their RSFC and cognitive performance using ICA. The results support our first hypothesis, showing that RSFC varies with age across different networks and regions. Consistent with previous research,[21,34,70] we found that RSFC can be higher or lower with increasing age. These findings highlight the complexity of brain aging and suggest that different networks may be affected in unique ways. Despite the variability in the effects observed, our data suggest a greater prevalence of negative RSFC changes overall, which might reflect age-related reduced large-scale network connectivity during rest. The clusters associated with negative age effects had a larger size than the positive ones (see Supplementary material Table S3.1). However, this interpretation is speculative, as we did not directly test this hypothesis. The potential causes of this decline remain unknown, but it may be related to different hallmarks of brain aging, such as cortical atrophy, white matter hyperintensities and the reduction in synaptic density.[71-73]

Our second hypothesis was that both singers and instrumentalists would exhibit fewer age-related RSFC changes. This hypothesis was also partially supported. The analyses revealed that both singers and instrumentalists significantly differed from the control group in certain small clusters, with no significant differences between singers and instrumentalists. This suggests that musical activities, whether vocal or instrumental, influence RSFC in specific regions within certain RSNs.

The age-by-group interactions on RSFC revealed complex relationships. In the controls, for DMN regions, RSFC was lower with aging. For non-DMN regions, the impact of age varied depending on the region. In visual and premotor areas, RSFC was higher with aging; in occipital, orbitofrontal, and striate cortex, RSFC was lower with aging. For the singers, RSFC was higher with aging in DMN, which suggests increased network communication in older singers compared to controls. RSFC was lower with aging in non-DMN regions in regions including premotor network, visual network, DAN and FPN compared to the other two groups. For the instrumentalists, in the DMN regions, there was no significant age effect, while in the non-DMN regions, RSFC was slightly higher with aging in FPN and DAN.

These findings suggest that for the DMN—which is associated with self-referential thinking, memory retrieval/consolidation and internal thought process— singers exhibit higher RSFC with aging, suggesting a singing-related consolidation of these processes with age. In contrast, controls' RSFC in the DMN weakened with aging, while instrumentalists did not exhibit significant age-related effects. These findings suggest that while playing instruments may protect RSFC from age-related decline (compared to the controls), mainly in the form of network maintenance, it may

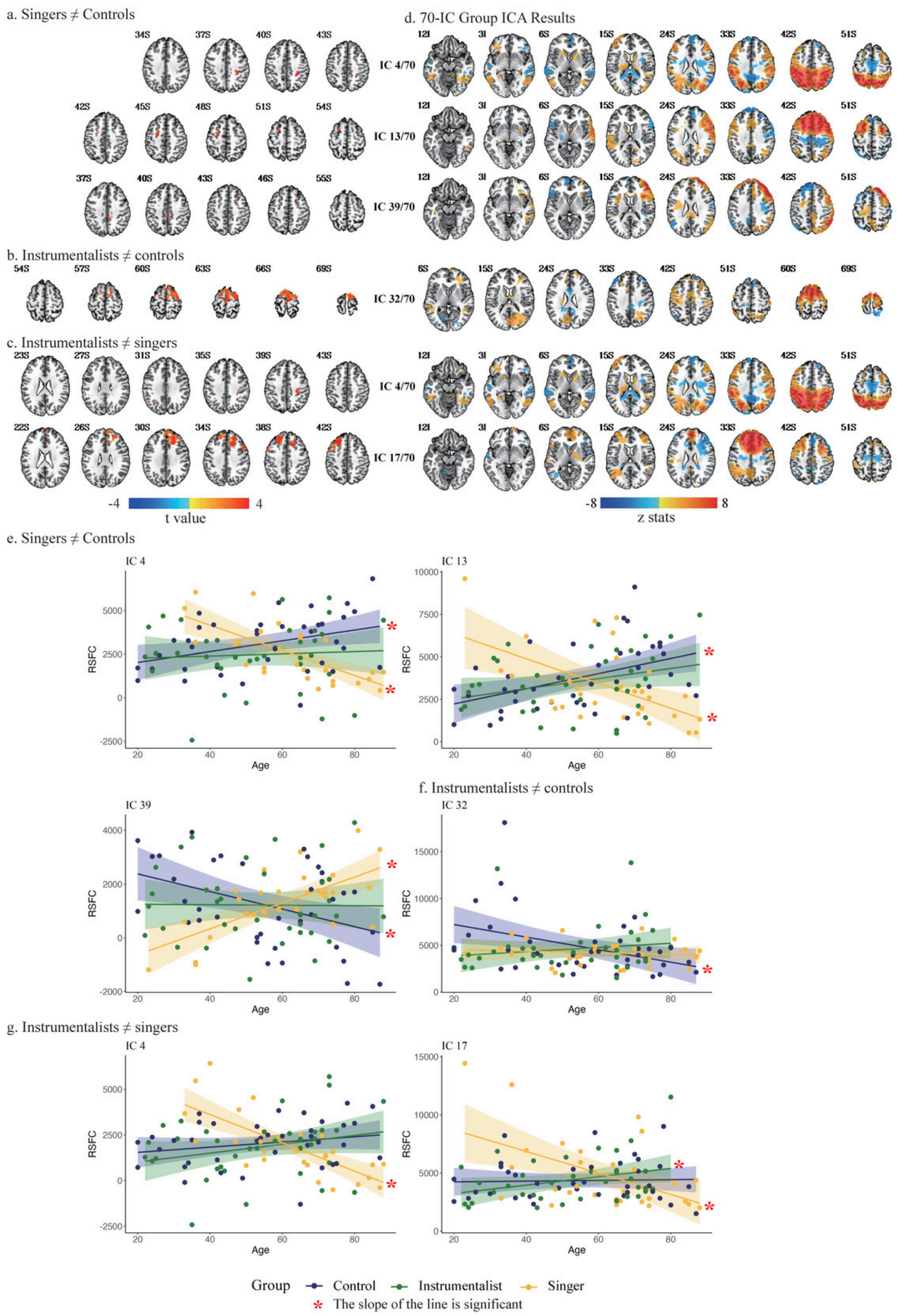


**Figure 6. a) Singers and controls showed different relations between RSFC and age. Singers (yellow line) exhibited a less steep regression slope than controls (blue line) of RSFC and age in the red regions in IC #4, and 13. Singers exhibited a steeper association than controls between age and RSFC in the regions identified in red in IC #39. b) Instrumentalists and controls showed different relations between RSFC and age. Instrumentalists exhibited a steeper regression slope of RSFC and age than controls in the regions in red in IC #32. c) Instrumentalists exhibited a steeper regression slope of RSFC and age than singers in the red or orange regions in IC #4, and #17. d) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. e-g) The plots illustrate the interaction pattern. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines. The red star indicates that the slope of the linear regression line is significant ($p<0.05$).**

