Fitness-induced structural plasticity in the brain:

Longitudinal study with 7 Tesla MRI

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1. **Abstract**

2. **Introduction**
    a. Neurodegeneration and physical intervention
    b. Translational research
    c. Whole-brain investigation
    d. Effects of physical intervention on physiology and vascularisation
    e. Effects of physical intervention on brain function, cognition and mood
    f. The present study

3. **Materials and methods**
    a. Participants and experimental design
    b. Assessment
        i. Fitness assessment
        ii. Cognitive assessment
            Rey Complex Figure Test and Recognition Trial
            Spatial Navigation Task
            Pattern Separation
            Working Memory
            Beck Depression Inventory
    c. Intervention
    d. 7-Tesla high-resolution MRI

4. **Pre-processing and segmentation with CAT12 toolbox**

5. **Statistical analysis with SPM12**
    a. Statistical modelling
    b. ROI analysis

6. **Results**
    a. Training-induced fitness changes
    b. Training-induced structural brain changes
        i. Linear effects in structural time-courses
        ii. Quadratic effects in structural time-courses

Abstract

One of the main deleterious features of dementia is neuronal and structural degeneration in the brain. To face that, several attempts through cognitive and physical exercise intervention have been performed, but results are still heterogeneous and controversial. Physical exercise seems to be particularly promising since hippocampal structural plasticity has been shown to be mediated by regional increase in vascular perfusion following training. However, almost nothing is known about the effect of training intervention next to the hippocampus and in motor areas, nor what parameters really mediate training-induced plasticity. Furthermore, the difference between maintenance and actual growth effects are yet to be clearly disentangled.
We addressed these questions with a longitudinal approach where 40 young healthy but physically inactive participants (24 females and 16 males, age range: 19-34 years) were enrolled in an intervention study, randomly assigned to either Training (intense aerobic exercise training on a treadmill) or Control condition. 7-Tesla MRI data as well as fitness, cognitive and vascular measures were collected at three time points (baseline, one month and four months) and VBM estimation of local amount of grey matter was performed using CAT12 toolbox. Thanks to the recently developed Sandwich Estimator (SwE) as an innovative, fast and accurate longitudinal statistical model, we investigated linear and quadratic volumetric effects in relation with fitness and cognitive improvement.
Our ROI-analyses revealed significant growth on the right hippocampus, together with some interesting trends on the ventromedial side of the frontal cortex. As for cognition, we found significant volume increase in the right middle temporal gyrus associated to improvement in memory recall, along with other plasticity trends related to spatial navigation and working memory. Furthermore, whole-brain post-hoc analyses revealed additional trends towards tissue expansion spread on fronto-temporal networks.
Given our longitudinal approach with 3 time points and our young sample, these results cast some light on the causality of this mechanism, suggesting that brain plasticity might be driven by or at least associated to fitness improvement, and it might consequently benefit cognition. Such findings could have relevant implications for prevention and low-cost intervention available to a broader range of people.

Introduction

Neurodegeneration and physical intervention

Neurodegeneration and brain atrophy strongly contribute to unhealthy aging and dementia, including Alzheimer's and Parkinson's diseases. Neurodegeneration is defined as the progressive loss of structural or functional properties of neurons in the brain and/or spinal cord, ultimately resulting in neuronal death, brain atrophy (i.e. tissue shrinkage due to the loss of neurons and the connections between them) and cognitive impairment. Nowadays, although the prevalence of neurodegenerative diseases is constantly increasing, available therapies help dealing with symptoms, but cannot stop or slow down neuronal loss. Cognitive, physical, or combined types of intervention have been experimentally implemented in the attempt of inducing neural plasticity against shrinkage, yet with controversial or even contradictory outcomes. At the other end of the spectrum, neuroplasticity is the ability of the brain to re-wire itself, re-organise topographical mappings, change the strength of synaptic connections and – in relation to the present study – even change tissue volume.

Due to its direct relation to body physiology and anatomy and the difficulty in grasping the mechanisms underlying mental training, physical exercise seems to be particularly promising in modulating neuroplasticity (Lindenberger et al., 2017). Aerobic training increases regional Cerebral Blood Flow (CBF; i.e. the blood supply to the brain in a given period of time) and Cerebral Blood Volume (CBV; i.e. the volume of blood that is present at a given moment within the neocranium) as a proxy of vascular perfusion, and this increase in fitness level correlates with hippocampal volume (Maass et al., 2015). Since the hippocampus appeared to be extremely plastic compared to other brain structures, a recent systematic review (Firth et al., 2017) gathered, controlled, and summarised all the findings acquired to date on the volumetric effect of aerobic exercise specifically regarding the hippocampus. Results showed a significant positive effect selectively on the left hippocampus with a p value of 0.003, while neither its right portion nor its total volume survived the statistical threshold. Additionally, authors checked whether the choice of automated versus manual segmentation tool drove a relevant portion of the variance, but that was not the case.

This is highly relevant for neurodegeneration tackling since some regions, including hippocampus, undergo a particularly steep and rapid shrinkage as a function of age.

Other studies also demonstrated training-induced grey matter expansion in specific motor areas on precentral gyri; for instance, hand motor area appeared to be shaped by hand

excitability (Granert et al., 2011). This led to the idea that the same mechanism might be true for other motor areas as a consequence of training or stimulation.

Translational research

In rats indeed, the volume of motor cortex was even correlated to the total distance run by the animal (Sumiyoshi et al., 2014). Surprisingly, also in humans, these structural effects quite rapidly occur, even within one-hour time (Tauber et al., 2016). However, Wenger and collegues (2016) recently suggested that they are as rapid as transient, since they found motor cortex's partial normalisation after the fourth week of expansion.

Whole-brain investigation

Nevertheless, plasticity effects in regions and networks other than the hippocampus and motor areas have been poorly investigated and have not provided a univocal body of knowledge yet. In human research, there is some evidence of exercise-induced volume change spreading in frontal and parietal regions. Firstly, physical activity has been shown to be correlated with grey matter volume in prefrontal and cingulate cortex (Flöel et al., 2010). Secondly, Reiter et al. (2015) demonstrated cortical thickening in insula, precentral gyri, precuneus, posterior cingulate, as well as inferior and superior frontal cortices in elderly subjects undertaking a moderate intensity walking training. Thirdly, Boyke et al. (2008) showed comparable grey matter changes in the middle temporal area of the visual cortex (hMT/V5) in young and old participants engaged in juggling exercise. Interestingly, structural changes also correlated with performance improvements both in humans and nonhuman primates (Taubert et al., 2010).

Effects of physical intervention on physiology and vascularisation

A beneficial effect of training was found also in the cerebellum, where gray matter increase parallels Brain-Derived Neurotrophic Factor (BDNF) increase (Ben-Soussan et al., 2015).
As far as the underlying mechanism is concerned, there is quite some evidence regarding the benefit of aerobic exercise on physiological indices such as BDNF, insulin-like growth factor-1 (IGF-1) (Heisz et al., 2017) and Granulocyte Colony-Stimulating Factor (G-CSF) (Flöel et al., 2010).
Due to the close relationship between physical and cardiovascular fitness, the latter has been investigated as a plausible mediator of the neural and maybe even cognitive effects. In the model proposed by Maass et al. (2015), hippocampal volume and recognition memory performance are modulated by regional vascular perfusion. However, this model seemed to fit

selectively for the hippocampal region. At a lower level of analysis, additional environmental stimulation triggered cerebellar molecular layer's expansion, with constant vascular density. On the contrary, voluntary exercise yielded an increase in the density of capillaries along with constant molecular layers' volume, therefore causing vessels' component to prevail in the ratio. Since regional CBV correlated with neurogenesis rate, which has also been shown to be enhanced by exercise, the authors suggested that this vascular parameter could be used as a proxy of neurogenesis (Thomas et al., 2012). Indeed, aerobic exercise was directly associated to angiogenesis and less vessels' tortuosity next to neurogenesis (Cassilhas & Tufik, 2016). Angiogenesis is defined as the generation of new blood vessels stemming from pre-existing ones, whereas vessel tortuosity is a vascular anomaly that could obstacle blood flow leading to ischemic attacks. CBF impairment is known to be crucial also in Alzheimer's disease, causing brain hypo-perfusion especially in early stages when brain macro-structure was not yet affected (Mattsson et al., 2014). Nevertheless, there seems to be room for prevention, given that cardiovascular fitness in adolescents has been tested as a good predictor of cognitive performance at the end of adolescence, which in turn predicted educational achievements in later stages (Åberg et al., 2009).

Effects of physical intervention on brain function, cognition and mood

In this direction, grey matter volume change has also been associated to other neural and/or cognitive alterations, such as functional connectivity changes in prefrontal and supplementary motor areas after a dynamic balancing training (Taubert et al., 2011) as well as in cortico-basal ganglia circuits in dancers compared to non-dancers (Li et al., 2015). Cortical changes correlated with fitness improvement in Jonasson et al., 2017; specifically, the aerobic component appeared to be necessary in order to trigger volume alterations (Colcombe et al., 2006). It is worth noting that cortical thickening in older adults has the same beneficial connotation in terms of neuroprotection as its opposite (thinning) in young adulthood (Williams et al., 2017).

Niemann et al. (2014), Motl et al. (2015) and Becker et al. (2016) reported an association between fitness and basal ganglia volume increase, suggesting that it might mediate another promising association between fitness and executive control improvements.

Interestingly, this structural change was also correlated with functional changes in the right inferior frontal gyrus, possibly jointly mediating cognitive enhancement.

The literature provides some encouraging direct correlations between physical exercise and cognitive measures – especially executive functions-related (Colcombe & Kramer, 2003) –

particularly evident in women (Barha et al., 2017): early recall (Maass et al., 2015), faster reaction times parallel to increased N2 and P3 amplitudes (Olson et al., 2016), improved high-interference memory (Heisz et al., 2017) and visuospatial performance (Barha et al., 2017). Despite that, the effect of exercise on behavioural measures looked sometimes divergent and ambiguous. According to Olson et al. (2016), this heterogeneity might be accounted for by the dual-task condition (physical and cognitive exercise at the same time) thus leading to a state of transient hypo-frontality. Jonasson and collegues (2017) tried to disentangle the neuropsychological construct by relating several test batteries to aerobic training and brain structure, and found a broad rather than a specific cognitive improvement, along with correlations between training condition and hippocampal volume, and between baseline prefrontal cortical thickness and starting fitness level.

On the other hand, the consequences of exercise on mood looked more univocal, with clear improvement in depression scores after training s

essions (Gourgouvelis et al., 2017). Overall, physical fitness and depression seemed to have opposite effects on cognition, meaning that fitness promotes better cognitive performance whereas depressive state might parallel cognitive decline (Déry et al., 2013).

The present study

As suggested in the literature (Draganski et al., 2014; May, 2011), in this study we choose a longitudinal approach in order to be able to causally define the underlying mechanism and disentangle diverse biological factors contributing to exercise-induced structural plasticity. Structural effects in the human brain out of the hippocampus might take more time to be evident, therefore we acquire measurements at three points in time, in order to account for both slower and early-adaptive effects, with their potential re-normalisation (Wenger et al., 2016).

In our study we acquire high-resolution 7-Tesla MRI images. With the exception of a previous study on an older sample performed by the same group, only rats have been scanned in 7-Tesla MRI after physical exercise to this date (Sumiyoshi et al., 2014).

