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Qigong exercise enhances cognitive functions in the elderly via an interleukin-6-hippocampus pathway: A randomized active-controlled trial

Di Qi [a,b,1], Nichol M.L. Wong [a,b,1], Robin Shao [a,b,1], Idy S.C. Man [a,b], Clive H.Y. Wong [a,b], Lai Ping Yuen [a,*], Chetwyn C.H. Chan [c,*], Tatia M.C. Lee [a,b,d,*]

[a] State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Hong Kong
[b] Laboratory of Neuropsychology and Human Neuroscience, The University of Hong Kong, Hong Kong
[c] Applied Cognitive Neuroscience Laboratory, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
[d] Institute of Clinical Neuropsychology, The University of Hong Kong, Hong Kong

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ABSTRACT

Background: Evidence has suggested that exercise protects against cognitive decline in aging, but the recent lockdown measures associated with the COVID-19 pandemic have limited the opportunity for outdoor exercise. Herein we tested the effects of an indoor exercise, Qigong, on neurocognitive functioning as well as its potential neuro-immune pathway.

Methods: We conducted a 12-week randomized active-controlled trial with two study arms in cognitively healthy older people. We applied *Wu Xing Ping Heng Gong* (Qigong), which was designed by an experienced Daoist Qigong master, to the experimental group, whereas we applied the physical stretching exercise to the control group. The Qigong exercise consisted of a range of movements involving the stretching of arms and legs, the turning of the torso, and relaxing, which would follow the fundamental principles of Daoism and traditional Chinese medicine (e.g., Qi). We measured aging-sensitive neurocognitive abilities, serum interleukin-6 (IL-6) levels, and brain structural volumes in the experimental (Qigong, n = 22) and control groups (stretching, n = 26) before and after the 12-week training.

Results: We observed that Qigong caused significant improvement in processing speed ($t(46) = 2.03$, $p = 0.048$) and sustained attention ($t(46) = -2.34$, $p = 0.023$), increased hippocampal volume ($t(41) = 3.94$, $p < 0.001$), and reduced peripheral IL-6 levels ($t(46) = -3.17$, $p = 0.003$). Moreover, following Qigong training, greater reduction of peripheral IL-6 levels was associated with a greater increase of processing speed performance (bootstrapping CI: [0.16, 3.30]) and a more significant training-induced effect of hippocampal volume on the improvement in sustained attention (bootstrapping CI: [-0.35, −0.004]).

Conclusion: Overall, these findings offer significant insight into the mechanistic role of peripheral IL-6—and its intricate interplay with neural processes—in the beneficial neurocognitive effects of Qigong. The findings have profound implications for early identification and intervention of older individuals vulnerable to cognitive decline, focusing on the neuro-immune pathway.

The trial was registered at clinicaltrials.gov (identifier: NCT04641429).

1. Introduction

Population aging is accelerating globally (Khan, 2019). Associated with the aging is the risk of neurocognitive decline (Calandri et al., 2020) affecting independent living (Harada et al., 2013) and causing significant burdens on caregivers and society. Among the aging populations, declines in processing speed, working memory (Grady, 2012), and sustained attention (Fortenbaugh et al., 2015) are the most noteworthy. These cognitive functions form the foundation of the higher order executive capacities (Gao et al., 2020; Fortenbaugh et al., 2017; Hill et al., 2016; Redick et al., 2015) and therefore account for a considerable part of the functional deficit in older people (Deary et al., 2009; Kim, 2016; Reinhart and Nguyen, 2019). However, the underlying neurobiological basis is still elusive, and there is no known

* Corresponding authors.
E-mail addresses: neuropsy@hku.hk (L.P. Yuen), Chetwyn.Chan@polyu.edu.hk (C.C.H. Chan), tmclee@hku.hk (T.M.C. Lee).
[1] These authors contributed equally to this work

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pharmacotherapy for reversing this aging-related decline in cognitive functioning.

Converging evidence has suggested that this cognitive decline is partly explained by neurodegeneration (Mu and Gage, 2011) and impaired synaptic plasticity (VanGuilder et al., 2011) in key brain areas responsible for cognitive functions (Axmacher et al., 2010; Barbey et al., 2013; Papp et al., 2014; Nathan et al., 2017; Radel et al., 2018; Rosano et al., 2010; Rosenberg et al., 2016). For example, the dorsolateral prefrontal cortex (DLPFC) (Hedden and Gabrieli, 2004) and hippocampus (Erickson et al., 2011) have been consistently reported to undergo significant reduction of gray matter volume (GMV) in older adults in relation to their cognitive decline (Cabeza et al., 2018; Grady, 2012; Shaw et al., 2016). Furthermore, it has been speculated that neural changes in aging are related to the neuro-immune interaction. Preliminary evidence has suggested that aging increases the inflammatory responses in the brain (Chen et al., 2008) and that inflammatory mechanisms and immune responses (Michaud et al., 2013; Woods et al., 2012), especially interleukin-6 (IL-6) (Economos et al., 2013), might modulate the pathogenesis of cognitive dysfunction (Trapero and Cauli, 2014). Circulating IL-6 levels have shown to be inversely related to processing speed performance (Bettcher et al., 2014; Bott et al., 2017). Thus, increasing concentrations of IL-6 are postulated to trigger the central inflammatory changes through several immune-to-brain pathways (Engler et al., 2017), in turn leading to neurodegeneration and impaired cognitive function (Yirmiya and Goshen, 2011). However, the question of whether the neurobiological underpinnings and the immune response could be remediated to protect older people's cognitive functioning remains unanswered. Studies fully interrogating the neuro-immune interaction involved in aging-related cognitive decline may be especially important in developing strategies to preserve cognitive functioning in older people (Yirmiya and Goshen, 2011).

Currently, there is no known pharmacotherapy for cognitive decline, but various lifestyle strategies have been proposed. Among them, evidence has accumulated on the beneficial neurocognitive effects of exercise that protect against cognitive decline (Cotman et al., 2007; Erickson et al., 2011). However, the physical conditions of older people (Chang et al., 2011), as well as the recent lockdown measures for protection against the COVID-19 pandemic, have greatly limited older people's opportunities to engage in outdoor exercise. Identifying an indoor exercise regime that is slow paced and low in intensity and that can protect against cognitive decline would be extremely applicable. Here, we studied a Daoist's *Wu Xing Ping Heng Gong* (Qigong) (Chan et al., 2013; Ho et al., 2012), which is slow and mild and has evidence of beneficial effects for people suffering from chronic fatigue syndrome (Chan et al., 2013; Ho et al., 2012) .