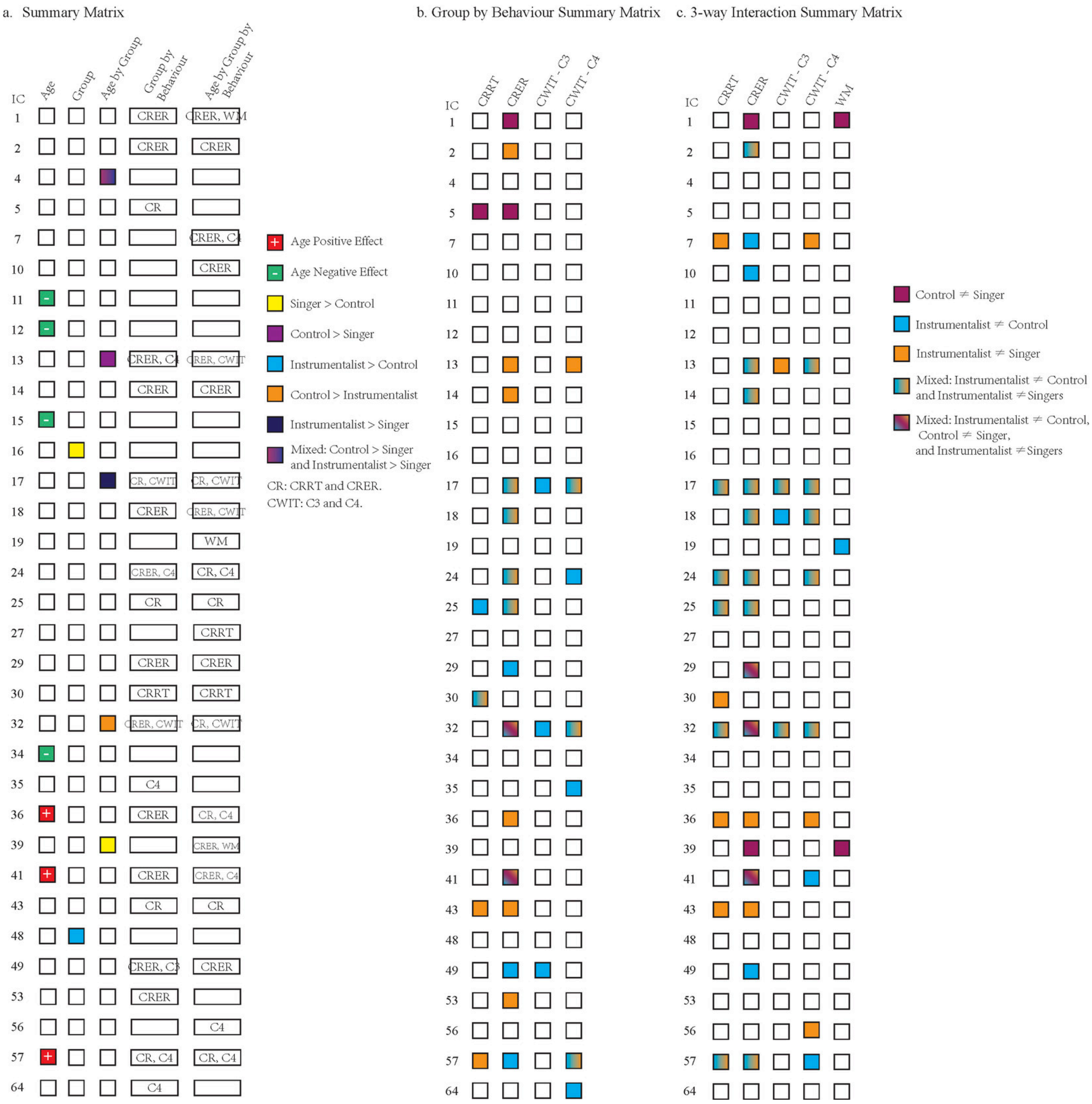


**Figure 7. Summary of the main findings of dual regression. a) Summary of all analyses: age association, group effect, age-by-group interaction, group-by-behaviour interaction, age-by-group-by-behaviour 3-way interaction on RSFC. b) Summary of group-by-behaviour interaction on RSFC. c) Summary of age-by-group-by-behaviour 3-way interaction on RSFC. CRRT = conflict resolution score based on reaction time. CRER = conflict resolution score based on error rate. CWIT—C3 = Colour-Word Interference Test—condition 3, inhibition. CWIT—C4 = Colour-Word Interference Test—condition 4, inhibition/switching. WM = working memory.**

not be as effective as singing, where a consolidation seems to occur.

For non-DMN, the RSFC of controls changed depending on regions and networks, which is consistent with other studies.[21,34,70] Singers showed lower RSFC with aging in non-DMN networks, including DAN and FPN, compared to the other two groups. Instrumentalists showed higher RSFC with aging in non-DMN networks compared to controls. This suggests that in non-DMN networks, singing and playing instruments protect RSFC from aging in different ways. The instrumentalists exhibited a similar RSFC aging pattern to that of the controls, but with a slower rate of change, which may suggest *preservation*, consistent with findings in the DMN.

In our previous study comparing amateur singers and non-singers (independent cohort), we found that RSFC increased with age in auditory, speech, and language networks, while it decreased in the default mode network (DMN) and dorsal attention network (DAN).[21] We observed positive aging effects in the left supramarginal gyrus, which is involved in various networks, including the auditory network. We found similar positive aging effects in the auditory network in the present study. However, the regions were not all the same between these two studies. The previous study exclusively compared singers to non-singers, which may have led to these discrepancies. Furthermore, a seed-based correlation approach was used, whereas here we employed a different method, potentially explaining the variations in findings. Studies comparing seed-based and

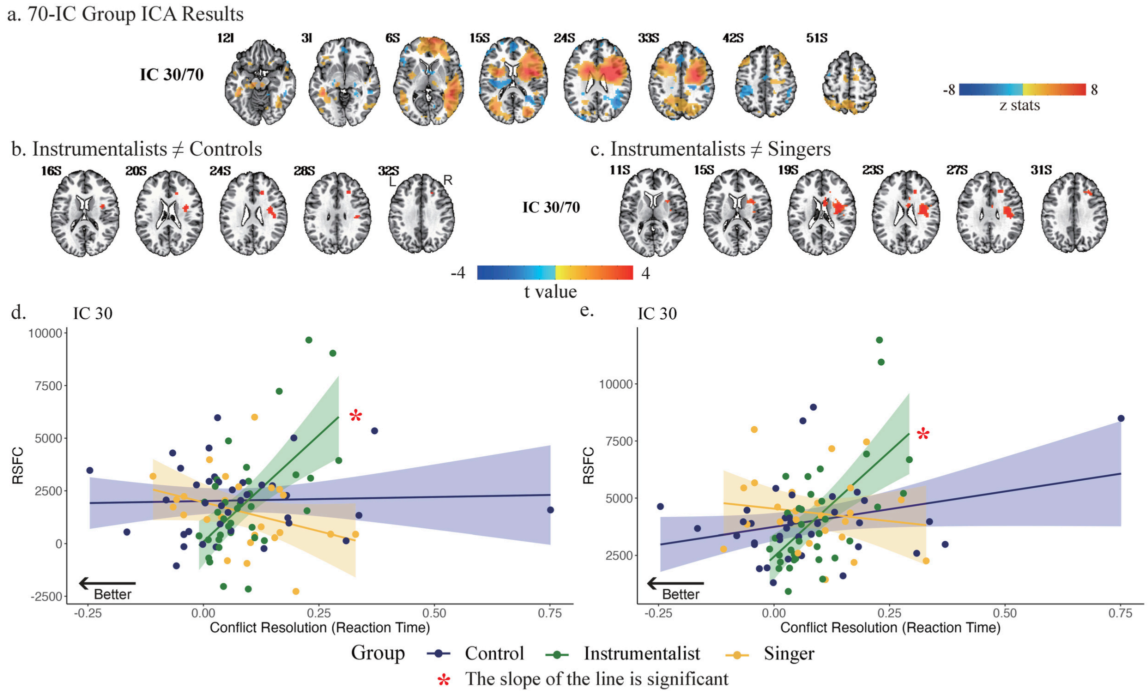


**Figure 8. a) IC#30 is shown on axial slices of the TT_N27 template in the MNI space. b) Group-by-CRRT interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and CRRT in IC#30. c) Group-by-CRRT interaction results show the regions where instrumentalists significantly differ from singers in the regression of average RSFC and CRRT in IC#30. d-e) The plots illustrate the interaction pattern. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines. The red star indicates that the slope of the linear regression line is significant (p<0.05).**