The choice of a young sample aims to help discerning between induced structural maintenance and actual growth in the brain, since other confounding factors linked to the aging process are ruled out isolating the intervention effect (Lövdén et al., 2013). We performed both ROI- and whole-brain analysis. ROIs analysis was grounded on the background literature reported above, leading to a hypothesised network that includes hippocampus, parahippocampal gyrus,

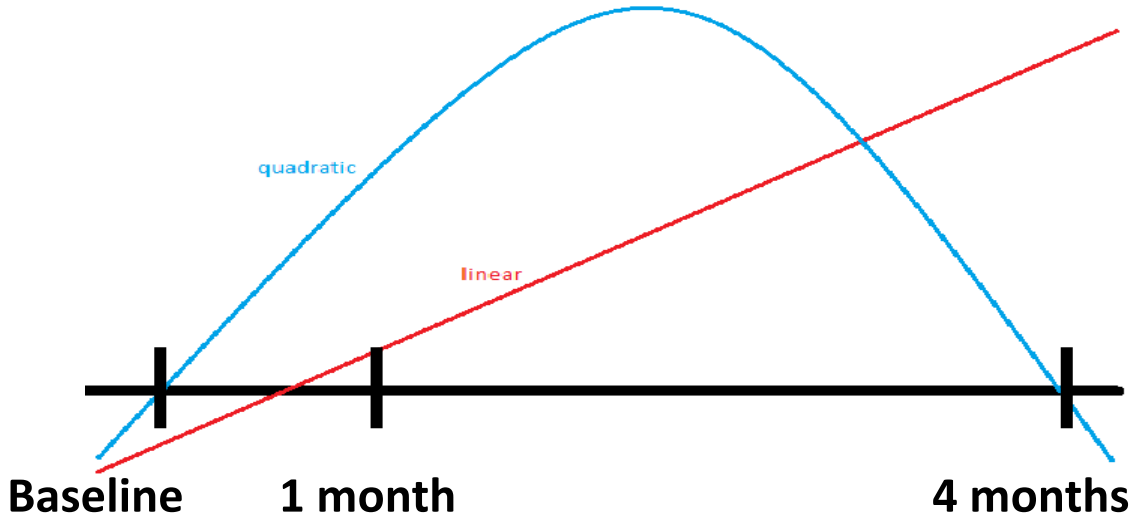
medial frontal cerebrum, anterior cingulate gyrus, subcallosal area, putamen, caudate, precentral gyrus and external cerebellum.

As a result, this study compares a physical intervention group versus a control group, under the hypothesis that fitness improvement can induce more within-subject change (both transient and persistent) in brain volume. Furthermore, we hypothesise that some fitness-induced cognitive improvement might be mediated by volume increase. Finally, we test whether vascularisation changes might mediate fitness-induced neuroplasticity and cognitive improvement.

Materials and Methods

Participants and experimental design

This longitudinal study consisted in a controlled 4-month aerobic exercise intervention, where 40 young participants (24 females and 16 males, age range: 19-34 years) were randomly assigned to either a Training or a Control condition. All subjects showed no signs of neurological or psychiatric illness and had normal or corrected-to-normal vision. Participants were selected and checked to be all healthy but physically inactive, so that their initial fitness level was kept controlled. Groups were matched on age, gender and Body Mass Index (BMI) covariates. Fitness, cognitive and vascular indices were acquired prior to the intervention to set the individual baseline, at the first time point after four weeks, and at the third time point after four months. We decided to test for three time points instead of two, to be able to account also for quadratic and/or re-normalisation effects. As for neuroimaging data, high-resolution 7-Tesla MRI scans were acquired at all time points. 7 subjects were excluded from the very beginning due to contraindication from magnetic resonance imaging (MRI). Out of the 44 recruited subjects, 2 failed the 7 Tesla (T) scanning, one was rejected due to higher physical activity levels and another one dropped out, leading to a final sample of 40 subjects, 19 trainers and 21 controls. All the participants signed written informed consent and received monetary compensation for participation. The study was approved by the ethics committee of the Faculty of Medicine of the University of Magdeburg.



Assessment

Fitness assessment

Their starting fitness level was assessed through an activity questionnaire, including information regarding frequency and type of physical activity per week, as well as a self-rating score of their fitness level from 0 to 5. In case of high fitness self-rate or participation to sports that could improve their cardiovascular fitness, they were excluded from the study. Moreover, their fitness levels were further corroborated by initial maximal oxygen consumption (VO_2 max) in an incremental fitness test on treadmill ergometer (HP COSMOS, QUARK CPET), using VO2 consumption at Ventilatory Threshold (VT) and Respiratory Compensation (RC) as parameters for fitness status.

For the sake of simplicity and clarity, a fitness composite score was calculated by combining the mean of the inverse z-score of blood lactate level change in percentage, and the z-score of VO_2 change in percentage. In order to test for exercise-induced effects, differences in fitness values (post minus pre) and spectral power values were calculated and subsequently tested for bivariate (Pearson) correlations.

In addition, dehydration status was estimated through plasma osmolality, namely body's electrolyte-water balance. High plasma osmolality can be considered a marker of dehydration, whereas low plasma osmolality indicates increased fluid levels. As a consequence, dehydration would decrease Cerebral Blood Flow (CBF) due to lower water content in the blood (Trangmar et al., 2014).

Cognitive assessment

A comprehensive cognitive assessment was performed around one hour prior to the VO2 max fitness test at all time points, with the aim of getting information about multiple cognitive functions. In addition, subjects completed the Beck Depression Inventory (BDI-II) (Beck et al., 1996), to test also for the mood dimension. The cognitive and affective assessment consisted of the below-described standardised indices.

Rey Complex Figure Test and Recognition Trial

The Rey complex figure test and recognition trial (RCFT) (Meyers and Meyers, 1995) was employed to assess a range of executive functions such as visuospatial abilities, memory, attention, planning and working memory. It consisted of four separate tasks: initially participants were shown a complex figure and asked to copy it on a blank sheet of paper (1). The time needed was recorded. After three minutes of unrelated verbal activity without the possibility of seeing the image, subjects completed an immediate recall test by trying to draw the image again only based on memory (2). A delayed recall trial was performed following a longer interval of 30 minutes (3). Accuracy was scored based on image reproduction and placement of 18 structural items of the original image. Right after the recall tests, participants underwent a recognition trial composed of 24 geometric items, half being actually present in the stimulus figure and half being incorrect options. The subjects had to indicate which ones they recognised from the original figure (4).

For the sake of this study and based on our initial hypothesis, only the delayed recall task (3) of RCFT was taken into account.

Spatial Navigation Task

We used a virtual environment navigation task including 4-way intersection to assess spatial navigation abilities. In the encoding phase, subjects pressed the keyboard spacebar to approach an intersection. In the following retrieval phase they approached that same intersection a second time from a different direction, meaning from a different perspective, and were supposed to identify the starting position of the previous trial. Reaction times for retrieval were calculated from correctly answered trials (with permission from Wayfinding Research Center, Bernmouth, UK).

Working Memory Task

We used a Delayed Match-to-Sample (DMS) task to assess working memory functioning. The DMS task consisted of 120 trials divided into six blocks in total, where two stimuli were presented for three seconds with a delay of 5 seconds. The former was the sample stimulus to be encoded, while the latter was the probe for retrieval. Subjects

had to judge whether the stimulus represented a public or a private space, plus trying to memorise it. Afterwards, they were asked whether the probe was identical to the sample (i.e. repeat) or not (i.e lure). Changes needed to be pointed out by the participant even if subtle or hard to detect. Half of the items were repeats and half lures. As a consequence of this experimental design, encoding, retention, and retrieval of contextual information were maximized during the DMS task.

Beck Depression Inventory

We administered the Beck Depression Inventory (BDI-II). It is composed of 21 multiple-choice questions, in the form of a self-report inventory informing on the depressive state of the person. It includes items related to depressive symptoms such as hopelessness and irritability, cognitive aspects such as guilt or punishment, along with physical aspects such as fatigue, weight loss, and lack of interest in sex. Questions refer to the past week. The subject is asked to define his/her status based on four possible responses that trace a gradual intensity range (e.g. (0) I do not feel sad, (1) I feel sad, (2) I am sad all the time and I can't snap out of it, (3) I am so sad or unhappy that I can't stand it). Scoring is based on a value from 0 to 3 assigned to each item, and the total score is then interpreted according to a standardised scale (i.e. 0–9: minimal depression, 10–18: mild depression, 19–29: moderate depression, 30–63: severe depression).

Intervention

Consumption of oxygen at ventilatory threshold ($VO_{2\ VT}$) was assessed by graded maximal exercise testing on treadmill ergometer. Intensity and steepness were both gradually increased: the former every 2 minutes from an initial speed of 3km/h up to a maximum of 6.5 km/h, the latter from 0% to 18%. The upper level was individually defined by exhaustion criteria of the participant, meaning when Respiratory Exchange Ratio (RER) was higher than 1.1. Exhaustion criteria were defined by oxygen uptake at respiratory compensation ($VO_{2\ RC}$) rather than max exhaustion (VO2 max), and none of the subjects performed at maximal heart rate (HRmax) as a precaution against health complications. In addition, blood samples from the ear lobe were collected at resting state, then every 2 min during the test, and 2 minutes following maximum intensity to assess lactate levels (EKF DIAGNOSTIC, Magdeburg). All the subjects received individually optimized treadmill training for a period of 16 weeks under the supervision of a sport scientist. Individual training intensities were determined by target

heart rates, according to Karvonen method (Karvonen et al. 1957), and verified to heart rate levels at the individual anaerobic threshold, based on lactate levels.
The training group (TG, N = 19) performed 3 sessions per week of intense aerobic exercise training at 70-90% HRmax for 45-75 min, including a 5-minute warm-up and 5-minute cool down.
The control group (CG, N = 21) instead performed only 2 sessions per week of walking for 10-15 min with breaks in between at 50% HRmax. For them, the maximum treadmill incline was 3% and the maximum walking speed was 4.5 km/h. This condition allowed to isolate the effect of cardiovascular fitness on the training group, by controlling potentially confounding variables such as motivation, scheduling and social interaction. Indeed, setting and monitoring procedures were kept constant between groups.
Due to the enormous variability in brain structure and plasticity, as well as in the responsiveness to training, a longitudinal intervention study is the only protocol allowing causal inference on the individual change. The optimal intervention parameters in the context of physical exercise research have yet to be precisely determined. However, a recent meta-analysis (not available at the time of our data acquisition) reports that moderate-intensity continuous training (MICT) along with resistance training (RT) are effective in modulating hippocampal plasticity (Feter et al., 2018). Being our training protocol rather intense (although always below the exhaustion level) but still well-handled by a group of healthy young adults, this study can help adjusting the definition of optimal parameters for training research.

7-Tesla high-resolution MRI

High-resolution structural T1-weighted images (whole-head, sagittal orientation) were acquired at pre- and post-intervention using a 7 Tesla MRI scanner (Siemens Magnetom, Erlangen, Germany; 32-channel head coil). Resolution was isotropic with a voxel size of 0.6x0.6x0.6 mm^3, slice thickness of 0.60 mm and Field of View (FoV) for read and phase encoding direction respectively 230 mm and 100.0%. The relative Signal-to-Noise ratio (SNR) was 1.00. The 288 slices were acquired in a single shot (no multi-slice) and interleaved with TR of 2500 ms, TE of 2.8 ms, TI of 1050 ms and a flip angle of 5 degrees, for a total acquisition time of 14:02 min. The sequence was encoded with a bandwidth of 150 Hz/Px, echo-spacing of 8.2 ms and fast Radio-Frequency (RF) pulse and gradient mode.

Pre-processing and segmentation

First of all we visually checked for artefacts in raw images using FSL view option. As for segmentation, we performed a Voxel-Based Morphometry (VBM) estimation of the local amount of gray matter using SPM software. A set of longitudinal 7-Tesla data was segmented using the SPM extension CAT12 toolbox (Gaser & Dahnke, Jena University Hospital), which is more sensitive to small expected structural changes, as in the present case of young healthy brains. Up to this date, it is the preferred segmentation tool for longitudinal structural studies in healthy people in the literature, and it has been optimised in order to be more sensitive and less conservative than the classical SPM pipeline. This is because no complex transformations are applied and only the between-subjects modulation is included. Since we were mainly interested in gray matter, we used only the tissue probability map of this tissue created by SPM, and affine regularisation to European brains. We set rough affine pre-processing (APP), medium strength of local adaptive segmentation, strength of skull-stripping and strength of final clean up. As for spatial registration, an optimised shooting template was preferred to the Dartel template, since the former is a more accurate average of the actual brains in question. Normalised images had a voxel size of 1 mm. We did not obtain surface and thickness estimation nor forward deformations, but only the modulated version of grey matter segmentation as well as the affine DARTEL export of average images. The strength of SPM inhomogeneity correction was set as strong having 7-Tesla data. A smoothing kernel (FWHM: [6 6 6]) was applied to the modulated warped pre-processed images.

Given the quality of the sample, small and subtle changes were expected, hence the choice of using CAT12 toolbox to segment tissues. However, besides being faster, CAT applies simpler linear transforms (i.e. rotations) decreasing conservativeness but also accuracy.