In this study, we incorporated cognitive, immunological, and neuroimaging measures to quantify Qigong's anti-aging effects and its potential neurobiological mechanisms. We hypothesized that Qigong training, relative to the control condition of physical stretching training, would improve the performance of processing speed, working memory, and sustained attention. Furthermore, on the basis of the literature, we speculated that Qigong would reduce the peripheral IL-6 levels and increase the volumes of the DLPFC and hippocampus, which would mediate the beneficial training effect on the cognitive performance in older people. Finally, we explored the potential neuro-immune interplay in determining the beneficial neurocognitive effect of Qigong.

2. Materials and Methods

2.1. Participants

The Institutional Review Board of the University of Hong Kong and Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB) provided ethical approval. The trial was registered at clinicaltrials.gov (identifier: NCT04641429). Participants were recruited from local communities through telephone calls, online posters, and word of mouth. We conducted an initial screening for all potential participants through phone by members of the research team using a standardized screening script. Those who passed the initial telephone screening were invited to visit our laboratory (Institute of Clinical Neuropsychology at Sassoon Road, Hong Kong, China) in order to participate in further screening and ensure they fulfilled our study criteria (see below). If eligible, they were invited to complete the neuropsychological assessments and were informed about the training schedule.

The recruitment criteria included the following: 1) age of 50 years old or above; 2) formal education of 6 years or above; 3) normal or corrected-to-normal vision and hearing; 4) right-handedness as measured by the Edinburgh Handedness Inventory (EHI) Short Form (Veale, 2014); 5) score of 22 or higher on the Montreal Cognitive Assessment Hong Kong version (HK-MoCA) (Wong et al., 2009); 6) no elevated anxiety or depressive features ($\geq$11 scores) as measured by the Hospital Anxiety and Depression Scale (HADS) (Snaith, 2003); 7) no current—or history of—neurological or psychological conditions or alcohol abuse that could affect cognitive function; 8) no regular practice of any forms of qigong, meditation, or another similar exercise within 6 months of this study; and 9) no regular practice of moderate- to high-intensity physical exercise within 6 months of this study. The operational definition of "regular practice" was 1) $\geq$ 30 min per time, 2) $\geq$ 3 times per week, and 3) the duration $\geq$ 3 months.

2.2. Study design and procedure

We conducted this study at the Institute of Clinical Neuropsychology at Sassoon Road, Hong Kong, China (September 2019 to March 2020). We first investigated whether Qigong was beneficial to the processing speed, sustained attention, and working memory of older people. We then examined how the effects of Qigong, if any, were mediated through longitudinal changes in the inflammatory responses and the structural brain changes resulting from the Qigong training. Since it has been widely acknowledged that IL-6 plays a key role in the immune-brain interaction and cognitive dysfunction (Trapero and Cauli, 2014), as well as in the anti-inflammatory effects of exercise (Gleeson et al., 2011), we specifically targeted the IL-6 as the inflammation maker tested in this study. We adopted a randomized active-controlled intervention trial design, with half of the recruited older people receiving the 12-week Qigong training and the other half receiving the physical stretching exercise training. We measured the three aforementioned cognitive abilities, the serum IL-6 levels, and the structural brain volumes of the older people participating in our studies, both before and after the 12-week training protocol. The participants were allocated to either a Qigong or a stretching exercise training group in a pseudo-randomized order, administered by one of the authors (D.Q.), and based on the recruitment orders and their availability. Prior to the first training session, participants had no knowledge of which type of training they were going to receive. After screening, all of the eligible participants underwent cognitive assessments and structural brain-imaging scanning. Screening, pre-training cognitive assessments, and scanning took place within 5 weeks of the start of training. All of the participants completed blood sampling between 12 and 2p.m. on the first training day, just before the training session. Participants then received 12 weeks of either Qigong or stretching exercise training, as detailed below. Within 5 weeks following the completion of training, participants underwent post-training cognitive assessments, structural brain-imaging scanning, and blood sampling. The cognitive assessments and brain-imaging scanning followed the same protocols as the pre-training assessments. The blood sampling was always drawn between 12 and 2p.m., in accordance with the time period of the pre-training blood drawing. We recorded global satisfaction and adversity ratings after training for each group. Details of the study flow are reported in the CONSORT Flow Diagram.

2.3. Interventions

Participants in the experimental group received group-based *Wu Xing Ping Heng Gong* (Qigong) exercise training, which was designed and taught by an experienced Daoist Qigong master (Yuen L. P.) with a traditional Chinese medicine background and >30 years of experience in Qigong practice. It is a mind–body exercise consisting of warm-up movement and nine movements involving the stretching of arms and legs, the turning of the torso, and relaxing (Chan et al., 2013). Similar to other forms of qigong exercise such as tai chi and Baduanjin, *Wu Xing Ping Heng Gong* (Qigong) is a slow-pace and low-intensity practice that is suitable for older people to engage in regularly. Our lab has a well-established protocol for the *Wu Xing Ping Heng Gong* (Qigong) exercise, and we previously showed the beneficial effects of this exercise in alleviating chronic fatigue symptoms and improving mental function (Chan et al., 2013; Ho et al., 2012). Participants in the control group received group-based stretching exercise training conducted by a retired teacher of physical education with >30 years of experience in teaching. The 12-week intervention consisted of 18 sessions of Qigong or stretching exercise for the participants. We scheduled two sessions for the first 6 weeks and one session for the last 6 weeks with each session lasting 2 h, from 2 to 4p.m. Each session of training was led by the instructor and three assistants (2 females and 1 male). The first 10 sessions of each training consisted of two parts, with the first half being a theoretical lecture and the second half involving exercise training. For the experimental group, the lecture contents of the first 10 sessions were introductions to the basic theories of traditional Chinese medicine and Qigong and precautions in Qigong exercise (Chan et al., 2013; Ho et al., 2012). For the control group, the first 10 sessions' lectures were about life in aging, including potential hearing loss, diets and nutrition, brain health and sleep, and knowledge and precautions related to stretching and warming up. The last eight sessions of each training group comprised only each type of exercise practice. During each of the 18 sessions, the instructors also answered questions and concerns raised by the participants about each exercise practice. Participants were instructed to practice for at least 30 min every day on the days without a training session and to keep a personal log for daily practices.