ICA methods have shown that both techniques can identify targeted networks,[74,75] but the results do not completely overlap.[76]

Changes in connectivity can be difficult to interpret from a functional perspective, however. Lower RSFC could reflect reduced within and between network communication and reduced integration, which can lead to cognitive impairment. Conversely, higher RSFC indicates more robust within and between network connections, suggesting enhanced communication and potentially better functional integration. Lower RSFC has been associated with neurodegenerative diseases, particularly Alzheimer's Disease (AD) and Parkinson's Disease (PD) for a review, see.[77] For AD patients, lower RSFC is observed in several networks, in particular the DMN, but also in the motor and prefrontal networks, compared to healthy controls.[78-80] For PD patients, in contrast, lower RSFC is predominantly observed in motor networks and the limbic system, which is related to emotion and memory, compared to healthy controls for a review, see.[77] Lower RSFC in the DMN is also reported in PD patients compared to healthy controls, but this is less common.[77] Hence, evidence from neurodegenerative diseases suggests that lower RSFC is associated with functional decline. Further, tau pathology and amyloid beta (Aβ) accumulations, which are features of AD and are present in other neurodegenerative disorders, are found within regions of the DMN, including the posterior cingulate cortex and medial temporal cortex, which exhibits reduced RSFC.[81-83]

Our findings of higher RSFC in both amateur singers and instrument players suggest that musical activities have a protective effect on the RSFC. This could contribute to delaying the onset of age-related decline in brain function. Musical activities, therefore, might provide a form of cognitive enrichment that supports brain health and resilience over time. This protective effect could be attributed to the complex cognitive, emotional and sensory-motor demands of musical activities, which may preserve or even enhance neural connectivity. Engaging in musical activities may offer a valuable strategy to mitigate some of the effects of aging on brain connectivity, reinforcing the idea that cognitive, emotional and sensory stimulation through music can play a significant role in maintaining brain health. However, longitudinal evidence is needed to fully understand the impact of musical activities on RSFC.

## RSFC AGING AND COGNITION

The analysis of RSFC-behaviour relationships revealed several patterns, supporting, to some extent, our third hypothesis that RSFC is related to cognitive performance and that this relationship varies with networks and regions. Multiple networks demonstrated interactions with cognitive performance, suggesting a broad interplay between functional connectivity at rest and cognitive performance. Addition-

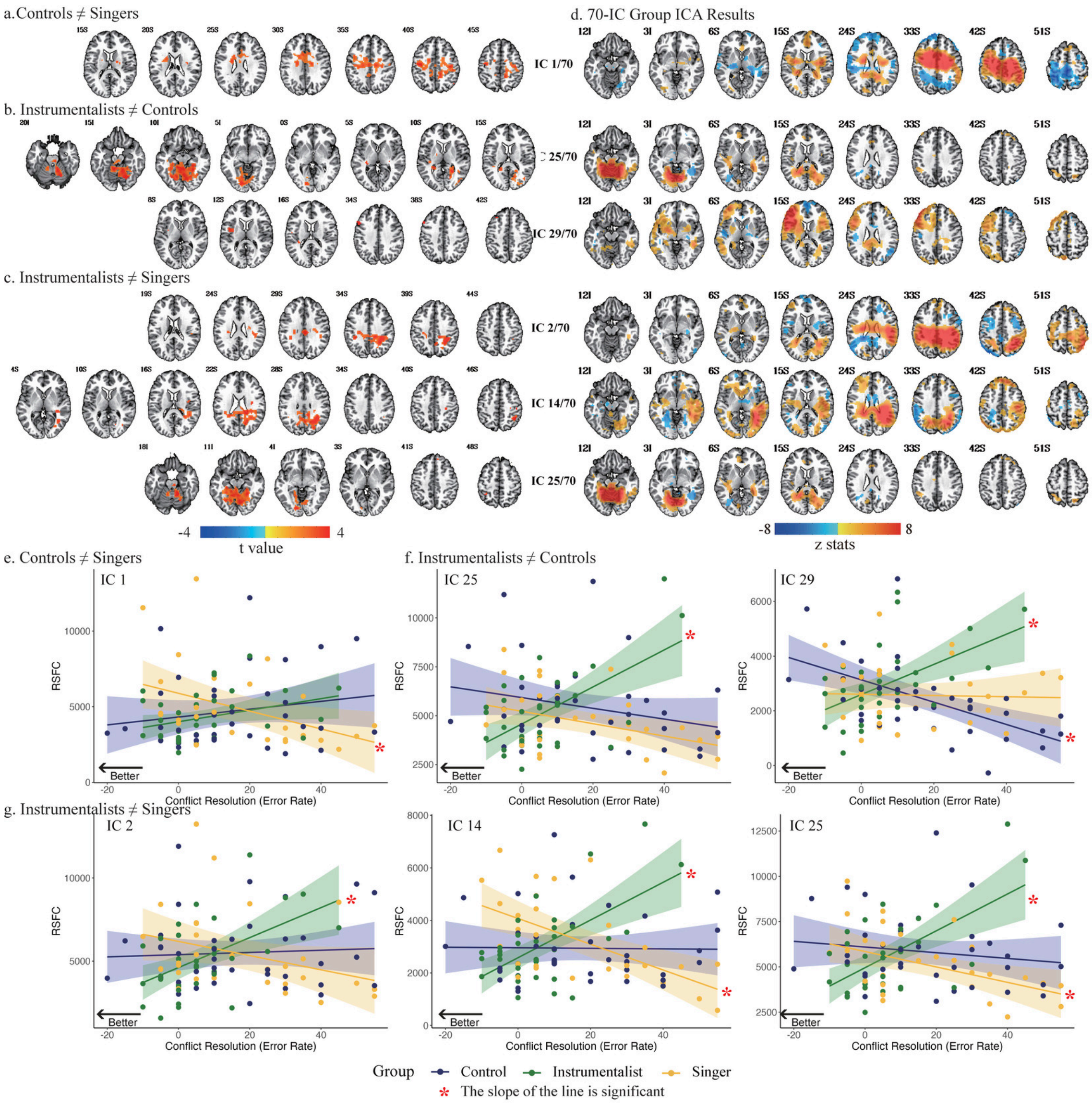


**Figure 9. a) The group-by-CRER interaction results show the regions where singers significantly differ from controls in the regression of average RSFC and CRER in IC #1. b) The group-by-CRER interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and CRER in IC #25 and #29. c) The group-by-CRER interaction results show the regions where instrumentalists significantly differ from singers in the regression of average RSFC and CRER in IC #2, #14, and #25. d) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. e-g) The plots illustrate the interaction patterns. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines. The red star indicates that the slope of the linear regression line is significant (p<0.05).**

ally, regions with significant group differences showed no clear lateralization, with neither hemisphere appearing more prominently involved than the other. The RSFC and behaviour analyses found that RSFC was related to cognitive performance to some extent.