Therefore, we also run segmentation using classical SPM pipeline as a double-check. It differs in the fact that it provides a measure of the individual warp across time and multiplies it by the average scan among the time points ("Generative Modelling Approach"). By performing these two different segmentation methods we aimed to investigate both their intrinsic risks: higher chance of overfitting with CAT one one hand, and higher chance of strong bias with SPM on the other.

CAT procedure allowed more insight into the data, however, since we double-checked with standard SPM segmentation method, we can assume that results are not driven by segmentation or registration errors. In both cases we applied a smoothing kernel of 4, 6 and 8 mm, to then find out that 6 mm was the optimal choice given the size of our ROIs.

Statistical analysis

Statistical modelling

As for statistical modelling, we applied the Sandwich Estimator Model (SwE), probably the most robust longitudinal model available at the time of the study. It first estimates the parameters of interest using a simple Ordinary Least Square model (OLS), followed by SwE to estimate covariates. In doing that, SwE accounts for within-subject/repeated measures correlation and it's robust even in case of misspecification of the covariance or severe heterogeneity of the variance (e.g. across time or across subjects). In addition, it is rather fast and easy to apply, since it does not require iterative modelling.

During the estimation of the parameters of interest, a marginal model is used, meaning that only fixed and random effects contribute to the estimation of the random error. Afterwards, covariates are split into between-subjects and within-subjects components; covariates are estimated per subject and then subjects are grouped in the so-called "superscripts" according to the degree of covariates' homogeneity (Guillaume et al., 2014).

We estimated 4 different models: our main model of interest investigated linear (i.e. persistent) time-by-group interactions. The second model explored quadratic (i.e. transient) relations between group and time; in both cases we hypothesised greater changes in the training group. The third and fourth more complex models addressed the question of whether the relation between longitudinal fitness and tissue changes could be modulated by training. Two physiological indices were used to measure fitness: lactate production delta change between the end and the beginning of the intervention (inversely related to fitness level) and oxygen consumption due to respiratory compensation at the sub-maximal level right before exhaustion (directly related to fitness level).

Variables such as age, gender and BMI were treated as confounders in all models.

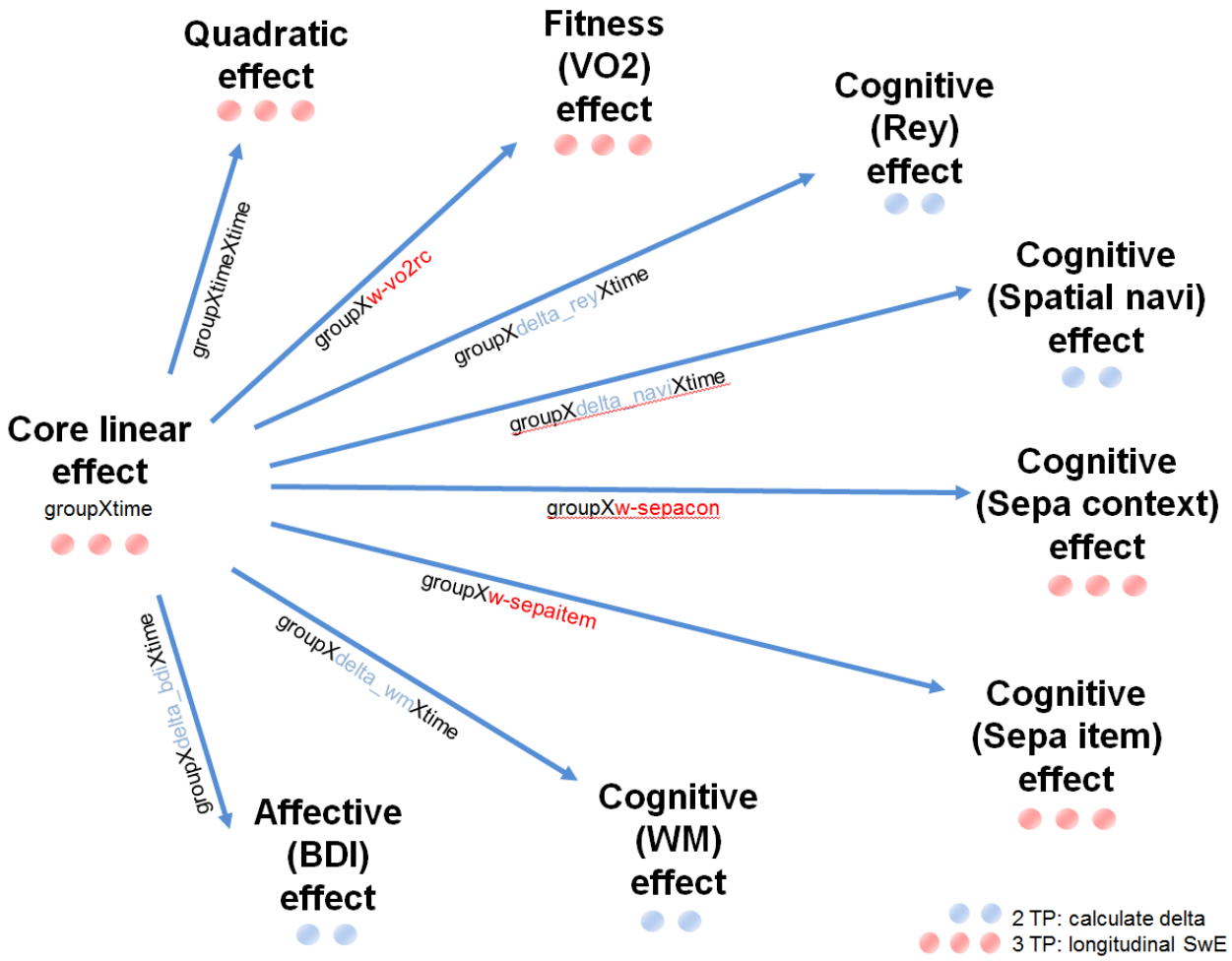
We applied Small Volume Correction (SVC) to correct for multiple comparisons for each ROI separately. We considered a voxel-based statistical threshold of $p < 0.05$ corrected for multiple comparisons using the Family-Wise Error (FWE) method implemented in SPM. Nevertheless, voxels surpassing the threshold but resulting from uncorrected analyses ($p<0.001$) are descriptively reported as statistical trends to inform future studies.

In a further step, we investigated cognitive-related volumetric change. To do that, we tested for group-by-time-by-cognitive change effects, where the type of cognitive variable varied across our three models: Rey figure, spatial navigation and working memory scores. For all of the cognitive measures, only two time points were available, therefore we computed a delta

value by subtracting the score of the initial time point to the score of the final one. In the first model, we introduced Rey figures' delta change. 3 participants had missing values in either the initial or the final time point or both, thus the analysis was run on a sample of 37 subjects. In the second model, we used delta scores of our spatial navigation task. In this case only one participant reported a missing value, hence the necessity of reducing the sample to 39. Finally, in the third cognitive model, our working memory delta score was computed and introduced. Here, 4 participants were removed due to missing values. In all the three models testing for cognition, a whole-brain grey matter mask was applied, and the same variables of non-interest were taken out.

Subsequently, cortical regions showing interesting results in the whole-brain analysis were tested for SVC, namely angular gyrus, middle temporal gyrus, posterior orbital gyrus and superior frontal gyrus (details are discussed in the Results section).

Overview of the models:



ROI analysis

The choice of the regions of interest (ROIs) was based on the background literature on fitness intervention and plasticity.

A great amount of research has shown that the hippocampus is one of the most sensitive regions to training-induced structural change, possibly mediated by local increase in vascular perfusion (Maass et al., 2015). However, given the often ambiguous findings, a recent systematic meta-analysis (Firth et al., 2017) took into account 14 different studies on training-induced hippocampal plasticity, revealing a significant increase in the left hippocampus only. As a result, the hippocampus was included among our ROIs.

Another group of studies (e.g. Granert et al., 2011 and Draganski et al., 2004) found structural change in motor cortices following physical training. Nonetheless, there is the possibility that this expansion is quite acute and rapid (Taubert et al., 2016) but normalises afterwards (Wenger et al., 2017).

A recent study by Müller et al. (2017) confirmed an effect on precentral gyri (corresponding to motor cortices) and suggested a potential implication of parahippocampal gyri, respectively associated with increased attention and verbal memory, hence our inclusion of both precentral and parahippocampal gyri as ROIs.

Furthermore, physical activity was associated with grey matter expansion in prefrontal and cingulate cortex by Flöel et al. (2010) and by Reiter et al. (2015). Prefrontal change even correlated with performance in Taubert et al. (2010) and with functional connectivity changes in prefrontal and supplementary motor areas in Taubert et al. (2011). Given this evidence, we included medial frontal cortices as a broad ROI, and subcallosal area as a more specific one.

We also added typical movement-related regions, namely cerebellum and basal ganglia – specifically putamen and caudate nucleus. Indeed, cerebellar volume was proved to increase as a function of physical training and BDNF rise (Ben-Soussan et al., 2015).

Basal ganglia structural changes along with executive functions' enhancement after physical exercise were found in Niemann et al. (2014), Motl et al. (2015) and Becker et al. (2016). Another study reported a lactate rise only on the contralateral side of basal ganglia in occurrence with finger movements, highlighting a direct relation between this regions and fitness measures, given that lactate can be used as a source of energy by neurons (Kuwabara et al., 1995).

Next to such previous findings, our choice was also further corroborated by the well-established key role of these structures in motor processing.

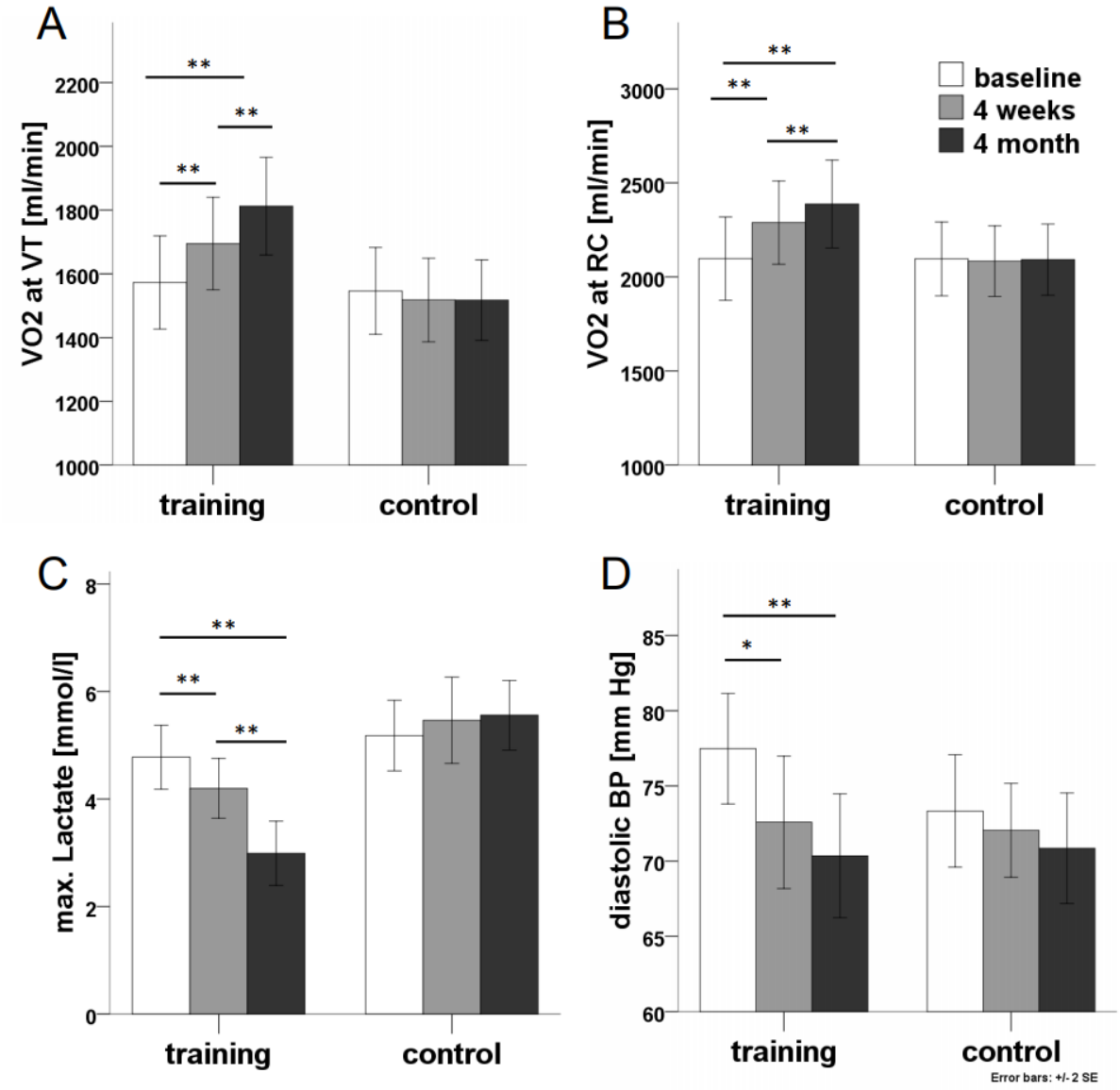
With the aim of referring to a more accurate template, we created a high-resolution sample-specific gray matter mask based on the difference between the overall mean image of the trainers and the overall mean image of the controls. To do this, we used spm_mean function to compute the means and imCalc to derive their difference.
We generated bilateral single-region masks based on the above-mentioned gray matter template and SPM12-provided neuromorphometric atlas. Eventually selected regions of interest were: caudate (corresponding to neuromorphometric atlas' numbers 11/12), putamen (29/30), hippocampus (21/22), parahippocampal gyrus (107/108), anterior cingulate gyrus (45/46), medial frontal cerebrum (79/80), precentral gyrus (119/120), subcallosal area (123/124), external cerebellum (13/14).