2.4. Cognitive assessment

Three well-trained members from our research team administered the cognitive assessments to all of the participants in both groups, and the allocation was random. The group identity of the participants was not disclosed to these assessors. We conducted the cognitive assessments following standard procedures.

Processing speed. The Symbol Digit Modalities Test (SDMT; A. (Smith, 1982) is a common tool to measure processing speed. In this test, participants were given 90 s to match specific numbers with corresponding geometric figures. This test has written and oral forms. We employed the oral form in this study, as it is less confounded by motor speed. The number of correct responses within 90 s was recorded as the SDMT score, and higher scores indicate better performance.

Sustained attention. The Conners Continuous Performance Test 3rd edition (CPT 3; (Conners et al., 2003) is a well-known and frequently used measure of sustained attention. Participants were required to respond as soon as possible whenever a letter appears on the screen, except when the letter "X" appears, in which case participants had to withhold their response. The total task consists of 360 trials and lasts for 14 min. We adopted detectability (d')—the sensitivity measurement of CPT that measures how well a respondent discriminates non-targets (i.e., the letter X) from targets (i.e., all other letters)—as the main index of sustained attention in this study (Chen et al., 2004; Rosenberg et al., 2016). Higher scores of d' indicate worse performance (i.e., poorer discrimination) as the test is reverse-scored.

Working memory. We used the Digit Span Backwards (DS-Backwards) of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) (Wechsler, 1997) to measure working memory. Participants first listened to a sequence of numerical digits at the rate of one per second and were then required to repeat the sequence in reverse order. The number of digit sequences increased with correct answers, and the longest number of digits that can be accurately recalled was recorded as the working memory capacity. Therefore, a higher score indicates better performance.

2.5. Serum interleukin-6 (IL-6) levels

Registered nurses drew 10 ml of peripheral venous blood from each participant at both the pre- and post-assessment and stored the samples in BD Vacutainer SST tubes (367955, BD Diagnostics, Franklin Lakes NJ, USA). After clotting, we separated the serum samples from whole blood samples by centrifugation at 3,000 g for 5 min at 18 °C and then stored them in four 1.5-ml aliquots at −80 °C. We measured serum IL-6 by commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (HS600C, R&D Systems, USA). We assayed all samples in duplicate and then log-transformed participants' IL-6 levels to obtain the normal distribution for analysis.

2.6. Structural imaging data acquisition and processing

We acquired participants' T1-weighted high-resolution anatomical scans with a 3 T Phillips scanner equipped with an 32-channel SENSE head coil, using a magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (137 contiguous sagittal slices, repetition time/echo time/flip angle = 6.6 ms/3.1 ms/9°, matrix = 256 × 200 mm^2, field of view (FOV) = 256 × 240 × 164 mm^3, voxel size = 1 × 1 × 1.2 mm^3). We processed and analyzed the data using the Computational Anatomy Toolbox (CAT12) (http://dbm.neuro.uni-jena.de/cat/) and Statistical Parametric Mapping (SPM12) (Wellcome Department of Cognitive Neurology, London, UK). We first affine-registered the reoriented, cropped, and bias-corrected T1 images using the customized age-specific tissue probability map generated with the CerebroMatic toolbox (Wilke et al.., 2017). We entered this customized tissue probability map as the prior probability for each of the six tissue classes used for the initial affine registration. Subsequently, we segmented and normalized the images on the basis of the Diffeomorphic-Anatomical-Registration-Through-Exponentiated-Lie-Algebra (DARTEL) procedure (Ashburner, 2007) using the customized unbiased normalized DARTEL template based on all of the participants' images, following the longitudinal registration pipeline of CAT12 (see Supplementary Material). We then smoothed the resulting modulated normalized gray matter (GM) images with an 8-mm full-width-at-half-maximum (FWHM) Gaussian kernel. We performed an automated image quality check following segmentation to ensure high-weighted average image quality for all participants.

We analyzed the smoothed, normalized, and modulated GM images at pre-training and GM *change* images (post-training minus pre-training) using the two-sample *t*-test in SPM, with the group (experimental vs. control) as the independent variable and total intracranial volume (TIV), age, and sex as covariates of no interest. We focused specifically on our a priori hypothesized regions of interest (ROIs), the bilateral DLPFC and the hippocampus. We defined the ROIs using the Wake Forest University (WFU) Pickatlas software and the Automated Anatomical Labelling (AAL) template. We first compared the two groups' GMV of the two ROIs at pre-training to ensure the GMV was matched at baseline. We then specifically examined the group differences in the changes of the GMV (ΔGMV) in the ROIs (i.e., group × time effect for GMV). We determined the statistical thresholds for all of the resulting t-contrast maps using the nonparametric method of threshold-free cluster enhancement (TFCE) (S. M. (Smith and Nichols, 2009) (see Supplementary Material). We evaluated the group differences of GMV at baseline and ΔGMV with 5,000 TFCE permutations and the family-wise error (FWE) corrected at $p < 0.05$ within each ROI. For the baseline comparisons, we tested whether

there were regions within the ROIs that showed significant group differences. We also extracted the mean GMV of each ROI and directly compared the GMVs between the two groups (see below). For the group differences of ΔGMV, we extracted the mean values of ΔGMV and GMV at both pre- and post-training from the significant cluster(s) within each ROI for subsequent analyses. For exploration, we performed whole-brain voxel-wise analysis to examine whether other brain regions' ΔGMV also showed a significant group difference (i.e., group × time effect for GMV).