For the TAiL, as detailed in our previous study,[1] instrumentalists had better accuracy and exhibited lesser age-related effects compared to controls and singers (Table 2). Consistent with these results, here instrumentalists showed RSFC patterns that differed from the other two groups . The instrumentalists showed an inverse relationship between RSFC and conflict resolution, with lower RSFC associated with better performance, especially in older and middle-aged subgroups. In contrast, in singers, higher RSFC was associated with better conflict resolution, especially in these same-age subgroups. In controls, only the older subgroup showed a positive association between RSFC and performance in IC#29 (bilateral SFG and left MFG) in the bilateral insula. Almost all the significant regions were in non-DMN regions. In our previous study,[21] we found that lower RSFC within the DMN was associated with better TAiL performance for both singers and controls. Since the DMN operates under a different mechanism compared to other networks—being deactivated to enhance task per-

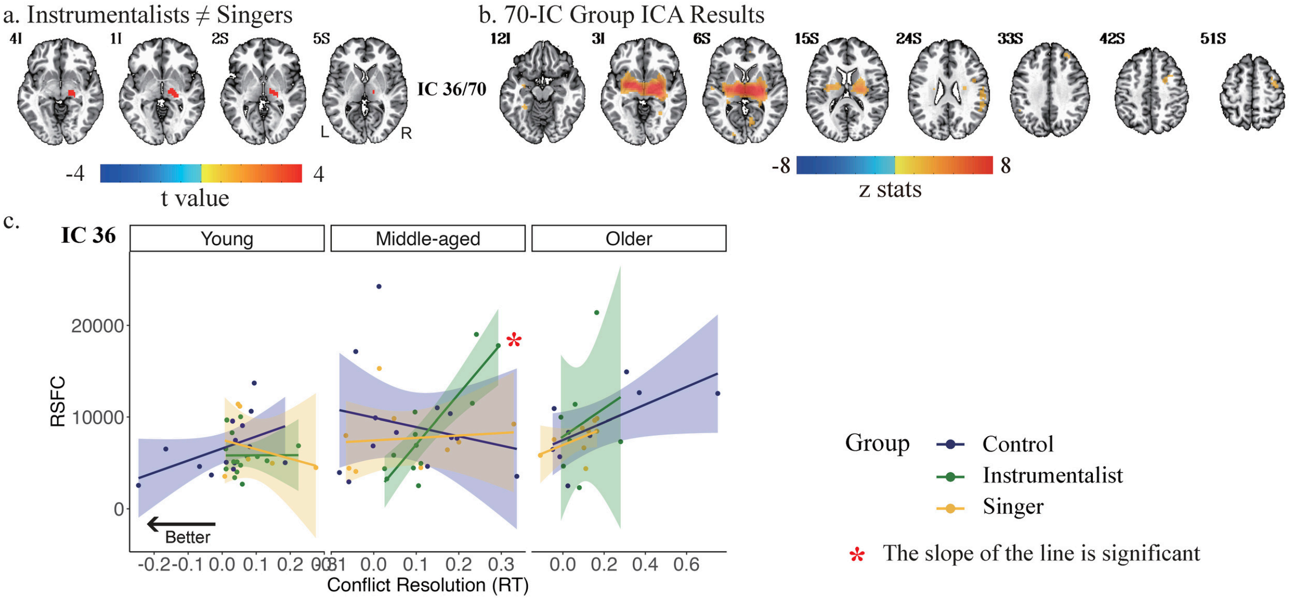


**Figure 10. a) The age-by-group-by-CRRT interaction results show the regions where instrumentalists significantly differ from singers in the regression of average RSFC and CRRT in IC #36. b) The age-by-group-by-CRRT interaction results show the regions where instrumentalists significantly differ from singers in the regression of the average RSFC and CRRT in IC #36. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space.**

formance while other networks are activated—our current findings of singers and controls are consistent with those from the previous study, but not for instrumentalists.

The CWIT evaluates selective attention and inhibition, which involves the prefrontal gyrus and the right cerebellum.[84] As detailed in our previous study,[1] musicians showed lower RT than controls in the C3 and C4 conditions. We thus expected to observe different RSFC patterns between musicians and controls, which was partially supported by the RSFC results. For the C3 condition, singers and instrumentalists did not show many significant differences. For the controls, especially the younger ones, higher RSFC was associated with better performance in non-DMN networks. Consistent with Okayasu et al.'s study, we found that regions that showed a relationship between RSFC and C3 performance were primarily located in the frontal and parietal gyri and there was also a small cluster in the right cerebellum.[84] Since these regions are involved in the CWIT task, the results suggest that the aging brain employs a preservation strategy, whereby it maintains cognitive functions by strengthening existing connections to sustain performance during aging. The other regions, such as the parietal and occipital gyri, are not typically related to the CWIT task, suggesting a *compensatory* mechanism to maintain performance in the C3 condition. For the C4 condition—the most challenging one—the findings were similar to those that were found for the TAiL. Like the controls in C3, singers exhibited higher RSFC that was associated with better performance primarily in the FPN, together with other regions that are not typically related to the task. Similar to the C3 task results, this result suggests that singers employ both preservation and compensation strategies, similar to the control group. In contrast, instrumentalists exhibited the opposite relationship whereby lower RSFC was associated with better performance. These results suggest that the mechanisms through which instrument-playing and singing affect RSFC in aging differ.

Turning now to auditory WM, which we measured using the digit span test, we found a few regions that showed a significant relationship between RSFC and auditory WM performance in the DMN and the visual networks. In DMN region (IC#39), for older controls, lower RSFC was associated with better WM performance. In non-DMN networks, specifically in the visual network, for young instrumentalists, lower RSFC was associated with better WM performance. Typically, WM engages FPN including the prefrontal, cingulate, and parietal cortices, as well as the cerebellum.[85] The reason might be that the visual network is not directly involved in the auditory WM task. The increased activation of the visual network could either represent compensation or reflect inefficiency in processing auditory WM tasks. However, the exact nature of the relationship between RSFC in the visual network and WM performance needs further investigation.