Results

Training-induced fitness changes

The estimated VO_2 mean score at time point one was 1578 (308) ml/min and blood lactate mean of maximum intensity was 4.97 (1.38) mmol/l. As shown in the bar graphs (see Figure 1), groups did not differ at baseline. In the training group, VO2 score (both at VT and RC) showed a linear increase after 1 month and 4 months, parallel to a linear decrease in blood lactate and diastolic blood pressure (BP), jointly confirming fitness improvement. This is consistent due to the known inverse correlation between these measures. Being these trends not visible in the control group, we can conclude that the fitness effect occurs selectively in the training condition.

Figure 1. Graph of fitness effects in terms of $VO_{2\ VT}$ (A), $VO_{2\ RC}$ (B), blood lactate (C) and diastolic BP (D). Fitness change was significant after 4 weeks and after 4 months compared to baseline, as well as between the fourth week and the fourth month (except in the case of diastolic BP). Significant results ($p<0.05$) are reported with an asterisk.



Training-induced structural brain changes

Linear effects in structural time-courses

We found a significant positive linear effect on the right hippocampus with a p value below 0.05 (Z=4.46, p=0.014, FDR), indicating significant volume increase after training (Figures 2-3).

Additionally, results show a linear trend towards volume increase also on the right medial frontal portion of the cerebrum, without however reaching significance (Z=3.24, p=0.074; Figures 4-5). Further slighter trends have been found on right parahippocampal gyrus (Z=3.66, p=0.596) and left exterior cerebellum (Z=3.10, p=0.868). Results are summarised in Table 1.

In order to control for hydration/de-hydration effects possibly temporarily altering brain atrophy, we introduced temperature inside the scanner and osmolality as confounding variables

in our linear model. Similarly, in order to eliminate compression effects in posterior regions due to sleeping posture, we controlled for scanning time during the day as a confounding variable. However, they didn't turn out to drive much variance, neither at the between- nor the within-subject level.

Figure 2. Projection map of significant linear effects on right hippocampus (Z=4.46, FDR=0.014). Z scores are superimposed to a grey matter template generated from T1 averaged images of the whole sample.

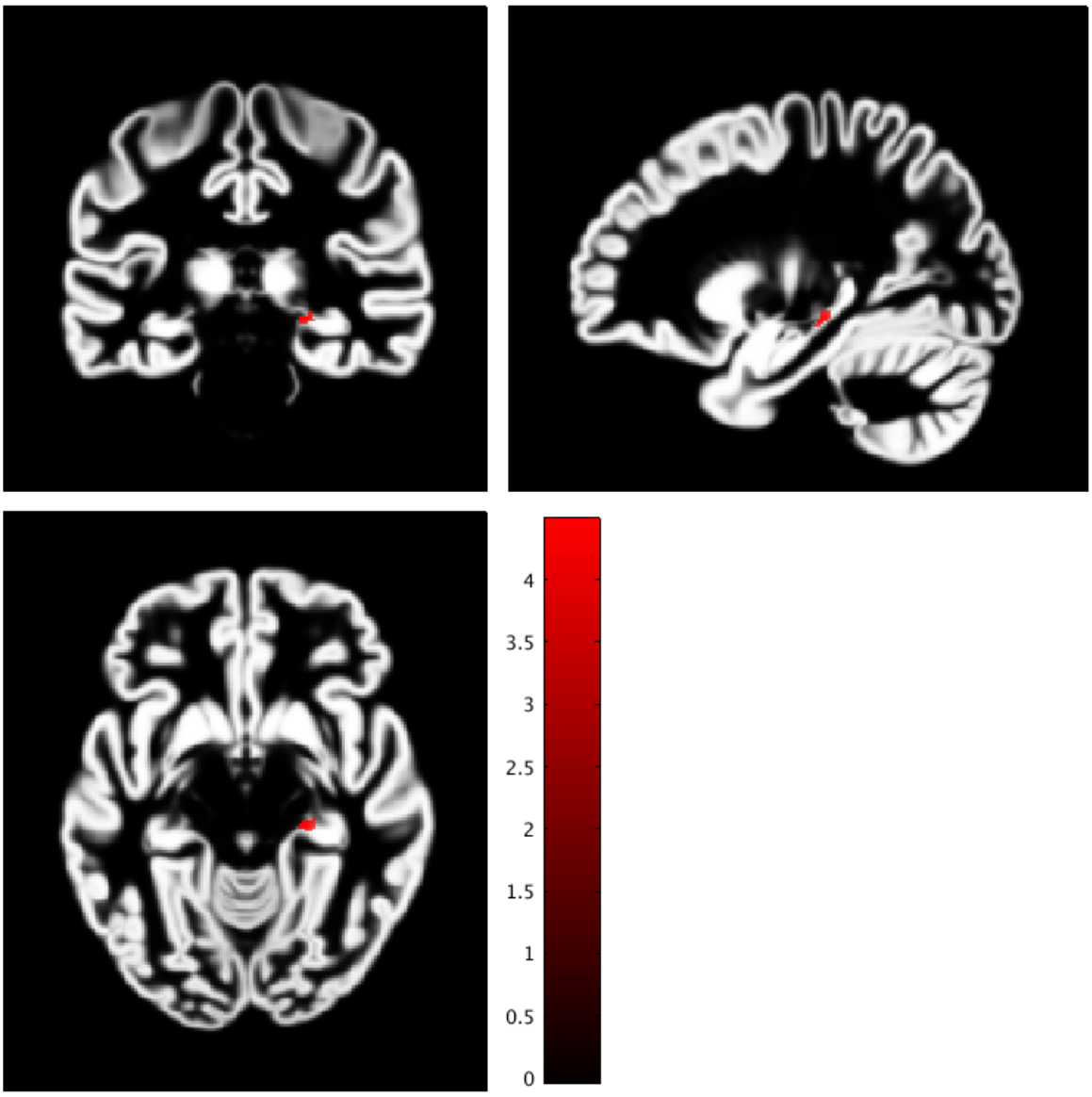


Figure 3. Plot of significant linear effects on right hippocampus. The plot represents adjusted data (age, gender and BMI were taken out as covariates of no-interest), with time in months on the x axis and grey matter volume (whole brain) on the y axis. The training group (red line) shows a steeper increase in grey matter volume compared to the control group (orange line), modelling raw data of the trainers (dark-grey lines on the background) and the controls (light-grey lines on the background).

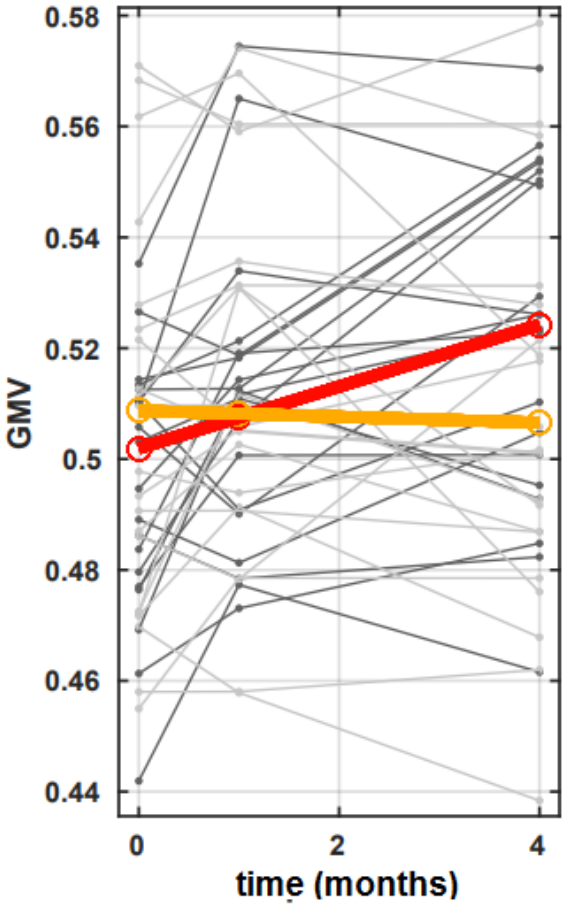


Figure 4. Projection map of main linear volumetric effects: additional statistical trends on right medial frontal cerebrum (Z=3.24, FDR=0.074), together with the already mentioned significant effect on right hippocampus. Uncorrected Z scores are superimposed to a grey matter template generated from T1 averaged images of the whole sample.