2.7. Statistical analysis

We performed all statistical analyses using SPSS version 26 (IBM Corp.). We examined the between-group differences in the demographic characteristics; scores on the SDMT, DS-Backwards and d'-CPT, and serum IL-6 level at the pre-training assessment; and the total and mean self-practice duration at home during the training period using independent-samples *t*-tests. We examined the sex difference between the groups using the chi-square test. For each cognitive measure and serum IL-6 level, we conducted a 2 (Group) × 2 (Time) linear mixed model (LMM) for repeated measures to test the effects of intervention. In this model, we specified time (pre vs. post) and group (experimental vs. control) as fixed effects, whereas between-participant mean differences (i.e., the intercepts) were modeled as random effects. We followed significant interaction effects by means of post hoc tests using paired-samples *t*-tests. In addition, we applied Holm-Bonferroni corrections to the results of the interaction effects on the three cognitive abilities measured. Pearson's correlation analyses were then performed between the changes of the serum IL-6 level and cognitive performance that showed significant time × group interaction effects. For significant intervention effects on the changes of cognitive measures that were correlated with the change in the serum IL-6 level (ΔIL-6) due to the intervention, we also investigated whether the group intervention effect on changes in cognitive performance was mediated by ΔIL-6. We performed the mediation analyses using PROCESS macro (Hayes, 2017) implemented in SPSS, with group as the independent variable, ΔIL-6 as the mediator variable, and changes in cognitive performance after the intervention as the dependent variable. In the mediation models, any mediating effect of ΔIL-6 would be reflected as the indirect effect. PROCESS macro was based on ordinary least-squares regression and adopted a nonparametric bootstrapping procedure (5,000 times), which gave rise to a bias-corrected confidence interval (CI) for effect size inference (Shrout and Bolger, 2002). A significant effect at $p < 0.05$ is indicated if zero is not included within the 95% CI (Preacher and Hayes, 2008).

We examined the baseline group differences of the mean GMV of the ROIs using linear regression models with TIV, age, and sex as covariates (Table 1). We examined the between-group differences in the GMV changes from baseline to post-training within the significant clusters of each ROI or the whole brain using LMM analysis by controlling for TIV, age, and sex. Then, within each group, we examined the changes in GMV of post-training compared to pre-training using paired-samples *t*-tests. We then performed Pearson's correlation analyses between ΔGMV and the changes of cognitive performance that showed significant time × group interaction effects. We set all of the statistical significance thresholds at $p < 0.05$, two-tailed. For significant intervention effects on the changes in cognitive measures that were correlated with the ΔGMV, we further investigated whether the group intervention effect on changes in cognitive performance was mediated by ΔGMV. The procedure of the mediation analyses was the same as that above, except with ΔGMV as the mediator variable.

Finally, accumulating evidence has shown that inflammation mechanisms could regulate the remodeling of neural circuits, neuroplasticity, and neurogenesis (Yirmiya and Goshen, 2011) and that brain morphology is a plausible pathway linking peripheral inflammation to neurocognitive functioning (Marsland et al., 2008; Marsland et al., 2015; Satizabal et al., 2012). Hence, we speculate that IL-6 might play an important modulating role in the training-induced neurocognitive changes. Therefore, we also explored whether ΔIL-6 would moderate any mediating effect of ΔGMV on the relationship between training group and cognitive changes by performing a moderated mediation analysis in PROCESS.

Table 1
Demographics, cognition, and gray matter volume (GMV) of participants at baseline.

	Total (n = 48)	Experimental (n = 22)	Control (n = 26)	Chi square / t value[a]	p value
Sex (females/males)	33/15	15/7	18/8	0.006	0.94
Age (years)	64.23 (4.22)	63.91 (4.06)	64.50 (4.41)	−0.48	0.63
Education (years)	13.00 (3.79)	13.14 (3.11)	12.88 (4.34)	0.23	0.82
MoCA	27.27 (1.53)	27.64 (1.56)	26.96 (1.46)	1.55	0.13
SDMT	57.75 (9.22)	59.64 (8.34)	56.15 (9.78)	1.31	0.20
d'-CPT	−3.60 (0.63)	−3.50 (0.58)	−3.68 (0.67)	1.13	0.27
DS-Backwards	6.06 (1.80)	6.64 (1.81)	6.00 (1.47)	1.23	0.22
GMV_Hippocampus[b]	0.24 (0.030)	0.24 (0.030)	0.24 (0.031)	0.075	0.94
GMV_DLPFC[b]	0.28 (0.023)	0.28 (0.020)	0.28 (0.026)	1.12	0.27

For Age, Education, MoCA, SDMT, d'-CPT, DS-Backwards, GMV_Hippocampus, and GMV_DLPFC, the values of each variable displayed here are the mean values and standard deviations (SDs, in bracket). [a] Between-group comparisons were conducted using a chi-square test for Sex, independent-samples t-tests for Age, Education, MoCA, SDMT, d'-CPT and DS-Backwards, and linear regression analyses for GMV_Hippocampus and GMV_DLPFC. MoCA, Montreal Cognitive Assessment Hong Kong version; SDMT, Symbol Digit Modalities Test oral form performance; d'-CPT, the d' measure of the Conners Continuous Performance Test 3rd edition; DS-Backwards, backward digit span; GMV_Hippocampus, mean GMV of the entire hippocampus ROI; and GMV_DLPFC, mean GMV of the entire dorsolateral prefrontal cortex (DLPFC ROI. [b] For these measures, the total sample size was 43 (experimental group: 21, control group: 22), and the results of the lineal regression models were obtained with total intracranial volume (TIV), age, and sex as covariates.

3. Results

3.1. Baseline demographics and cognition

We included 55 right-handed, native Cantonese-speaking, and cognitively healthy elderly adults in our study, with 28 in the Qigong exercise training (experimental) group and 27 in the stretching exercise training (control) group, the sample size of which is comparable to previous qigong studies on cognition (Duarte et al., 2020; Ladawan et al., 2017; Tao et al., 2017). They all provided written informed consent for participation in the study. No participants were absent from more than four out of the 18 training sessions. As for interleukin-6 (IL-6) analysis, we excluded five participants (all from the experimental group) because they were taking different anti-inflammatory medications at the baseline (pre-training) and post-training time points, which might have confounded the effect of training on the serum IL-6 levels. One participant from the experimental group who could not commit to the exercise most of the time due to lumbar disc herniation onset was also excluded from the data analysis. Furthermore, one participant from the control group was excluded because of minimal self-practice at home (i.e., 3 standard deviations (SDs) below the mean practice time of the group). Therefore, a total of 48 participants (males: females = 15: 33) aged between 53 and 69 years (mean = 64.23, SD = 4.22) who scored 22 or

higher on the Montreal Cognitive Assessment (MoCA) were included in the final analysis.