In sum, here we found group differences in RSFC that mainly aligned with the group differences in cognitive performance reported in a previous study from our group.[1] However, the results were not entirely consistent. For example, although we found several significant group differences in the relationship between RSFC and CRRT, no significant differences were reported in Joyal et al. Interestingly, for CRER, instrumentalists showed distinct RSFC patterns compared to singers and controls. This aligns with the cognitive finding that the age-related negative effects were more limited in instrumentalists compared to the other two groups. However, in the WM task, the RSFC differences we

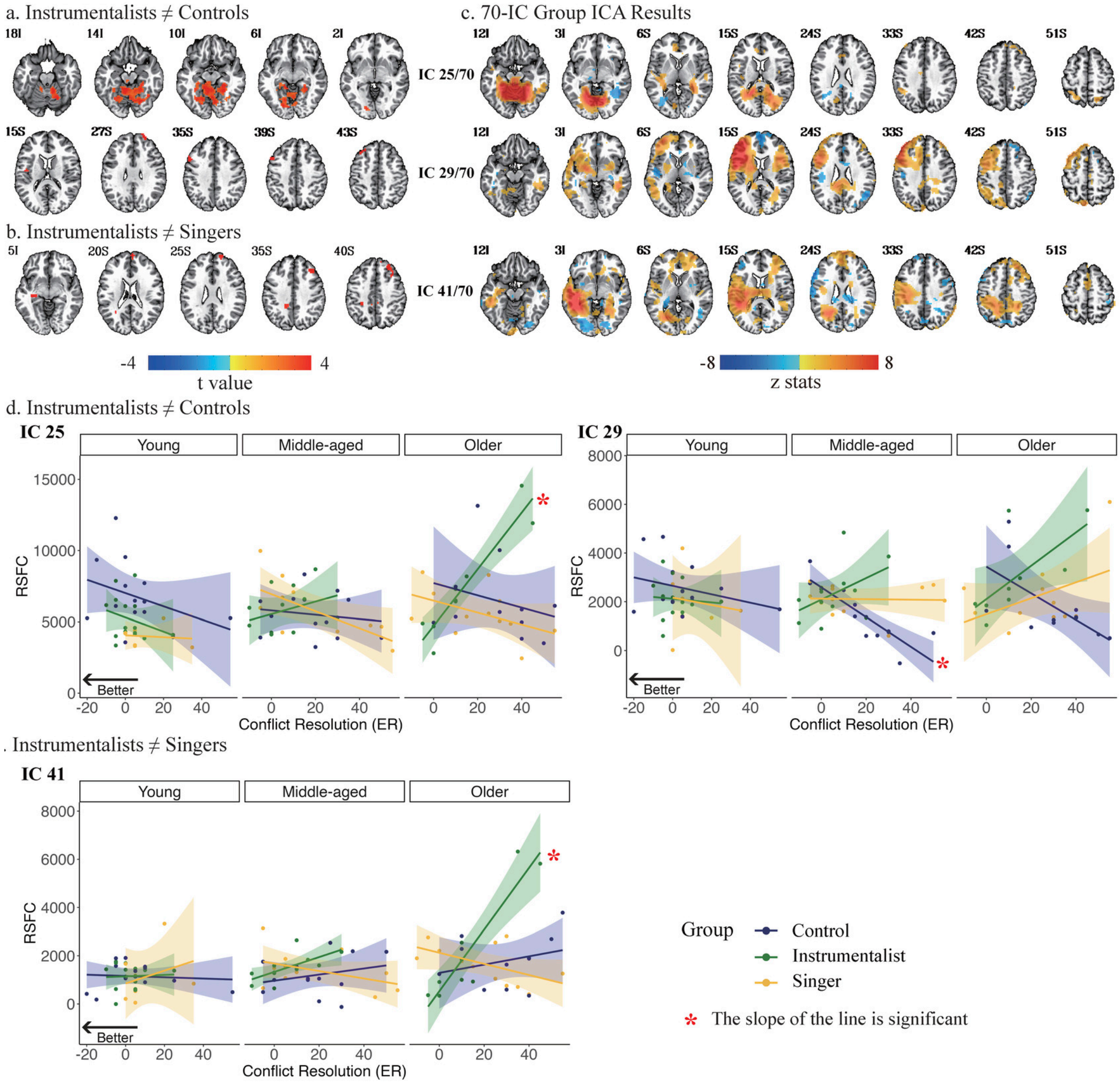


**Figure 11. a) The age-by-group-by-CRER interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and CRER in IC #25 and #29. b) The age-by-group-by-CRER interaction results show the regions where instrumentalists significantly differ from singers in the regression of average RSFC and CRER in IC #41. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. d-e) The plots illustrate the interaction patterns. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines. The red star indicates that the slope of the linear regression line is significant (p<0.05).**

identified did not match the cognitive findings from the previous paper. In terms of WM performance, we have previously shown that singers are at an advantage compared to controls and instrumentalists. Here we found no pattern of RSFC that was specific to singers. This discrepancy highlights the complexity of the relationship between RSFC and cognitive performance, suggesting that distinct mechanisms may underlie cognitive maintenance in singers and instrumentalists. While our findings reveal inconsistencies across tasks, they also suggest that musical training may shape brain aging differently depending on the type of practice. This aligns with our fourth hypothesis that singers and instrumentalists would exhibit distinct patterns of association with cognitive performance. Overall, our findings partially support this hypothesis. Singing and instrument-playing were associated with different patterns of results, with effects varying across different regions and networks. In general, for controls, similar to singers, higher RSFC was associated with better performance, with specific patterns depending on the task and age subgroup. This suggests that singing may affect brain aging through a *preservation* mechanism.[49] For instrumentalists, conversely, lower RSFC tended to be associated with better performance. This finding suggests that instrumentalists rely on a different mechanism to maintain their cognitive functions compared to younger singers and controls. As instrumentalists age, their brains may recruit additional neural resources to sustain cognitive function, suggestive of a *compensatory* mech-

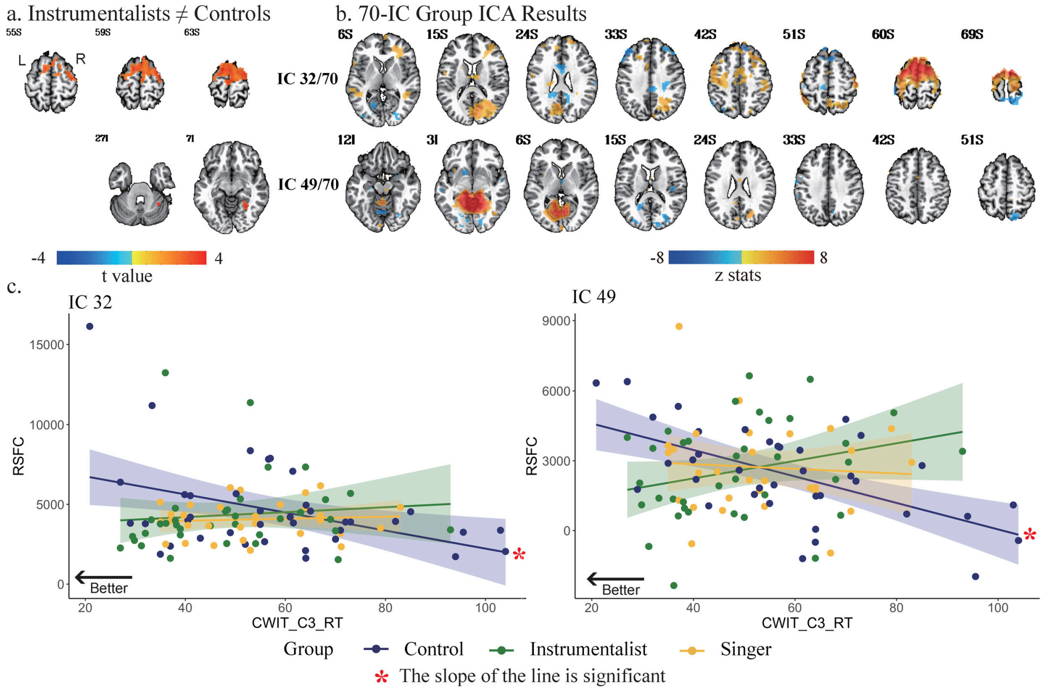


**Figure 12. a) The group-by-C3 interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and C3 in IC #32 and #49. b) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. c) The plots illustrate the interaction pattern. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines. The red star indicates that the slope of the linear regression line is significant (p<0.05).**

anism.[49] This result is consistent with the compensatory scaffolding or the Scaffolding Theory of Aging and Cognition (STAC-r) model.[49] Compensatory scaffolding involves the expansion of neural networks and the recruitment of additional neural resources to maintain cognitive functions as the brain ages, while also supporting cognitive processes through the reinforcement of alternative neural circuits. While instrumentalists exhibited lower RSFC associated with better performance, this does not imply a lack of neural resource recruitment for the task in the regions that showed a significant relationship between RSFC and cognition. Instead, it may suggest that they rely on more efficient neural communications or alternative regions or networks as compensation, which aligns with the STAC-r. Rather than simply expanding neural networks, instrumentalists might be optimizing their existing connections to preserve functions, while also enhancing cognitive function through the strategic use of alternative networks to compensate.