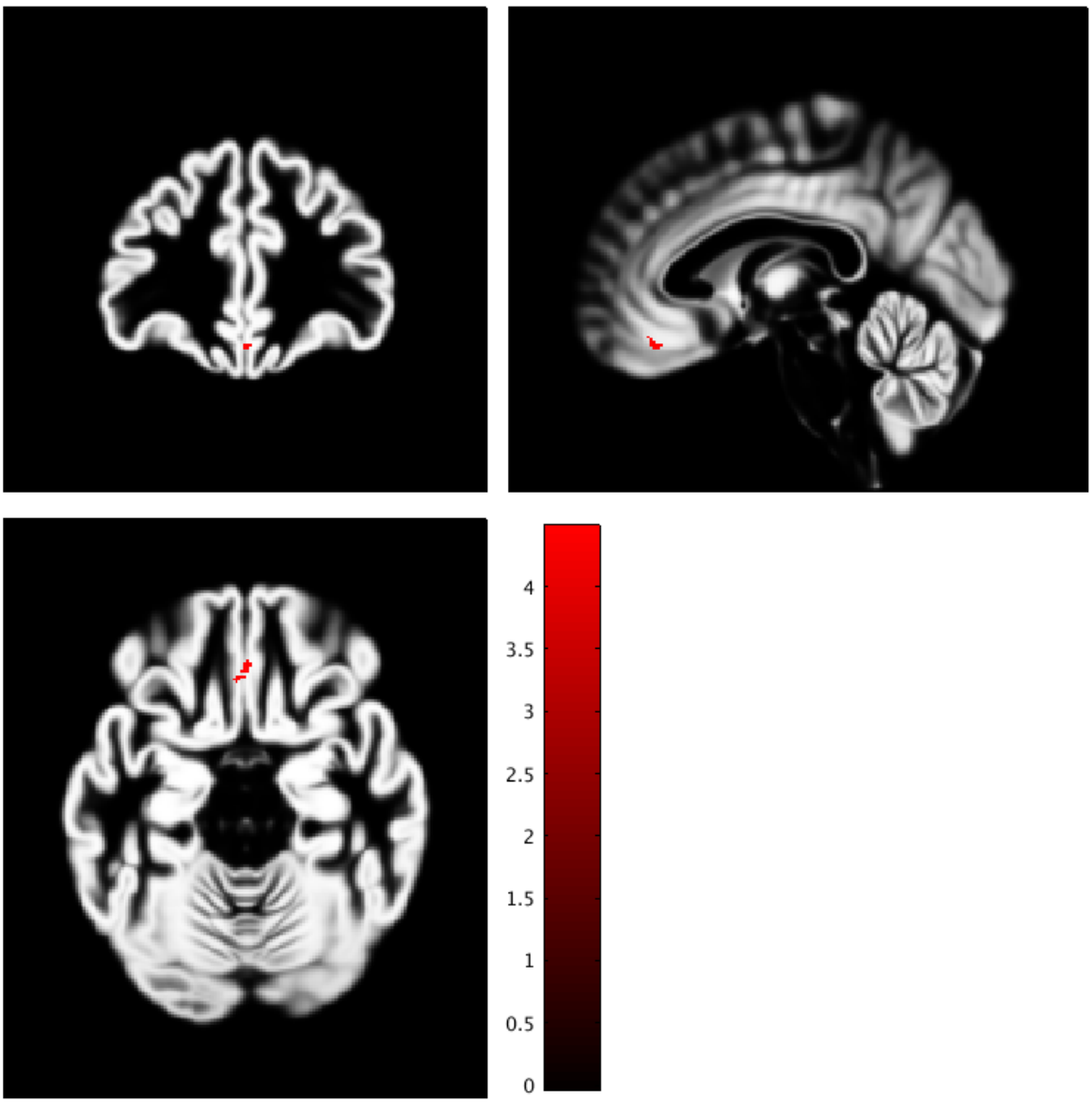
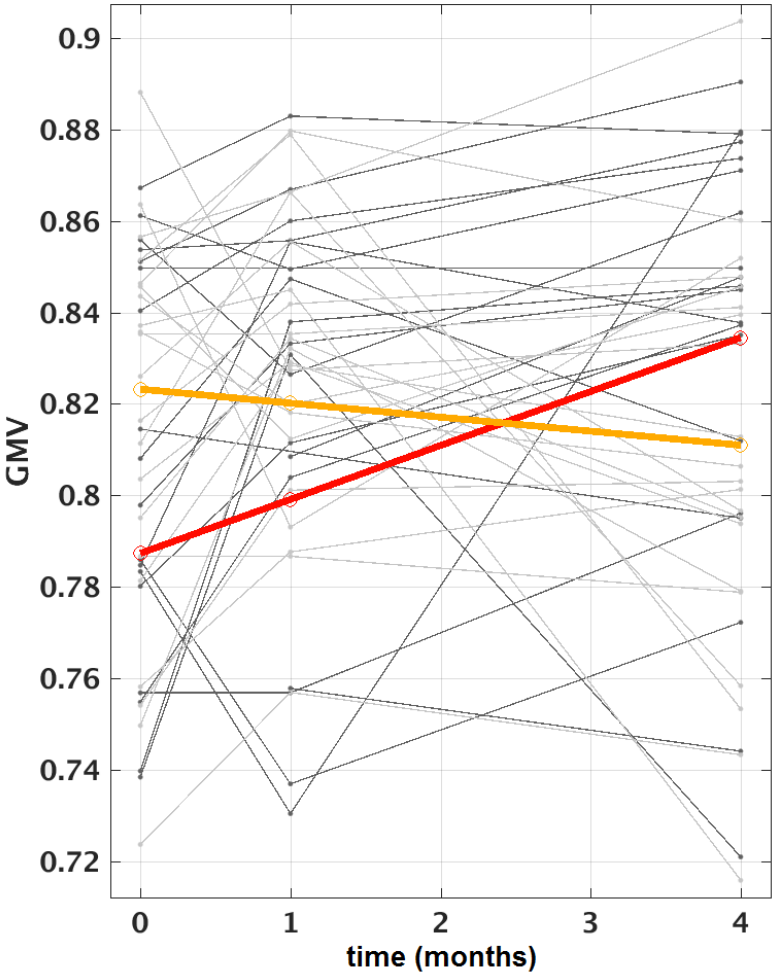


Figure 5. Plot of linear statistical trends. The plot represents adjusted data (age, gender and BMI were taken out as covariates of no-interest), with time in months on the x axis and grey matter volume (whole brain) on the y axis. The training group (red line) shows a steeper increase in grey matter volume compared to the control group (orange line), modelling raw data of the trainers (dark-grey lines on the background) and the controls (light-grey lines on the background).



Quadratic effects in structural time-courses

As shown in Table 1, the quadratic model revealed tissue expansion tendency on left caudate nucleus only, without surviving the statistical threshold (Z=3.17, p=0.259).

Fitness-associated brain changes

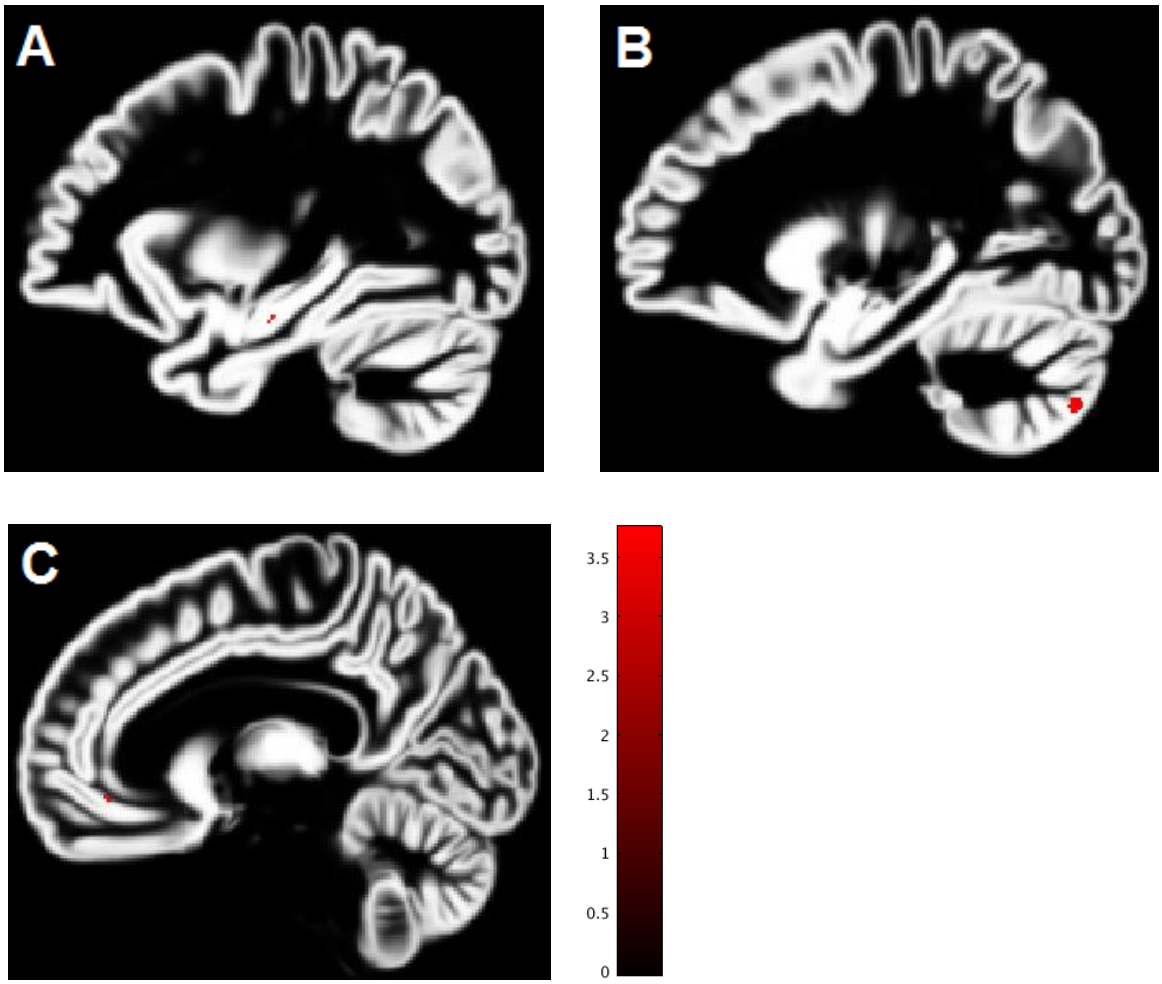
This model tested for individual variations in grey matter volume driven by within-subject change in oxygen consumption at respiratory compensation ($VO_{2\ RC}$), regardless the group condition. Our findings suggest that the relation between grey matter volume and fitness varies

depending on the grade of fitness change, specifically on left hippocampus (Z=3.74, p=0.150), left and right exterior cerebellum (Z=3.81; Z=3.35, p=0.364) and left anterior cingulate gyrus (Z=3.23, p=0.747; Figure 6). Despite not reaching significance, these results are overlapping with the structural linear group effect, therefore suggesting that most structural changes are induced by – or at least related to – fitness change. Trends' values are reported in Table 1.

Table 1. Linear, quadratic and fitness effects following SVC. Significant results ($p<0.05$) are reported with an asterisk.

ROI		Linear effects		Quadratic effects		VO_2-related effects	
		P value	Z value	P value	Z value	P value	Z value
Hippocampus	Left	--	--	--	--	0.150	3.74
	Right	0.014*	4.46	--	--	--	--
Medial frontal cerebrum	Left	--	--	--	--	--	--
	Right	0.074	3.24	--	--	--	--
Caudate	Left	--	--	0.259	3.17	--	--
	Right	--	--	--	--	--	--
Cerebellum exterior	Left	0.868	3.10	--	--	0.364	3.81
	Right	--	--	--	--	0.364	3.35
Anterior cingulate	Left	--	--	--	--	0.747	3.23
	Right	--	--	--	--	--	--
Parahippocampal gyrus	Left	--	--	--	--	--	--
	Right	0.596	3.66	--	--	--	--
Precentral gyrus	Left	--	--	--	--	--	--
	Right	--	--	--	--	--	--
Putamen	Left	--	--	--	--	--	--
	Right	--	--	--	--	--	--
Subcallosal area	Left	--	--	--	--	--	--
	Right	--	--	--	--	--	--

Figure 6. Projection map of main volumetric effects driven by fitness change in terms of $VO_{2\,RC}$ on (a) left hippocampus (Z=3.74, FDR=0.150), (b) left cerebellum (Z=3.81, FDR=0.364), right cerebellum (Z=3.35, FDR=0.364), and (c) left anterior cingulate gyrus (ACG, Z=3.23, FDR=0.747). Uncorrected Z scores are superimposed to a grey matter template generated from T1 averaged images of the whole sample (sagittal view).



Cognition-related brain structural changes

Our exploratory whole-brain analysis on the relation between improvement in the Rey figure task and tissue growth reveals remarkable effects spread on parietal and temporal regions. Given the well-known involvement of visuospatial processing in this task, such areas seem particularly consistent and promising.

As a consequence, we performed SVC on the main resulting peaks, namely angular gyrus and middle temporal gyrus for several cognitive measures, namely the RCFT, spatial navigation and visual working memory (delayed-match-to-sample).

For the RCFT we found a significant effect on right middle temporal gyrus (Z=4.43, FDR=0.043), as well as statistical tendencies on left middle temporal gyrus (Z=3.74, FDR=0.056) and right angular gyrus (Z=4.45, FDR=0.057; Figure 7). These findings speak in favour of a training-induced improvement in cognition that is mediated by structural change in grey matter tissue.

For spatial navigation delta change the preceeding whole-brain analysis indicated a volume increase of frontal structures. In particular, SVC was performed for posterior orbital and superior frontal gyri, approaching – yet not reaching – statistical significance. As summarised in Table 2, left posterior orbital gyrus reports values of Z=3.89 and FDR=0.057, whereas left superior frontal gyrus Z=3.69 and FDR=0.228 (Figure 8). Nonetheless, given the specificity of such volumetric effects, we argue that these trends should be taken into account for further research.

Similarly, our first exploratory whole-brain analysis on working memory change related to fitness suggests occipital and thalamic involvement. Deeper SVC uncovers volume increase trends on left thalamus proper (Z=3.72, FDR=0.059; Figure 9).

An overview of cognitive effects induced by training and mediated by structural plasticity is reported in Table 2.

Table 2. Cognitive effects following SVC. Significant results ($p<0.05$) are reported with an asterisk.