We matched the demographical characteristics of the two groups on age, sex, and years of formal education (all $p > 0.1$, Table 1). Also, the groups showed comparable levels of general cognitive ability, as measured by the HK-MoCA ($p > 0.1$). There was also no baseline difference between the two groups in the SDMT performance ($t(46) = 1.31$, $p = 0.20$), Digit Span Backwards (DS-Backwards) ($t(46) = 1.23$, $p = 0.22$), or the detectability (d', sensitivity) measure of the Conners CPT 3 ($t(46) = 1.13$, $p = 0.27$) (Table 1).

For participants' self-practice at home during the training period, the two groups' total self-practice duration ($t(46) = 0.59$, $p = 0.56$, experimental group: mean = 2692.59, SD = 1092.94, control group: mean = 2548.91, SD = 540.40) and mean self-practice duration ($t(46) = -0.26$, $p = 0.79$, experimental group: mean = 40.80, SD = 16.56, control group: mean = 41.79, SD = 8.86) were not significantly different, suggesting that they were both matched.

3.2. Intervention effects on processing speed and sustained attention

The results of the LMM analysis showed that the scores on the SDMT overall significantly increased after training (the main effect of time: $t(46) = -3.60$, $p = 0.001$), and the time × group interaction effect was also significant ($t(46) = 2.03$, $p = 0.048$). Specifically, there was a significant increase in scores on the SDMT for the experimental group after training ($t(21) = 4.25$, $p < 0.001$), whereas no significant increase was observed for the control group ($t(25) = 1.20$, $p = 0.24$) (see Fig. 1).

For the d' measure of CPT 3, the scores overall significantly decreased after training (the main effect of time: $t(46) = 3.51$, $p = 0.001$, indicating better performance at post-training), and the time × group interaction effect was significant as well ($t(46) = -2.34$, $p = 0.023$). Specifically, the experimental group's scores on d' significantly decreased after training ($t(21) = -4.67$, $p < 0.001$); however, no significant decrease was observed for the control group ($t(25) = -0.91$, $p = 0.37$) (Fig. 1). There was no significant effect of group, time, or time × group for the DS-Backwards score. Table 2 presents the details of the results. After Holm-Bonferroni corrections, the time × group interaction effects on SDMT and d'-CPT were marginally significant (corrected p values: 0.069, 0.096, respectively).

3.3. Intervention effects on the serum IL-6 level

For the serum IL-6 level, the LMM analysis showed that the main effects of group ($t(72.48) = 0.21$, $p = 0.84$) and time ($t(46) = 1.52$, $p = 0.14$) were not significant. However, a significant time × group interaction effect was observed ($t(46) = -3.17$, $p = 0.003$) (Table 2). Post hoc tests showed that the serum IL-6 level of the participants in the experimental group decreased significantly after training ($t(21) = -4.21$, $p < 0.001$) compared to the baseline, whereas the control group showed no significant change after training ($t(25) = 0.90$, $p = 0.38$) (Table 2, Fig. 1).

3.4. The change of the serum IL-6 level (ΔIL-6) mediates the intervention effect on processing speed

The change of the serum IL-6 level (ΔIL-6) was further found to be significantly correlated with the change of the SDMT performance after training (ΔSDMT) ($r(46) = -0.41$, $p = 0.004$) across groups but not with the change of d' of CPT 3 (Δd') ($r(46) = 0.23$, $p = 0.12$). Thus, we explored whether ΔIL-6 following the intervention mediated the group effect on ΔSDMT. We conducted mediation analyses with group as the independent variable, ΔIL-6 as the mediator variable, and ΔSDMT as the dependent variable. The analyses revealed that the mediating effect of ΔIL-6 was significant and positive (bootstrapping CI: [0.16, 3.30]), whereas the direct effect was not significant (bootstrapping CI: [-1.74, 4.92]). Specifically, group (experimental group coded as 1, control group coded as 0) had a negative effect on the ΔIL-6 (bootstrapping CI: [-0.47, −0.12]), which was consistent with the finding that the experimental group showed greater reduction of the serum IL-6 level following the intervention, which in turn had a negative effect on ΔSDMT performance (bootstrapping CI: [-11.06, −0.51]). Therefore, older people with greater reduction of IL-6 responses following the Qigong exercise training showed a greater increase in SDMT, reflecting higher processing speed (Fig. 2).

3.5. Intervention effects on the gray matter volume (GMV) of the hippocampus

Among the 48 participants, 43 of them attended scanning at 2 time points (experimental group: 21, control group: 22). First, at baseline, we confirmed that no hippocampal or DLPFC region showed significant between-group difference, and the two groups' mean GMV of the two ROIs (DLPFC and hippocampus) was matched (both $p > 0.1$, Table 1). Of the two ROIs, after controlling for age, sex, and TIV, we found a significant group difference in a cluster seated at the right posterior hippocampus (locus of maxima = 20, −36, 3; maximal $t = 3.54$, $P_{FWE} =$