## A FRAMEWORK FOR THE STUDY OF RSFC IN NEUROCOGNITIVE AGING

Here we propose a framework for understanding the relationship between healthy RSFC aging and cognition, shown in Figure 17. Our basic assumptions are that, consistent with the literature, higher RSFC is linked to better network functionality, particularly within individual brain networks. Lower RSFC, in contrast, is often associated to poorer behavioural performance in patients with neurodegenerative diseases, including AD.[77] In our model, we presume that higher RSFC is beneficial, whereas lower RSFC is considered detrimental. Our brain-behaviour analysis revealed three patterns of associations:

1. higher RSFC associated with better cognitive performance, and, conversely, lower RSFC associated with worse cognitive performance;
2. lower RSFC associated with better cognitive performance, and, conversely, higher RSFC associated with worse cognitive performance; and
3. no significant association.

The first pattern - a higher RSFC associated with better performance, interpretation varies as a function of the region or network expressing this relationship. If the region is part of task-related network, it suggests that the brain maintains performance through a *preservation* mechanism. In contrast, if the region is outside the task-related network, it suggests that the brain recruits additional neural resources to *compensate* for senescence and sustain cognitive performance. The second pattern -a higher RSFC associated with worse performance—suggests *dedifferentiation*, which is the process by which specialized neural networks become less distinct and more generalized in their activity.[86] When lower RSFC is associated with better performance, if the region is within the task-related network, it suggests that the network demonstrates increased neural efficiency. If the significant region is outside the task-related network, the interpretation is less straightforward. The recruitment of additional neural resources may indicate dedifferentiation. However, the phenomenon of lower

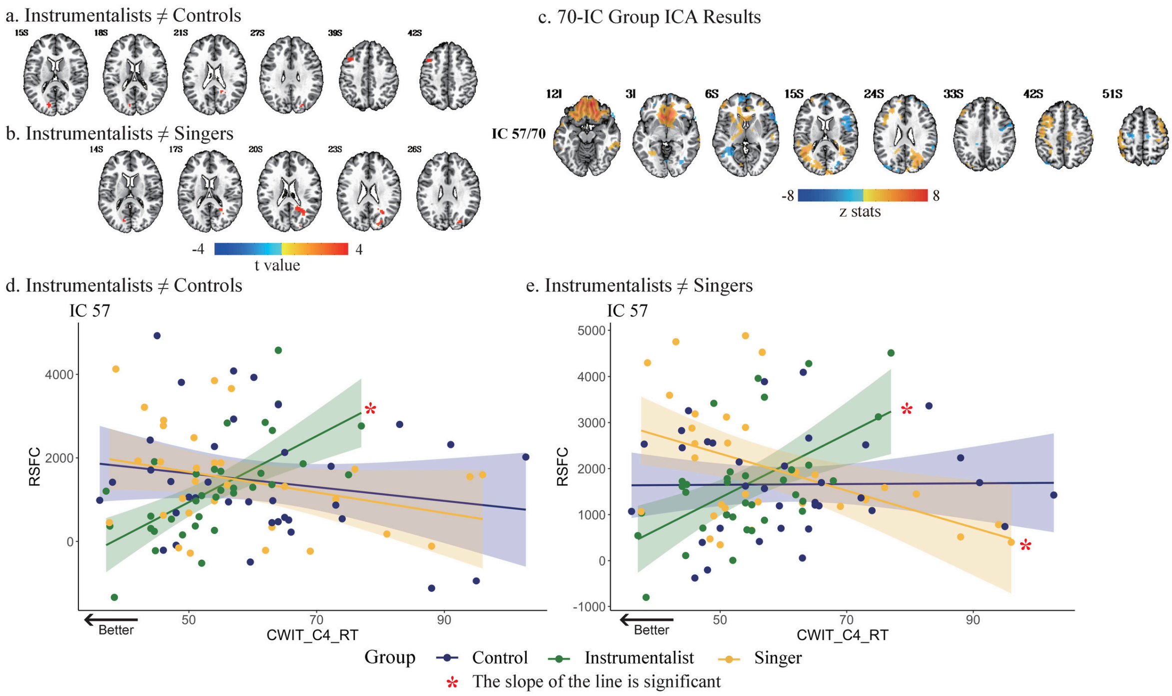


**Figure 13. a) The group-by-C4 interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and C4 in IC #57. b) The group-by-C4 interaction results show the regions where instrumentalists significantly differ from singers in the regression of average RSFC and C4 in IC #57. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. d-e) The plots illustrate the interaction patterns. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines. The red star indicates that the slope of the linear regression line is significant (p<0.05).**

RSFC being associated with better performance remains difficult to explain. The third pattern indicates that the relationships between RSFC and behaviour have no significant relation to specific cognitive task performance. This model applies to non-DMN networks, as the DMN operates under an opposing mechanism, and vice versa, this model can be applied to the DMN by reversing the direction of the RSFC trend.

### STRENGTHS AND LIMITATIONS

To our knowledge, this is the first study to compare RSFC aging in amateur singers and instrumentalists. One of the main strengths of this work is that unlike previous studies, we included an active control group: each person in that group was engaged in at least one regular cognitive-motor activity with a comparable amount of practice time as the musicians. Importantly, our three groups did not differ in age, education, cognition, self-reported health, and activity practice experience. Therefore, differences in RSFC and cognition among groups likely reflect an association with *musical* activities specifically rather than being associated with other aspects of musical activities, including their social component.

The main limitation of this study is that there was no passive control group. Future studies should strive to investigate the association between musical experience history, RSFC and behaviour. In our previous work, we showed that the impacts of musical practice on cognition were dose-dependent, that is, not all musicians exhibited the same benefits, as those were tied to their training and amount of practice.[87] However, in the detailed analyses of the cognitive performance of our groups that we previously published,[1] only minimal effects of experience were observed. Given the complexity of RSFC aging and its relationship with cognition, we therefore chose not to include experience as a variable in this paper.

## CONCLUSIONS

Our findings suggest that connectivity is generally lower with aging, though some regions exhibit increased connectivity with increasing age. Musical activities is associated with neuroplastic changes within several functional systems. For singers, higher RSFC is associated with preserved cognitive function, whereas for instrumentalists, lower RSFC is linked to better performance, suggesting distinct neural mechanisms at play. While further research is needed to elucidate the underlying neurobiological processes and age-related changes in resting-state activity, our results align with the notion that amateur musical practice can drive experience-dependent brain reorganization across the lifespan.