ROI		Rey figure		Spatial navigation		Working memory	
		P value	Z value	P value	Z value	P value	Z value
Angular gyrus	Left	--	--	--	--	--	--
	Right	0.057	4.45	--	--	--	--
Middle temporal gyrus	Left	0.056	3.74	--	--	--	--
	Right	0.043*	4.43	--	--	--	--
Posterior orbital gyrus	Left	--	--	0.057	3.89	--	--
	Right	--	--	--	--	--	--
Superior frontal gyrus	Left	--	--	0.228	3.69	--	--
	Right	--	--	--	--	--	--
Middle occipital gyrus	Left	--	--	--	--	0.350	3.88
	Right	--	--	--	--	--	--
Thalamus proper	Left	--	--	--	--	0.059	3.72
	Right	--	--	--	--	--	--

Figure 7. Projection map of main volumetric effects driven by cognitive change in Rey figures performance. (a) Significant effects ($p<0.05$) have been found on right middle temporal gyrus (rMTG, Z=4.43, FDR=0.043). (b) Additional statistical trends approaching significance are reported on left middle temporal gyrus (lMTG, Z=3.74, FDR=0.056) and right angular gyrus (rANG, Z=4.45, FDR=0.057). Z score are superimposed to a grey matter template generated from T1 averaged images of the whole sample (sagittal view).

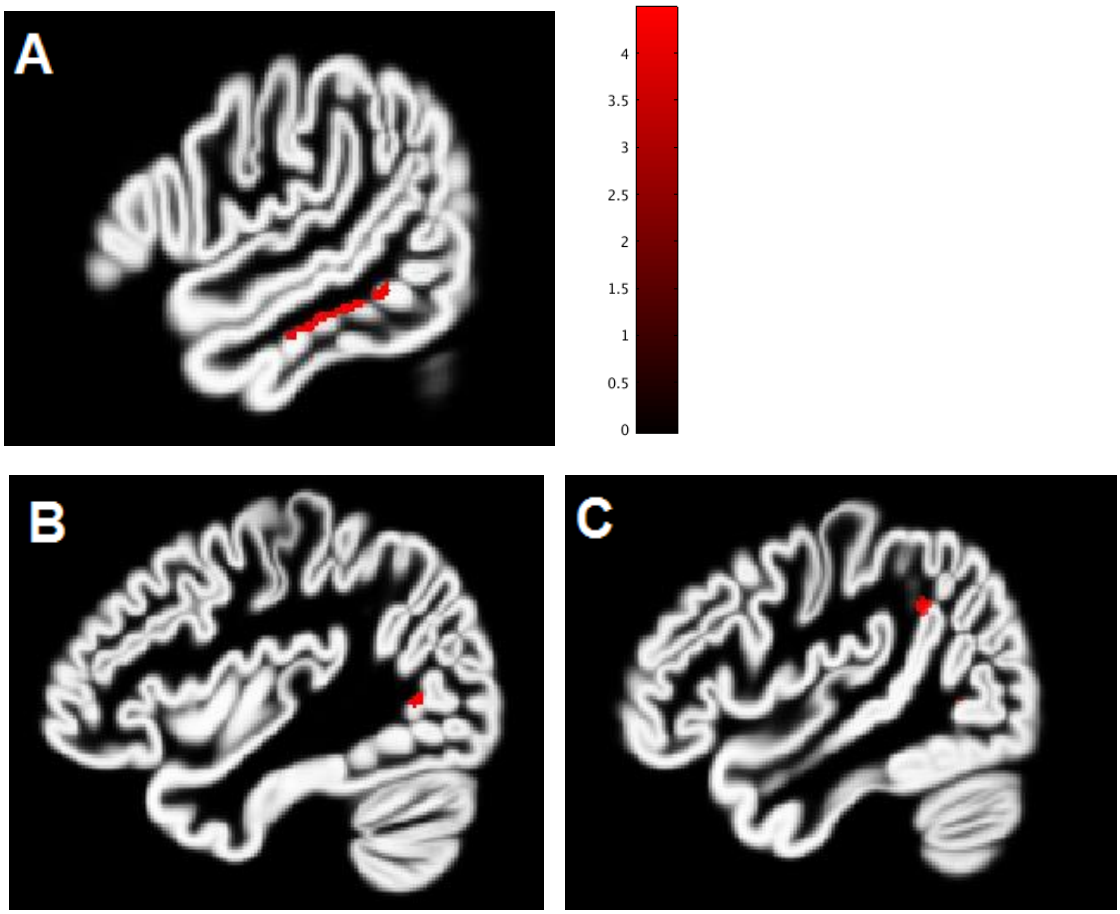


Figure 8. Projection map of main volumetric effects driven by cognitive change in Spatial Navigation performance on (a) left posterior orbital gyrus (Z=3.89, FDR=0.057) and (b) left superior frontal gyrus (Z=3.69, FDR=0.228). Uncorrected Z scores are superimposed to a grey matter template generated from T1 averaged images of the whole sample (sagittal view).

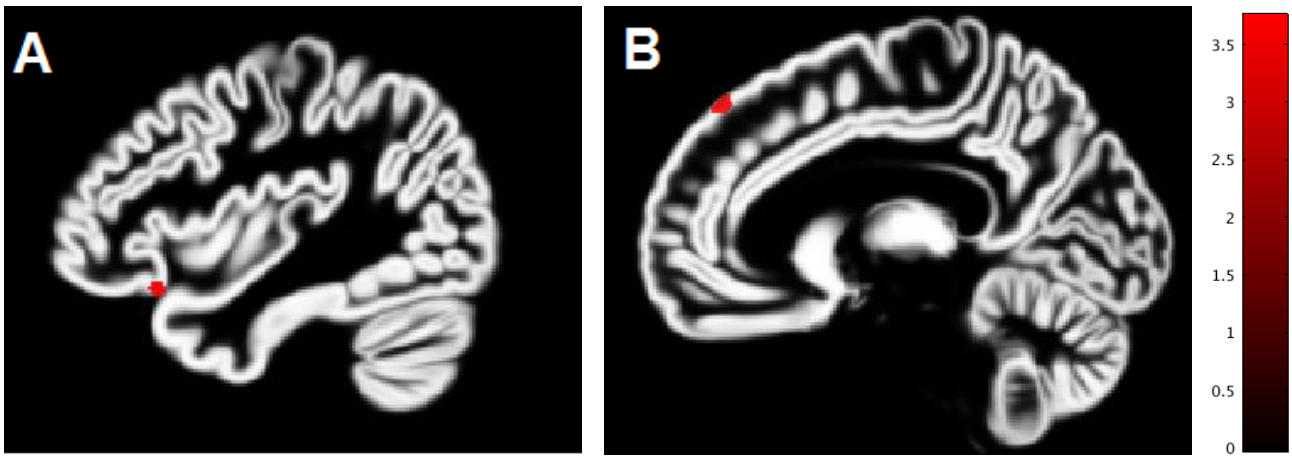
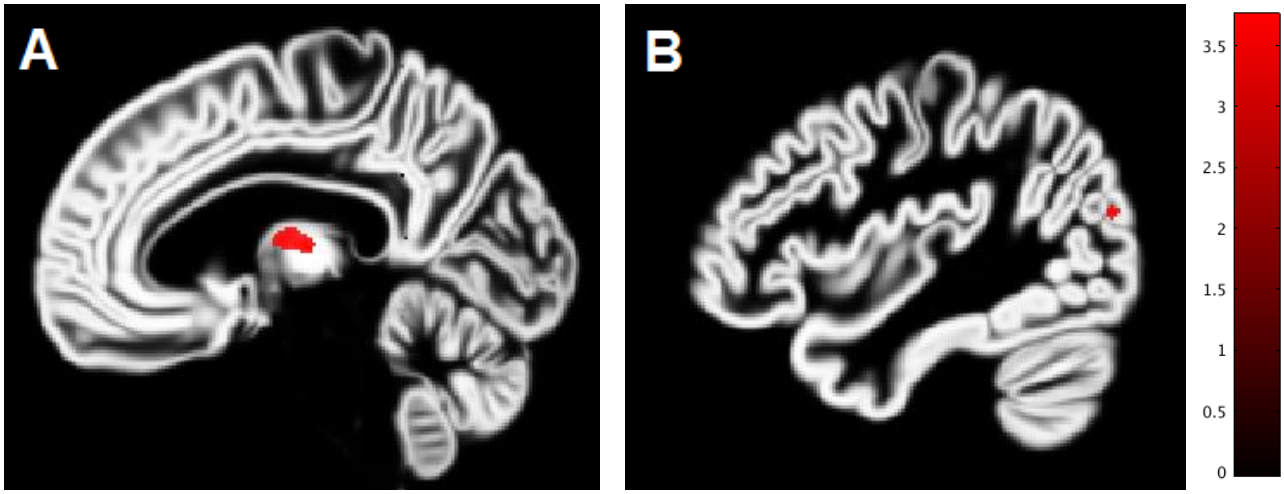


Figure 9. Projection map of main volumetric effects driven by cognitive change in Working Memory performance on (a) left thalamus proper (Z=3.72, FDR=0.059) and (b) left middle occipital gyrus (Z=3.88, FDR=0.350). Uncorrected Z scores are superimposed to a grey matter template generated from T1 averaged images of the whole sample (sagittal view).



Post-hoc whole-brain analysis

As a further step, we performed exploratory post-hoc analysis with no a priori ROIs but instead a single whole-brain grey matter mask, in order to check for unconsidered and unexpected changes out of our hypothesided network (see 5.b section "ROIs analysis").
Along with the predicted above-mentioned regions, we found positive linear trends on left middle occipital gyrus (Z=3.47), right and left angular gyrus (Z=3.46; Z=3.42), left mediotemporal gyrus (Z=3.33) and right superior frontal gyrus (Z=3.33). Transient quadratic effects were found on left precuneus (Z=3.59), left and right angular gyrus (Z=3.58; Z=3.54), right superior parietal lobule (Z=3.52), left middle frontal gyrus (Z=3.46) and left temporal pole (Z=3.36; Figure 10).
Group-dependent VO2-related volumetric trends outside our a priori network were encountered with quite high Z values on right inferior temporal gyrus (Z=4.10), left thalamus (Z=4.07), right lingual gyrus (Z=4.04), vermis and exterior cerebellum (Z=3.86; Z=3.81; Figure 11).
Altogether, post-hoc results on whole-bain gray matter suggest spread trends of tissue expansion on temporo-parietal regions specific for the training group, yet without reaching statistical significance.
We then performed the same procedure with a whole-brain white matter mask, with a view to testing for other tissues' effects on top of grey matter ones. Surprisingly, we found significant

white matter expansion especially in posterior regions such as right and left cuneus (Z=5.22, FDR=0.032; Z=4.79, FDR=0.038), right cerebral white matter (Z=4.62, FDR=0.043), left superior parietal lobule (Z=4.53, FDR=0.044) and right medial precentral gyrus (Z=4.49, FDR=0.044). See Table 3 for an overview of whole-brain results; Figure 12 for plotted visualisation.

Table 3. Whole brain analysis. Statistic results for time varying linear, quadratic and fitness-induced effects. Significant results ($p<0.05$) are reported with an asterisk.

	GM	p	Z	WM	p	Z
Linear	L middle occipital	0.986	3.47	L inferior temporal	0.501	4.41
	R angular	0.986	3.46	R middle frontal	0.501	4.04
	L angular	0.986	3.42	R temporal pole	0.568	3.88
	L middle temporal	0.986	3.33	R cerebellum	0.568	3.83
	R superior frontal	0.986	3.33	L superior frontal	0.576	3.69
				L temporal pole	0.590	3.62
Quadratic	L precuneus	1.000	3.59	R precuneus	0.314	4.20
	L angular	1.000	3.58	R lingual	0.314	4.00
	R angular	1.000	3.54	R cerebral WM	0.314	3.95
	R superior parietal	1.000	3.52	L parahippocampal	0.314	3.88
	L middle frontal	1.000	3.46	R calcarine	0.314	3.73
	L temporal pole	1.000	3.36	R lingual	0.314	3.73
VO2	R inferior temp	0.788	4.10	R cuneus	0.032*	5.22
	L thalamus	0.788	4.07	L cuneus	0.038*	4.79
	R lingual	0.788	4.04	R cerebral WM	0.043*	4.62
	vermis	0.788	3.86	L superior parietal	0.044*	4.53
	L cerebellum	0.788	3.81	R medial precentral	0.044*	4.49

Figure 10. Projection maps from post-hoc grey matter whole-brain analyses, later further investigated through Small Volume Correction (SVC). Quadratic effects appeared to be spread on parietal and temporal regions, specifically (a) left and right angular gyrus (Z=3.58; Z=3.54), (b) left precuneus (Z=3.59), (c) right superior parietal lobule (Z=3.52), (d) left middle frontal gyrus (Z=3.46), (e) left temporal pole (Z=3.36). Resulting Z values (uncorrected p<0.001) have been superimposed to a grey matter template originated from the average of the sample.