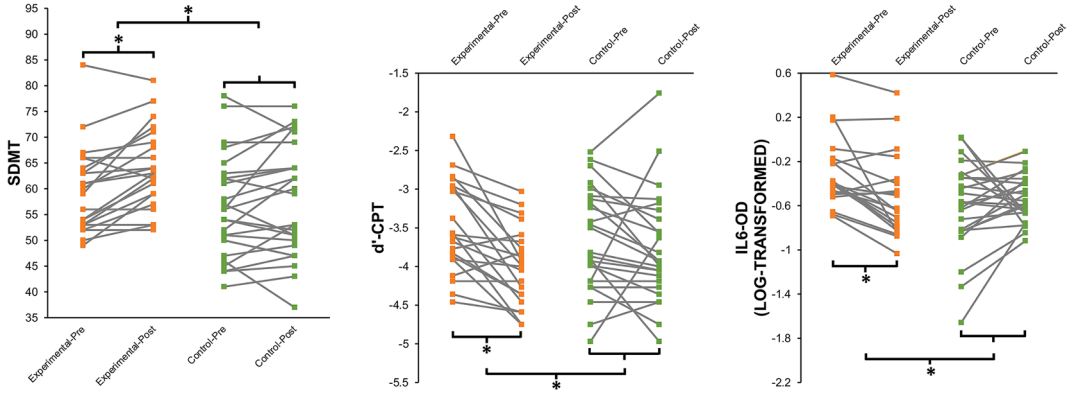

Fig. 1. Intervention effects on the processing speed (left), sustained attention (middle) performance, and serum IL-6 level (right). Significant group × time interaction effects were found for all of these measures. The experimental group's SDMT and d'-CPT performance improved, and the serum IL-6 level decreased significantly after training (all $p < 0.001$), whereas the control group's changes were all non-significant. Each data point represents the value of one participant. (The pre- and post-values of the experimental group are in orange, and the pre- and post-values of the control group in green.) Each line between two points represents the value change of each participant after the intervention. *$p < 0.05$. SDMT: Symbol Digit Modalities Test oral form performance; d'-CPT: the d' measure of the Conners Continuous Performance Test 3rd edition; and IL6-OD (log-transformed): log-transformed optical density (OD) values of the serum IL-6 level. Note that for d', high values indicate worse performances. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary of mean scores and standard deviations (in parentheses) and results of mixed linear model analyses and within-group comparisons on cognitive performance, IL-6 measures, and hippocampal GMV of participants for the pre- and post-training assessments.

Outcomes	Experimental (n = 22)		Control (n = 26)		Linear mixed model comparisons						Within-group comparisons (Post - Pre)			
	Pre-	Post-	Pre-	Post-	Group		Time		Group × Time		Experimental		Control	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	t value	p value	t value	p value	t value	p value	t value	p value	t value	p value
SDMT	59.64 (8.34)	63.91 (7.82)	56.15 (9.78)	57.42 (10.76)	−2.40	0.02*	−3.60	0.001*	2.03	0.048*	4.25	<0.001*	1.20	0.24
d'-CPT	−3.50 (0.58)	−4.00 (0.50)	−3.68 (0.67)	−3.79 (0.70)	1.15	0.26	3.51	0.001*	−2.34	0.023*	−4.67	<0.001*	−0.91	0.37
DS-Backwards	6.64 (1.81)	6.41 (1.76)	6.00 (1.47)	5.77 (1.82)	−1.28	0.21	−1.23	0.22	−0.009	0.99	1.00	0.33	0.81	0.43
IL6-OD (log-transformed)	−0.31 (0.31)	−0.55 (0.36)	−0.59 (0.40)	−0.53 (0.22)	0.21	0.84	1.52	0.14	−3.17	0.003*	−4.21	<0.001*	0.90	0.38
GMV of the hippocampal cluster[a]	0.30 (0.039)	0.31 (0.045)	0.33 (0.034)	0.33 (0.035)	1.63	0.11	−4.05	<0.001*	4.26	<0.001*	4.18	<0.001*	−1.55	0.14

SDMT, Symbol Digit Modalities Test oral form performance; d'-CPT, the d' measure of the Conners Continuous Performance Test 3rd edition; DS-Backwards, backward digit span; IL6-OD (log-transformed), log-transformed optical density (OD) values of the serum IL-6 level; and GMV of the hippocampal cluster, the GMV of the hippocampal cluster showing a significant group × time interaction effect. [a] For this measure, the total sample size was 43 (experimental group: 21, control group: 22), and the results of the linear mixed model comparisons were obtained after controlling for total intracranial volume (TIV), age, and sex. * $p < 0.05$.

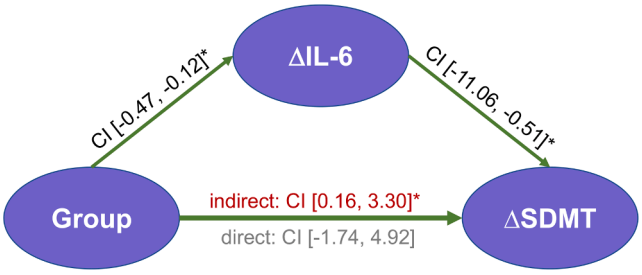

Fig. 2. The serum IL-6 level change (ΔIL-6) due to the intervention mediates the group's effect on SDMT performance change (ΔSDMT). Group was the independent variable (experimental = 1, control = 0), ΔIL-6 was the mediator variable, and ΔSDMT was the dependent variable. Beside each arrow is the bootstrapping confidence interval (CI) of each effect. *p < 0.05.

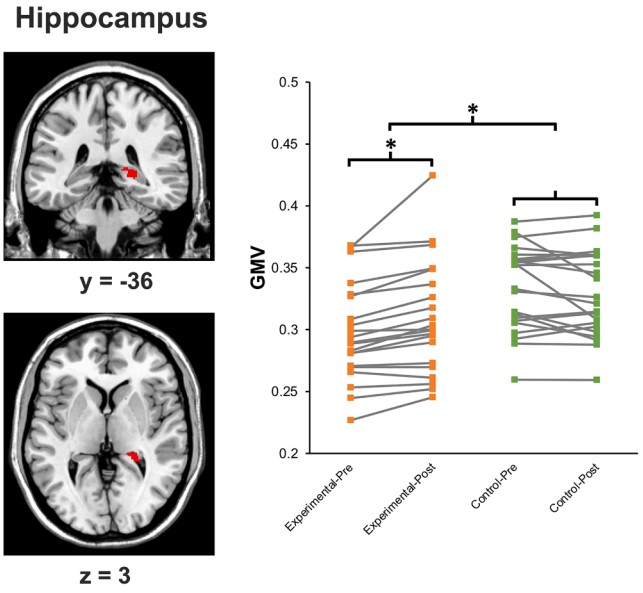

Fig. 3. Intervention effects on the gray matter volume (GMV) of the hippocampus. Left panel: the hippocampal cluster (in red), of which the GMV showed a significant group × time interaction effect, is overlaid on the gray matter ch2 template. Right panel: the line chart of the group × time interaction effects on the hippocampal cluster's GMV. The experimental group's GMV of the cluster improved significantly after training ($p < 0.001$), whereas the control group's GMV did not show significant change. Each data point represents the GMV value of one participant. (The pre- and post-values of the experimental group are in orange, and the pre- and post-values of the control group in green.) Each line between two points represents the GMV change of each participant after the intervention. *p < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.011, number of voxels = 113). The results of the LMM analysis showed that the GMV of the hippocampal cluster overall significantly increased after training (the main effect of time: t (41) = -4.05, $p < 0.001$), and the time × group interaction effect was significant as well (t (41) = 4.26, $p < 0.001$) (Table 2). Post hoc paired-samples *t*-tests showed that the GMV of the cluster increased significantly after training in the experimental group (t (20) = 4.18, $p < 0.001$), but there was no significant change in the control group (t (21) = -1.55, p = 0.14) (Table 2, Fig. 3). For the whole-brain analysis, none of the region's ΔGMV showed a significant group difference (i.e., group × time effect for GMV) after FWE correction at $p < 0.05$.