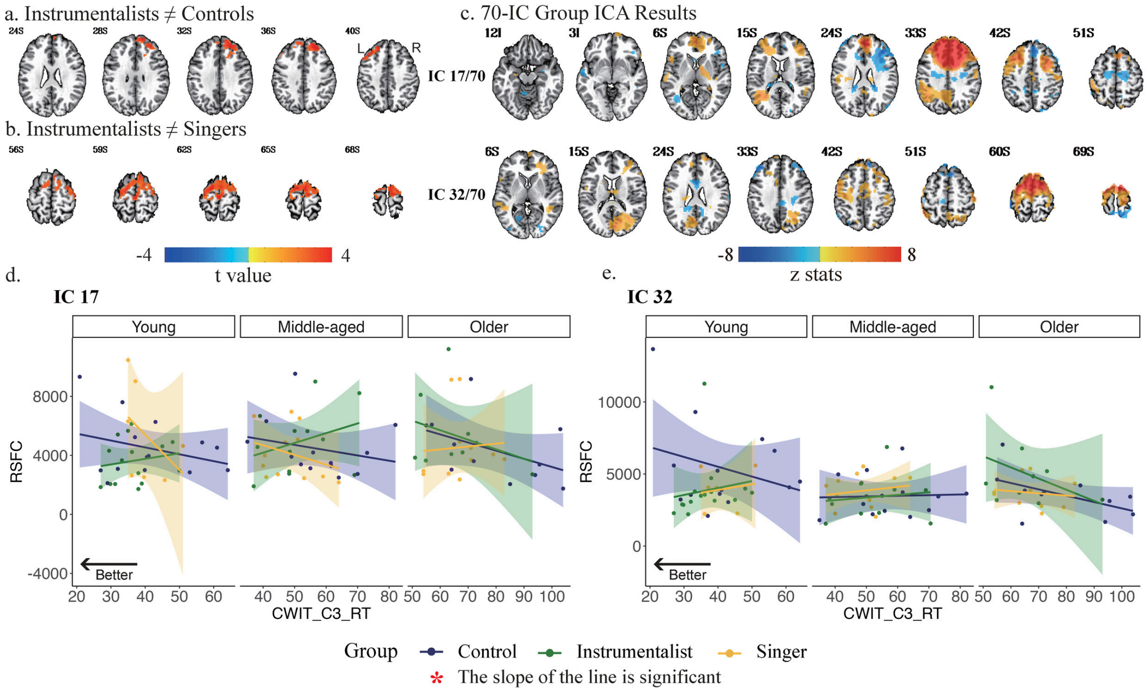


**Figure 14. a) The age-by-group-by-C3 interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and C3 in IC #17. b) The significant regions that showed differences between instrumentalists and singers in IC#32. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. d-e) The plots illustrate the interaction patterns in IC #17 and #32. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines.**

## DATA AND CODE AVAILABILITY

The raw datasets generated during the current study cannot be made publicly available because participants did not consent to public data sharing at the time. However, the group MRI data will be available on Borealis, the Canadian Dataverse Repository (https://doi.org/10.5683/SP3/PCGLCP) upon publication, along with aggregated behavioural data.

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## CONFLICTS OF INTEREST

The authors declare that they have no competeing interests.

## SUPPLEMENTARY MATERIAL

The supplementary material of this article includes:

Supplementary material S1, Test of Attention in Listening (TAiL) Scores Calculation

Supplementary material S2, Group ICA results.

Supplementary material S3, Age-by-group regression results.

Supplementary material S4, Group-by-behaviour results.

Supplementary material S5, Age-by-group-by-behaviour results.

Supplementary material S6, Full summary matrices.

Submitted: October 23, 2024 CDT. Accepted: February 20, 2025 CDT.

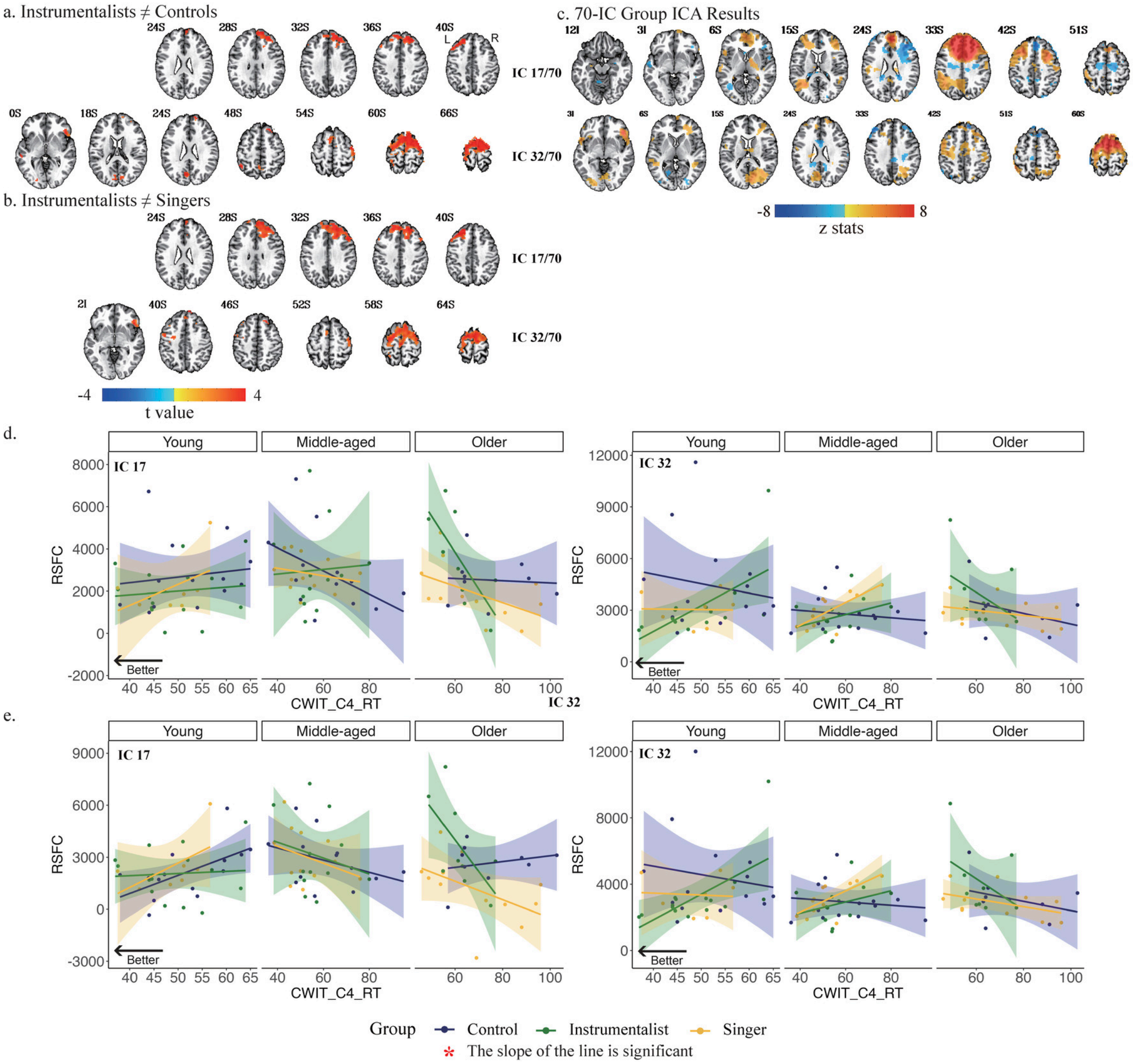


**Figure 15. a) The age-by-group-by-C4 interaction results show the regions where instrumentalists significantly differ from controls in the regression of average RSFC and C4 in IC #17 and #32. b) The age-by-group-by-C4 interaction results show the regions where instrumentalists significantly differ from singers in the regression of average RSFC and C4 in IC #17 and #32. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. d-e) The plots illustrate the interaction patterns. Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines.**