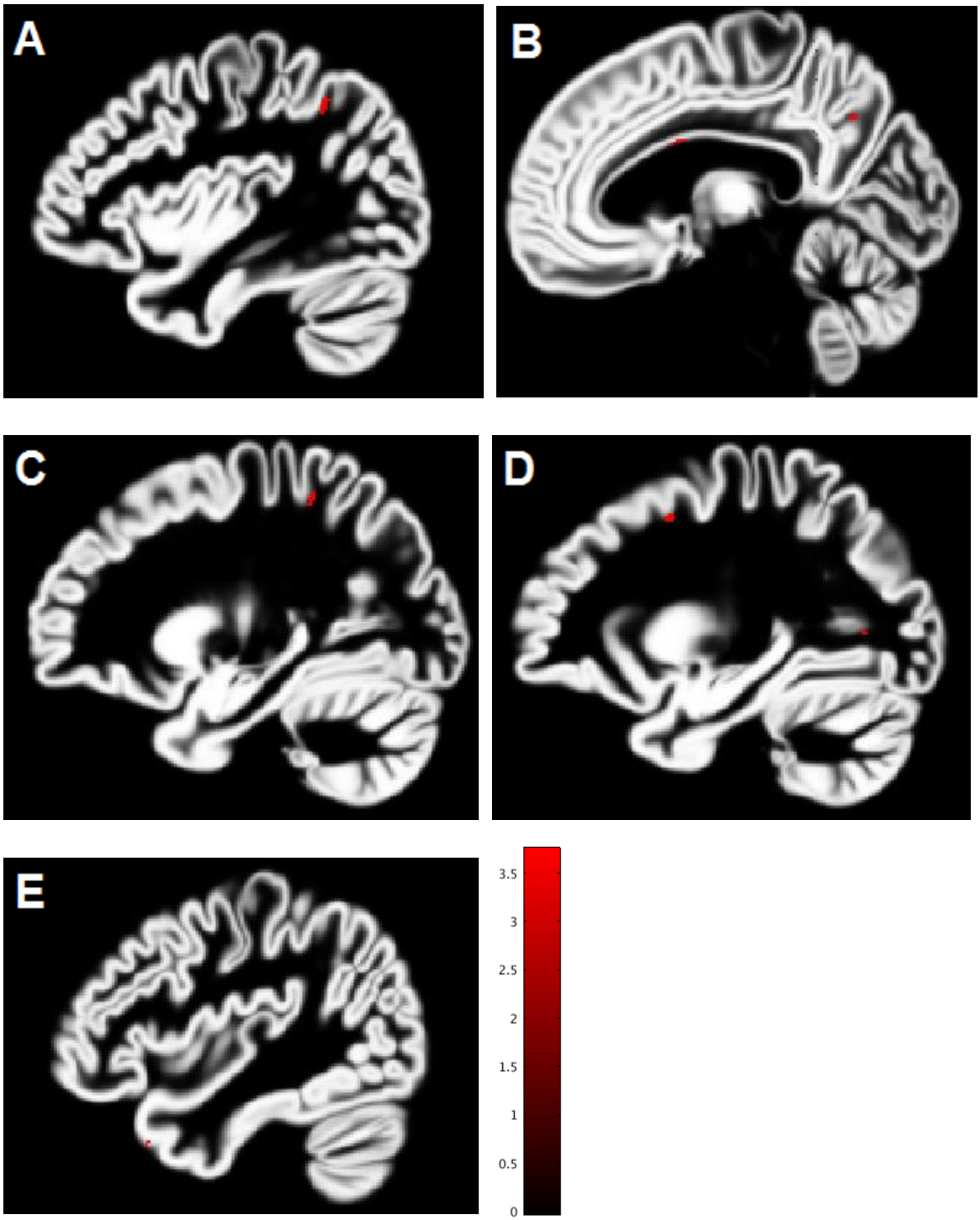


Figure 11. Projection maps from post-hoc grey matter whole-brain analyses, later further investigated through Small Volume Correction (SVC). Fitness-related (VO2) structural effects have been found specifically on (a) right lingual gyrus (Z=4.04), (b) vermis (Z=3.86) and (c) left external cerebellum (Z=3.81), (d) right inferior temporal gyrus (Z=4.10), (e) left thalamus proper (Z=4.07). Resulting Z values (uncorrected $p<0.001$) have been superimposed to a grey matter template generated from T1 averaged images of the whole sample.

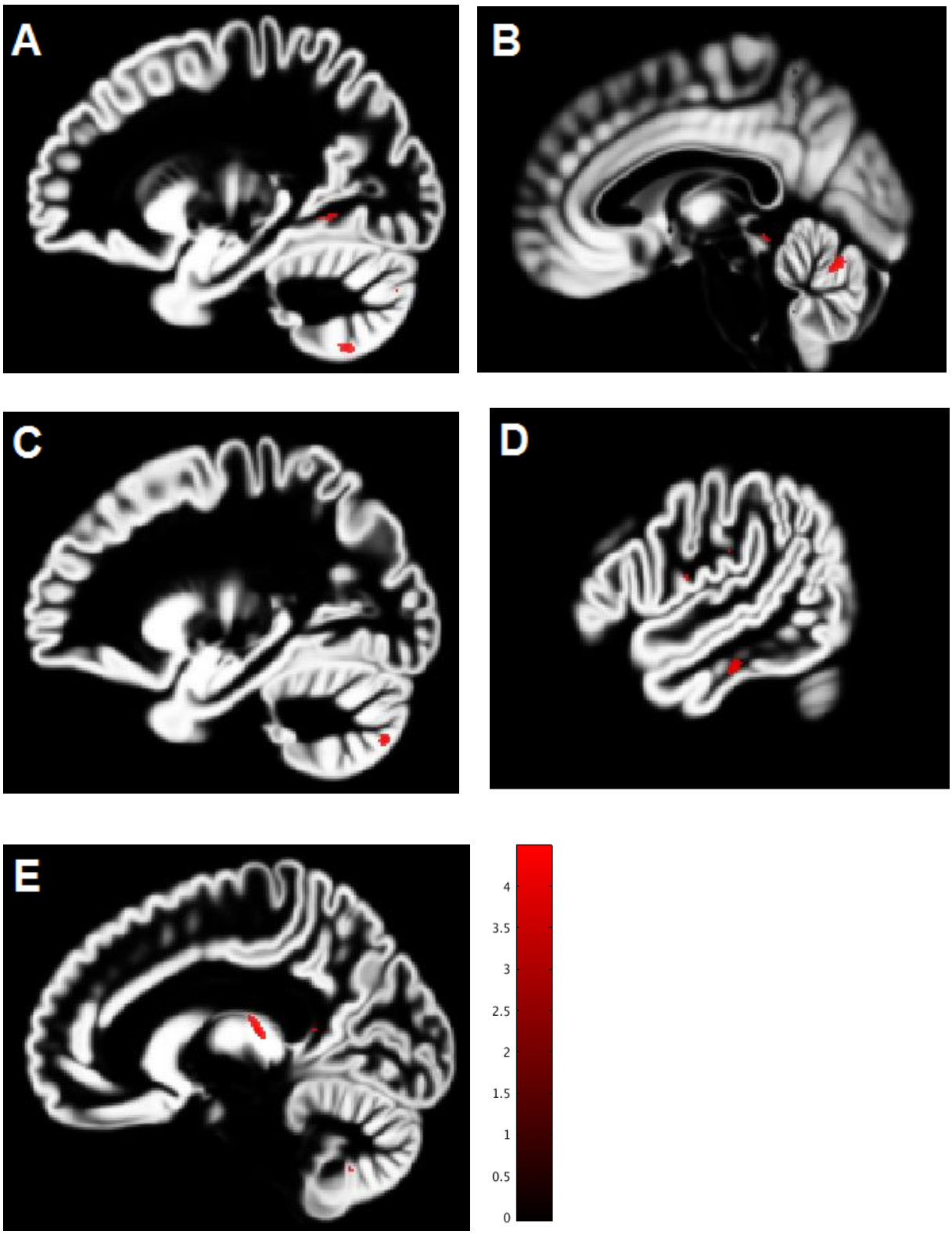
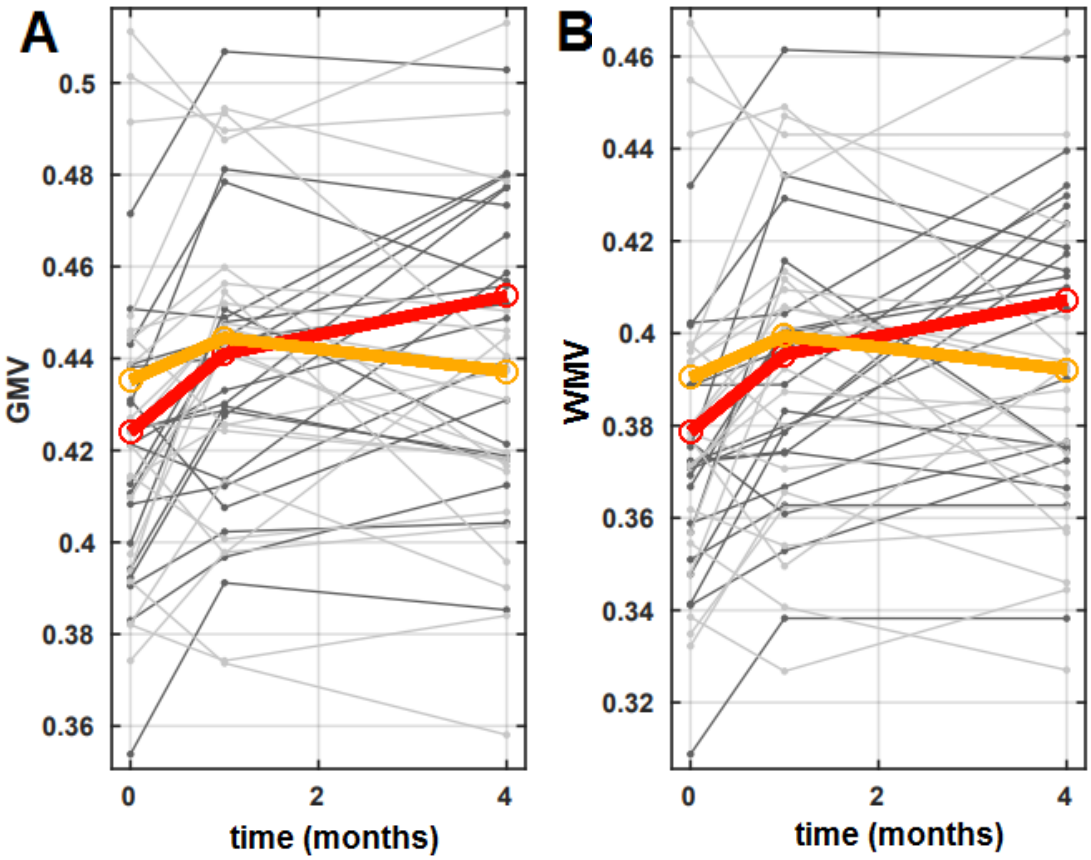


Figure 12. Plots of whole-brain quadratic effects on (a) grey matter (GMV) and (b) white matter volume (WMV). The plots represent adjusted data (age, gender and BMI were taken out as covariates of no-interest), with time in months on the x axis and tissue volume (whole brain) on the y axis. The training group (red line) shows a greater quadratic increase in both tissue types' volume compared to the control group (orange line), modelling raw data of the trainers (dark-grey lines on the background) and the controls (light-grey lines on the background).



Discussion

We observed significant linear volume increase on the right hippocampus after intervention. Since this effect is group-dependent, occurring only in trainers, we can conclude that it is induced by aerobic physical training. Except lateralisation, this finding is aligned with previous literature, where hippocampus seems to be particularly susceptible to structural plasticity regardless of age and clinical status (Firth et al., 2017).