3.6. Hippocampal ΔGMV mediates the intervention effect on sustained attention

We found a significant correlation of the ΔGMV of the significant cluster in the hippocampus with Δd' (r (41) = -0.31, p = 0.04) but not with ΔSDMT (r (41) = 0.11, p = 0.50). We thus further tested whether the ΔGMV mediated the group intervention effect on Δd'. The results showed that the mediating effect of the ΔGMV was significant and negative (bootstrapping CI: [-0.27, −0.0065]), whereas the direct effect was not significant (bootstrapping CI: [-0.50, 0.12]). Specifically, group (experimental group coded as 1, control group coded as 0) had a positive effect on the ΔGMV (bootstrapping CI: [0.0091, 0.025]), consistent with the finding that the experimental group showed increased GMV in the hippocampal cluster following the intervention, which in turn had a negative effect on the Δd' (bootstrapping CI: [-18.61, −0.43]). Therefore, participants with a greater increase in GMV of the hippocampal cluster following the Qigong exercise training showed a greater reduction in their d' scores, reflecting better sustained attention (Fig. 4).

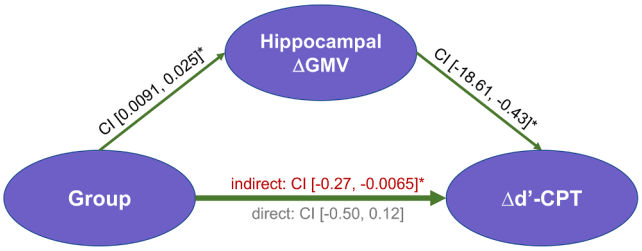

Fig. 4. The gray matter volume change (ΔGMV) of the cluster within the hippocampus, which has a significant group difference, mediates the group's effect on d' performance change (Δd'-CPT). Group was the independent variable (experimental = 1, control = 0), ΔGMV was the mediator variable, and Δd'-CPT was the dependent variable. Beside each arrow is the bootstrapping confidence interval (CI) of each effect. *p < 0.05.

3.7. ΔIL-6 moderates the mediation effects of hippocampal ΔGMV on the intervention effect on sustained attention

Finally, we explored whether the mediating effect of hippocampal ΔGMV was moderated by ΔIL-6 by further including the ΔIL-6′s moderation effects in the mediation model. Our results showed that the indirect effects of group on Δd' were dependent on the level of ΔIL-6. Specifically, when ΔIL-6 was more negative (i.e., 1SD below the mean, or −0.45), the indirect effect was significant (bootstrapping CI: [-0.35, −0.004]). However, when ΔIL-6 was more positive (i.e., 1SD above the mean, or 0.26), the indirect effect was not significant (bootstrapping CI: [-0.22, 0.033]), signaling no significant mediation effect (Fig. 5). In other words, only among participants with greater reduction of IL-6 levels did hippocampal ΔGMV significantly mediate the beneficial effect of Qigong on sustained attention.

4. Discussion

This study demonstrated the neurobiological effect of a 12-week Qigong training on processing speed and sustained attention in older people. We showed that the Qigong training reduced older people's peripheral IL-6 responses and increased their right hippocampal GMV. Importantly, IL-6 reductions following training mediated the training effect on processing speed. Moreover, hippocampal GMV increases mediated the training-induced increase in sustained attention performance, which was further found to be moderated by the level of IL-6 changes. Thus, changes in the peripheral IL-6 levels played significant roles in the mechanistic pathway underpinning the observed neurocognitive improvement after the Qigong training.

We found that Qigong training improved processing speed and sustained attention in older people. Previous studies have reported the beneficial neurocognitive effects of other types of qigong training for younger adults (processing speed: (Ladawan et al., 2017) and adolescents (sustained attention: (Duarte et al., 2020). Thus, our study converges with previous findings that neurocognitive improvement after qigong could be consistently observed across various age groups and types of qigong. On the other hand, our findings indicated that the Qigong effect might not extend to other important cognitive functions such as working memory, possibly because the intervention was of insufficient duration (Erickson et al., 2014).

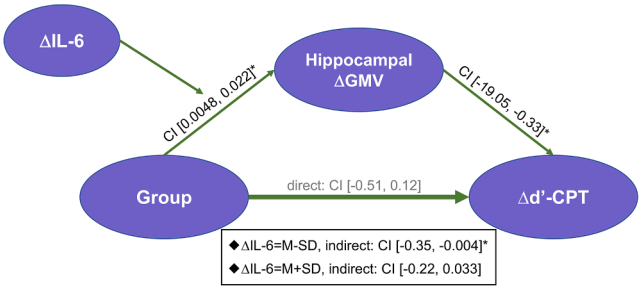

Fig. 5. The moderated mediation model linking ΔIL-6 to the mediation effects of hippocampal ΔGMV on the intervention effect on d' performance change (Δd'-CPT). Group was the independent variable (experimental = 1, control = 0), ΔGMV was the mediator variable, Δd'-CPT was the dependent variable, and ΔIL-6 was the moderating variable. Beside each arrow is the bootstrapping confidence interval (CI) of each effect. M and SD are the mean and standard deviation values of ΔIL-6. *p < 0.05.