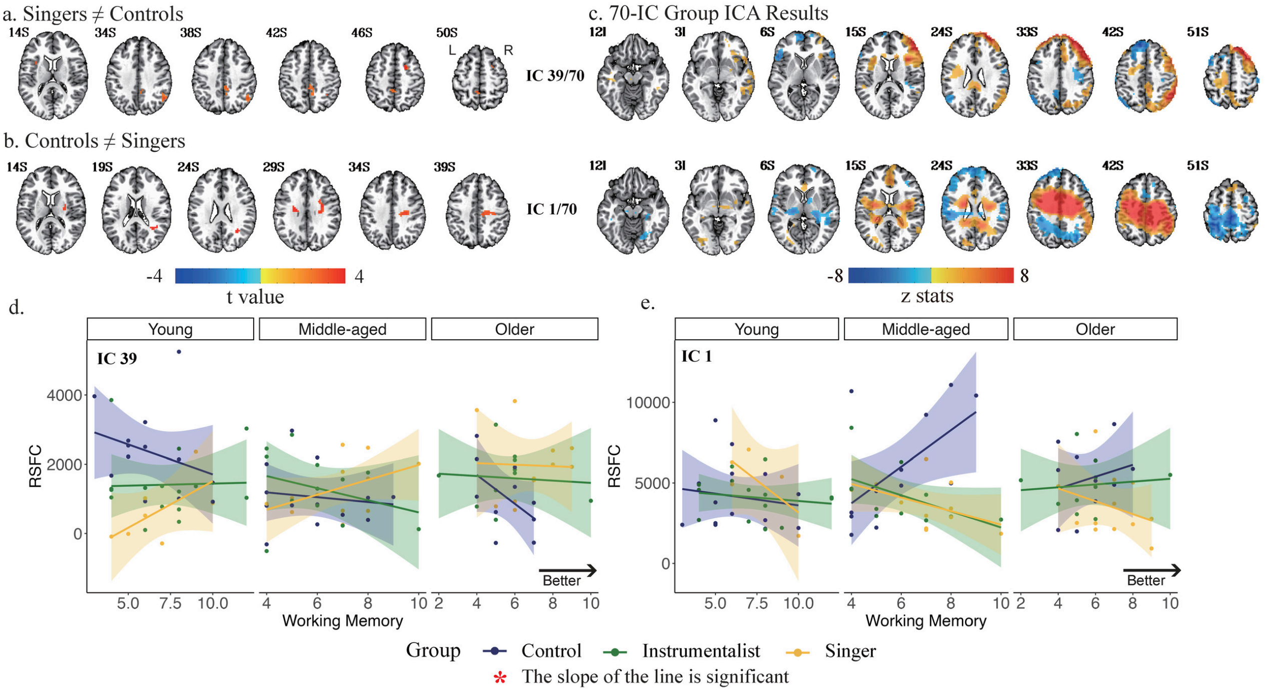


**Figure 16. a-b) The regions where singers are significantly different from controls in age-by-group-by-WM 3-way interaction. In (a), the region where singers have steeper slopes than controls are shown. In (b) the region where singers have less steep slopes than controls are shown. c) The corresponding networks are shown on axial slices of the TT_N27 template in the MNI space. d-e) The plots illustrate the interaction patterns in IC #39 (d) and IC #1 (e). Each dot represents one participant. The shadows around the lines represent the confidence intervals of the regression lines.**

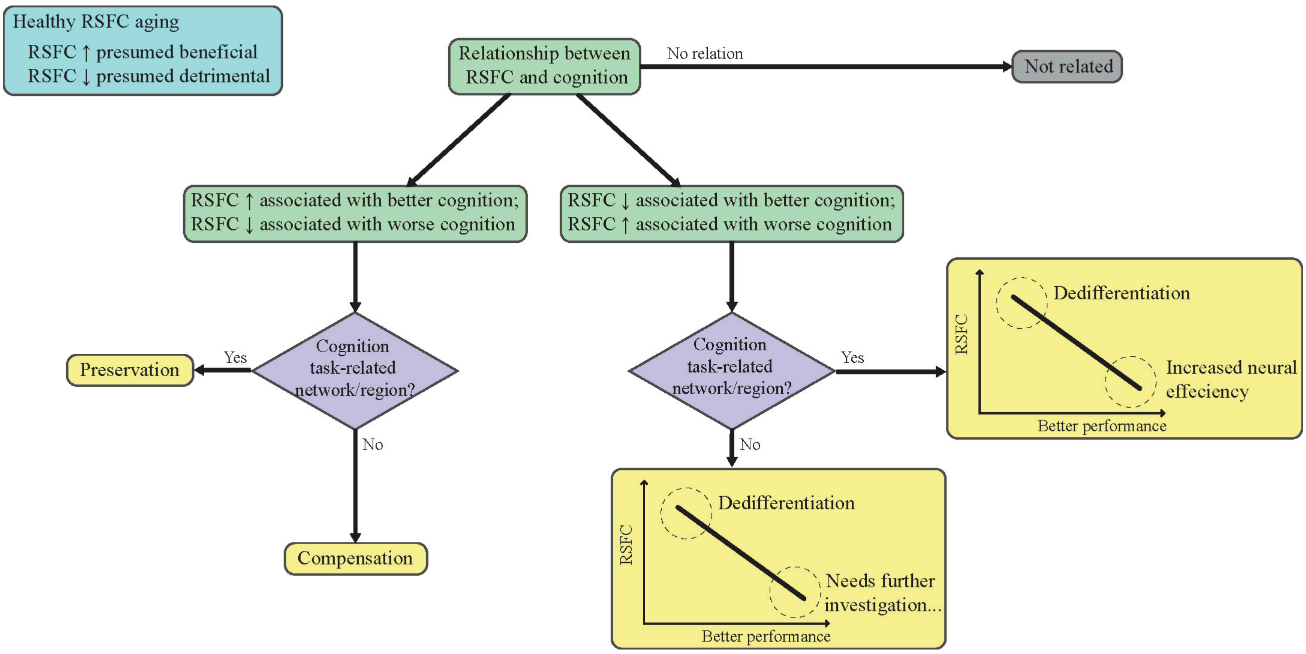


**Figure 17. The framework for the interpretation of RSFC patterns in neurocognitive aging.**

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# SUPPLEMENTARY MATERIALS

## Supplementary Materials

Download: https://apertureneuro.org/article/130919-aging-of-resting-state-functional-connectivity-in-amateur-singers-instrumentalists-and-controls/attachment/268594.docx?auth_token=spxTD0deXd9RtK-BvsGH