In contrast, our other regions of interest did not reach significance level. Nonetheless, we believe that some additional above-threshold statistical trends should be taken into account for further investigation, considering the tiny effect size one could expect from such intervention studies on younger healthy subjects with structural plasticity as a dependent variable. In fact, these VBM studies are typically over-conservative due to the multiple comparison problem of the voxel-based approach. To our view, the most promising one is the linear effect on the right medial frontal cerebrum. We also controlled for within-subject changes in temperature experienced inside the scanner and plasma osmolality measures, as a proxy of potential de-hydration mechanisms which might in turn temporarily alter brain volume. This good practice is also reported and advised in the literature (Biller et al., 2015). Additionally, we checked whether the scanning time during the day introduced a bias in the results, possibly due to compression effects. Our main linear effects were confirmed even when including these variables in the model, demonstrating that neither de-hydration nor compression drive relevant pieces of variance.

Fitness effects

The attempt of this study was also to assess how much of the within-subjects variance is directly driven by fitness-related physiological parameters associated to sport, such as oxygen consumption and lactate production. Between the two, the individual change in the amount of oxygen consumption at the time of respiratory compensation appears to be a much better predictor, based on higher Z values on those same areas showing linear volumetric effects, namely hippocampus and medial frontal cortex. These results are highly promising given the functional and clinical relevance of the regions involved. The hippocampus is well-established as the beholder of short-term and long-term episodic and spatial memory, thus being massively investigated by researchers and clinicians interested in dementia and neurodegenerative disorders involving mnemonic deficits. Its atrophy is a reliable and powerful marker of

Alzheimer's dementia onset, yet to which degree depends on age (Van de Pol et al., 2006; Henneman et al., 2009).

Ventromedial regions are crucial in cognitive control, inhibition and associations between somatic or visceral markers and emotions or mental objects, which will then be recalled in decision making processes. In fact, according to Hänsel and Känel (2008), activity in ventromedial prefrontal cortex (especially in the right hemisphere) modulates parasympathetic and negatively arousing responses, possibly mediated by vagal nerve. Consistently, depressive scores are usually linked to inefficient vmPFC top-down regulation of amygdala. If this is the case, vmPFC could be crucial in stress reaction, which might be down-regulated by physical activity and fitness. One possibility is that the volume increase that we attribute to fitness could also be indirectly mediated by emotional and stress relaxation. Indeed, animal models demonstrate chronic stress-induced volume loss, dendritic atrophy and decreased vmPFC inhibition, which in turn yield autonomic and behavioural dysfunction.

Given the linearity of our core model, these changes are supposed to show up gradually across the intervention period. Interestingly, our experimental set-up demonstrates that persistent volumetric changes are possible and visible already after four months of training, which is a relatively short period.

Quadratic component

We observed even faster and more acute volumetric effects in movement-related regions such as caudate nucleus belonging to basal ganglia, but not in a stable fashion in the long run. This pattern nicely aligns with previous animal and human research: Becker et al. (2016) demonstrated the possibility of neuroplasticity in basal ganglia following physical exercise, thanks to its influence on their molecular architecture and metabolic capacity. Indeed, basal ganglia structures are known to be crucial not only in motor initiation and execution but also in cognitive functions such as mental flexibility, task-switching and cognitive control. Consequently, neurophysiological disruption of these circuits (e.g. in Parkinson's disease) comes together with cognitive decline. According to the literature, within basal ganglia system, caudate nucleus and putamen show a particularly steep volume change across lifespan (Becker et al., 2016), therefore it wouldn't be surprising that they are more prone to be affected by intervention. From a clinical point of view, experience-dependent neuroplasticity (i.e. functional and structural neuronal modulation through exercise intervention and motor learning) has been proved to benefit animal models of Parkinson's disease, by stimulating adaptive changes in basal ganglia circuits in terms of neurotransmitters and neurotrophic

factors (Petzinger et al., 2011). Nonetheless, in contrast to Motl et al. (2015) reporting significant associations between all basal ganglia structures (i.e. caudate nucleus, putamen and globus pallidum) volume and VO_2 peaks, our basal ganglia-related effects were specific for caudate nucleus only, whereas putamen did not show any change.

We also did not find any effect on precentral gyri, in disagreement with Granert et al. (2011), Draganski et al. (2004) and Müller et al. (2017), that reported training-induced volumetric changes in motor cortices. Importantly, in these papers participants were asked to perform motor tasks that were more specific than our general treadmill fitness activity.

Although our quadratic results did not survive statistical threshold, they point to the potential presence of very early-adaptive changes. Out of speculation, our suggestion is that basal ganglia might be more easily plastic but less stable, therefore motor programs encoded in them might result efficient in the learning process of daily specific activities but not as long-term therapeutic strategy or prevention. In contrast, hippocampus and ventromedial regions of the brain need more time to rearrange their structure in response to training, however, given their stable trend and their central role in cognitive control and learning, they could be promising as potential intervention targets.

To our knowledge, only Woost et al. (2018) investigated fitness-induced structural time-courses at high-field strengths in humans, and few other studies provided three time points instead of only two. As a consequence, we highly encourage upcoming repetition of such approach, in order to better trace a temporal frame of cerebral plasticity trigger and duration.

Cognitive effects

On top of between-group tissue expansion driven by exercise intervention, we found within-group variability spread on fronto-temporo-parietal networks driven by cognitive performance. Specifically, we observed significant volume increase on right middle temporal gyrus associated to executive functioning improvement in the Rey figure test. Growth on the left side of middle temporal gyrus and right angular cortex were close to significance level.

Collectively, these data suggest that training can benefit cognitive performance by modulating brain structure. These findings are particularly meaningful since Rey figure task has been previously shown to reflect posterior temporal and parietal cortex functioning, due to the visuospatial abilities involved (Melrose et al., 2013). Even though we selectively accounted for delayed memory recall in our analysis, Rey figure test seems to rely not so tightly on the hippocampal formation, but rather on a broader temporo-parietal network. Next to its traditionally-attributed role in episodic memory, this network has already been proved to

sustain also visuospatial working memory, mental imagery and viewpoint transformations. These include allocentric representations, of which Rey figure test might be an example (Dhindsa et al., 2014).

According to a recently published review, spatial navigation recruits a network-like functional organisation in the human brain. Together with the traditionally associated hippocampal, prahippocampal and entorhinal cortices, as well as the more recently discovered retrosplenial cortices, frontal lobes were proved to contribute especially during routes' planning process (Epstein et al., 2017). In alignment with this body of knowledge, our results show tendencies of training-induced volume increase of frontal regions in relation to spatial navigation improvement, in particular on the posterior side of left orbital cortex.

Finally, consistently to recent evidence confirming the role of the thalamus in working memory processes (Peräkylä et al., 2017), we also observed a similar growth trend of left thalamus selectively associated to working memory improvement. We thus hypothesise that structural plasticity might parallel functional one in specific areas recruited in the task of interest.

Post-hoc whole-brain analysis

Surprisingly, our exploratory whole-brain results indicate relevant volumetric adjustments of both grey and white matter tissues, particularly striking in the latter.

Fitness-induced whole-brain effects have not been sufficiently addressed yet, as it was not the main focus of this study, but our findings encourage further investigation. So far indeed, the research in this field has usually narrowed its focus of interest to a number of brain regions, most likely the hippocampus or specific motor areas. In contrast, these diffuse effects suggest the hypothesis that fitness training might promote a general benefit in the brain, rather than a local one.

In addition, the fact that these effects are mainly present on posterior regions and that they occur in a quadratic fashion (i.e. early-adaptive) might be explained by an improvement in vascular brain perfusion following training. Nevertheless, some additional esploratory analyses seem to indicate that vascular indices don’t explain the totality of these changes. However, we believe this is not enough to exclude an important role of vasculature, which should be therefore further investigated, together with neurogenesis and other potential contributing factors.

As a consequence, in light of these unexpected findings, we encourage a reasoning shift in future studies, considering general rather than local effects, and white matter together with grey matter.

Collectively, according to our findings, physical aerobic exercise affects medial rather than dorsal cortex, but it remains unclear whether its effect is mainly shifted towards anterior or posterior regions, or rather uniformly widespread.
Our interpretation of some inconsistencies with other studies refers to the excessive variety of approaches to this topic in the literature, both in terms of statistical analysis and experimental design. We therefore encourage scientific repetition and agreement on fitness intervention protocols as well as MRI data analyses, with a view to reducing multiplicity. Longitudinal research should address this issue in the upcoming years, by integrating and testing multiple approaches into a hierarchical model, instead of selectively choosing one, thus leading to overfitting and repetition problems. Two pitfalls to be taken into account within the framework of this study are the relatively small sample size (n=40) lowering power, and CAT automatic segmentation's downsides increasing the probability of inaccuracies.

Underlying mechanisms

Up to now, it is not clear which physiological variations really underlie training-induced plasticity. It has been traditionally believed that the molecular mechanism leading from fitness benefit to cerebral volume increase was a rise in the level of growth- and stimulating-factors such as Brain-Derived Neurotrophic Factor (BDNF), insulin-like growth factor-1 (IGF-1) (Heisz et al., 2017) and Granulocyte Colony-Stimulating Factor (G-CSF) (Flöel et al., 2010), ultimately yielding neurogenesis. In spite of previous evidence on correlations between neurogenesis rate and cerebral perfusion (Thomas et al., 2012), later findings stressed the presence of angiogenesis boost (Cassilhas & Tufik, 2016). Angiogenesis can happen through splitting or sprouting of branches, probably due to the augmented pressure on capillaries' walls jointly with training-induced mild hypoxia. Physical exercise is able to increase capillary density (whereas environmental enrichment influences capillary number), thus resulting in an advantage of vasculature components over other tissues. Aerobic exercise-associated increase in the vessel diameter has also been seen in magnetic resonance angiography. Given the magnitude of this effect, it seems that this process alone could account for the whole cerebral tissue expansion (Thomas et al., 2012). Latest evidence, indeed, are rising doubts on this widespread assumption on grey matter-specific neurogenesis involving neuronal cell bodies,

demonstrating that (hippocampal) neurogenesis actually drops enormously already during the first few years of life in primates and humans, to then stop completely or almost completely in adult life (Sorrells et al., 2018). According to this cutting-edge perspective, vascularisation constitutes the most accredited neurophysiological cascade linking increase in fitness level and increase in tissue volume (Maass et al., 2015; Thomas et al., 2012; Cassilhas & Tufik, 2016). The crucial beneficial mechanism behind fitness might be an increase in regional cerebral blood volume, acting as a mediator between fitness parameters and tissue growth. According to our findings, the fitness parameter of interest is likely to be oxygen consumption. There are two main patterns suggesting this theory: firstly, the presence of early-adaptive (i.e. quadratic) changes tells that this plasticity potential is extraordinarily rapid, therefore it must stem from an as much rapid physiological process. Cerebral blood flow displacement, if repeatedly merged towards the same brain areas through the same activity for weeks (aerobic training in this case) could possibly promote a plasticity-stimulating environment.

Secondly, the occurrence of tissue expansion found to be spread almost everywhere in the brain speaks in favour of vasculature mediation role. Brain perfusion is indeed one of the most widespread (neuro)biological mechanisms, thus everything affecting blood stream is very likely to be encountered potentially at each point down its way. Importantly, it is tightly and directly associated to oxygen consumption.

If this is the case, aerobic exercise would constitute an even more tailored strategy against Alzheimer's disease, where cerebral blood flow impairment is known to be crucial, yielding brain hypo-perfusion especially at early stages when macro-structural damage is not yet detectable (Mattsson et al., 2014).

Conclusions

In conclusion, our belief is that exercise might induce both active plastic improvement and passive preventive neuroprotection against neurodegeneration, probably mediated by a systemic "cocktail" of beneficial changes.

In this regard we would like to second Kempermann's quote claiming that "Physical activity might thus be much more than a generally healthy garnish to leading "an active life" but an evolutionarily fundamental aspect of "activity," which is needed to provide the brain and its systems of plastic adaptation with the appropriate regulatory input and feedback" (Kempermann et al., 2010).

Conflict of interest

The authours declare no conflicts of interest.

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Author contributions

AB and ED conceived the project and designed the experiment. AB performed the experiment and supervised data acquisition. GL and GZ analysed the data. GL wrote the manuscript; AB, GZ and ED commented on it. All authors discussed the rational and the results, as well as scientific and clinical implications.

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