The increase in hippocampal volume after Qigong training is broadly similar to the training effects observed in other types of qigong. Previous findings have revealed a similar increase in hippocampal GMV in older adults after 12-week exercises of other types of qigong (i.e., Tai Chi Chuan and Baduanjin) as compared to the control group that received just basic health education (Tao et al., 2017). This increase is also evident in older adults with mild cognitive impairment (MCI) (Tao et al., 2019). While the hippocampus is mainly involved in episodic memory, its role in sustained attention has been demonstrated in previous clinical and lesion studies (e.g., (Sax et al., 1999; Zhang et al., 2009)) and in a recent study on patients with amnestic mild cognitive impairment (Nathan et al., 2017). These findings supported the functional role of the hippocampus in sustained attention. The specific increase in right hippocampal volume can be reconciled with literature showing that the right hippocampus is more sensitive to experience and learning (Shinohara et al., 2013; Koch et al., 2016). Erickson et al. (Erickson et al., 2014) reviewed the associations among physical activity, cardiorespiratory fitness, and GMV in older adults and concluded that both physical activity and cardiorespiratory fitness were associated with greater GMV in the hippocampus and the prefrontal cortex, but we did not observe a significant increase in GMV in the DLPFC. Given that neither the existent literature on the effects of qigong exercises on neural structure nor our current study found a Qigong-induced increase in the DLPFC GMV, we speculate that practicing qigong may take longer before an effect on the DLPFC GMV can be observed (Erickson et al., 2014).

It is unclear how neuro-immune interactions might be crucial in protecting against aging-related cognitive decline. Accumulating evidence has shown that qigong exercise reduces inflammation (Oh et al., 2010, 2012; Liu et al., 2017), including IL-6 levels (Chen et al., 2006). This resonates with our finding that Qigong was effective at reducing the peripheral inflammation as indexed by IL-6 levels in the older adults. The chronic increases in peripheral inflammation accompanying aging could increase the inflammatory responses in the brain and thus likely contribute to the pathogenesis of aging-related cognitive decline (Marsland et al., 2015). Hence, inflammation in the brain was expected to decline as a result of decreases in peripheral inflammation after the Qigong exercise, which would protect the brain from neurodegeneration, facilitating brain function and efficient information transfer in the brain (Curtin et al., 2019). Consequently, this would result in the enhancement of processing speed and modulation of the mediation effect of the hippocampal volume on sustained attention improvement. Some initial research on depressed individuals has suggested that a higher IL-6 level is related to smaller hippocampal volume (Kakeda et al., 2018) and that reducing one's IL-6 level following electroconvulsive therapy would correlate with an increase in hippocampal volume (Belge et al., 2020). Therefore, our results converge to highlight the critical role of inflammation in the hippocampal region and the homeostasis of the central nervous system and extend to support IL-6′s mechanistic role in the beneficial neurocognitive effects of the Qigong regime.

Our finding that IL-6 changes moderated the mediating role of hippocampal volume changes in the beneficial effect of Qigong on sustained attention is particularly intriguing. On the basis of literature supporting the key impacts of immune reactions on both generic and circuit-specific neurocognitive processes, neural plasticity, and neurogenesis (Yirmiya and Goshen, 2011), we speculate on the following possibilities. First, it is possible that under relatively high levels of IL-6 concentrations and

strong neuro-immunological reactions, hippocampal neurogenesis (i.e., GM increase) mostly compensates for the generic adverse effect of excessive immune reactions on neurocognitive processes and thus may not be accompanied by significant neurocognitive improvement, as was observed under relatively low IL-6 levels. Second, it could be that high IL-6 levels disrupt the functional processes in the hippocampus, such that the hippocampal functional level may not increase linearly with its increase in grey matter. Such functional disruption could occur for multiple cellular mechanisms involving microglia and neurotrophic factors (Yirmiya and Goshen, 2011). Third, high IL-6 levels may disrupt the functional interactions between the hippocampus and other important neurocognitive circuitries (Leal and Yassa, 2015; Tao et al., 2016). Human functional MRI studies are needed to further delineate the relationship between inflammation responses and functional interactions among neural networks.

Our findings have important implications for improving the brain health of older populations. Specifically, the findings strongly implicate the critical importance of immunological processes, reflected by peripheral IL-6 levels, in determining both the improvement of processing speed and whether hippocampal volume increase will lead to the improvement of sustained attention following a short-term Qigong intervention. Thus, older individuals with long-term immune-related diseases and heightened chronic inflammatory reactions may be particularly vulnerable to neurocognitive decline. These individuals could be the target for alternative interventions, such as Qigong, that we demonstrated to both reduce IL-6 levels and improve neurocognitive function. Reducing IL-6 levels is also a prerequisite for any beneficial effect of neurogenesis in the hippocampus on improving certain cognitive processes. Whether such a pattern extends to other learning and memory-dependent processes demands further research, although some supporting evidence already exists (Yirmiya and Goshen, 2011).

There are several limitations in the current study. First, the sample sizes were modest because of the space constraint and the strict recruitment criteria. However, one strength was that it allowed a more supportive and focused training environment for our participants, which increased the strength of the neurocognitive and neurobiological effects. Second, we did not include an aerobic exercise group and could not confirm whether the neurocognitive effects of Qigong would be superior to those of the aerobic exercise. Notwithstanding, our aim in this study was to test Qigong's specific effects on cognitive functioning relative to a simple stretching exercise, and future study could further test the Qigong regime against other types of physical exercises. Third, after multiple comparison corrections, the group × time interaction effects on processing speed and sustained attention were just marginally significant. This could be due to the small sample size and short training period; thus, future larger-scale and longer follow-up intervention studies are needed to corroborate Qigong's effects on these cognitive domains. Last but not least, although we measured the fundamental cognitive domains, we did not include a full set of neurocognitive tests and could not confirm whether Qigong's protective effect on aging would extend to other cognitive functions. Nonetheless, the cognitive measures that we included, namely processing speed, sustained attention, and working memory, were considered to form the fundamentals of other higher order cognitive functions. Future intervention studies may incorporate measures of other cognitive domains with a longer follow-up duration.

5. Conclusions

Our findings have far-reaching implications on the potential value of Qigong exercise as an effective intervention for slowing or even reversing aging-related cognitive decline in older people, specifically when outdoor exercise is not encouraged due to lockdown measures imposed during the COVID-19 pandemic. This study is the first to demonstrate Qigong's neurocognitive effects and the underlying neuroinflammatory pathway. It provides potential modulating targets for future intervention development and lays the groundwork for promoting Qigong exercise to the broad elderly population for healthy aging.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.04.011.

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