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PII: S0166-4328(18)31321-4
DOI: https://doi.org/10.1016/j.bbr.2018.12.041
Reference: BBR 11713

To appear in: *Behavioural Brain Research*

Received date: 13 September 2018
Revised date: 28 November 2018
Accepted date: 20 December 2018

Please cite this article as: Ruotsalainen I, Renvall V, Gorbach T, Syväoja HJ, Tammelin TH, Karvanen J, Parviainen T, Aerobic fitness, but not physical activity, is associated with grey matter volume in adolescents, *Behavioural Brain Research* (2019), https://doi.org/10.1016/j.bbr.2018.12.041

Aerobic fitness, but not physical activity, is associated with grey matter volume in adolescents

Ilona Ruotsalainen[a], Ville Renvall[b,c], Tetiana Gorbach[d,e], Heidi J. Syväoja[f], Tuija H. Tammelin[f], Juha Karvanen[e], Tiina Parviainen[a]

a Department of Psychology, Centre for Interdisciplinary Brain Research, University of Jyväskylä, Jyväskylä, Finland
b Department of Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland
c AMI Centre, Aalto NeuroImaging, School of Science, Aalto University, Espoo, Finland
d Umeå School of Business, Economics and Statistics, Umeå University, Umeå, Sweden
e Department of Mathematics and Statistics, University of Jyväskylä, Jyväskylä, Finland
f LIKES Research Centre for Physical Activity and Health, Jyväskylä, Finland

Corresponding author:

Ilona Ruotsalainen, MSc
Department of Psychology
University of Jyväskylä
Kärki,
Mattilanniemi 6
FI-40014 Jyväskylän yliopisto
Finland
email: ilona.p.ruotsalainen@jyu.fi
phone: +358503699727

Declarations of interest: none

Abbreviations: eTIV, estimated intracranial volume; MVPA, Moderate-to-vigorous intensity physical activity; ROI, region of interest

Highlights

- We analyzed how both aerobic fitness and physical activity associate with frontal, motor and subcortical grey matter volume in adolescents.
- The association between aerobic fitness and gray matter volume was specific and only seen in the left superior frontal cortex and in left pallidum.
- Moderate-to-vigorous intensity physical activity did not associate with any region of interest.

Abstract:

Higher levels of aerobic fitness and physical activity are linked to beneficial effects on brain health, especially in older adults. The generalizability of these earlier results to young individuals is not straightforward, because physiological responses (such as cardiovascular responses) to exercise may depend on age. Earlier studies have mostly focused on the effects of either physical activity or aerobic fitness on the brain. Yet, while physical activity indicates the amount of activity, aerobic fitness is an adaptive state or attribute that an individual has or achieves. Here, by measuring both physical activity and aerobic fitness in the same study, we aimed to differentiate the association between these two measures and grey matter volume specifically. Magnetic resonance imaging scans were used to study volumes of 30 regions of interest located in the frontal, motor and subcortical areas of 60 adolescents (12.7–16.2 years old). Moderate-to-vigorous intensity physical activity (MVPA) was measured with hip-worn accelerometers and aerobic fitness was assessed with a 20-m shuttle run. Multiple regression analyses revealed a negative association between aerobic fitness and left superior frontal cortex volume and a positive association between aerobic fitness and the left pallidum volume. No associations were found between MVPA and any brain region of interest. These results demonstrate unequal contribution of physical activity and aerobic fitness on grey matter volumes, with inherent or achieved capacity (aerobic fitness) showing clearer associations than physical activity.

Keywords: physical activity; cardiorespiratory fitness; magnetic resonance imaging; accelerometer; adolescence

1 Introduction

Aerobic fitness, also referred to as cardiorespiratory fitness, and physical activity are factors that are known to be associated with brain health. Studies have demonstrated that physical activity and aerobic fitness levels are positively associated with several structural properties of grey matter e.g. [1–3]. However, the majority of the research in this field has focused on older adults, and the generalizability of these results to other age groups is not straightforward. The integrity of the neural network in later life is strongly influenced by the developmental processes during the first decades of life, highlighting the importance of exercise-related effects on the brain during this time period. Importantly, research so far has focused on the influence of *either* physical fitness *or* physical activity on brain measures rather than comparing the effects between them. In order to understand the role and significance of exercise-related measures on brain structure, it is crucial to compare the contribution of physical activity behaviour vs. the level of aerobic fitness to grey matter volumes in adolescents.

Physical activity and aerobic fitness are distinct concepts. Caspersen et al. [4] defined physical activity as “any bodily movement produced by skeletal muscles that results in energy expenditure” (p.126). On the other hand, physical fitness is a condition or adaptive state that an individual has or achieves. Aerobic fitness is typically measured either directly by measuring oxygen consumption during maximal exercise test or indirectly by using submaximal tests or field tests, such as the maximal 20-m shuttle run. Physical activity can be determined subjectively using questionnaires or objectively with measurement devices such as accelerometers. However, self-reported physical activity assessments are considered less valid, because they are influenced by recall biases and other factors [5].

Even though measures of physical activity and aerobic fitness provide different information, they are related to each other to a certain extent. There seems to be a dose-response relationship between physical activity and aerobic fitness, concerning both the intensity and the amount of physical activity. In other words, the higher the intensity and amount of exercise training, the larger the improvements in aerobic fitness [6–8]. Importantly however, during adolescence the relationship between objectively measured physical activity and aerobic fitness is suggested to be only low to moderate [9–11]. Besides individual’s own actions, aerobic fitness is influenced by inherent properties, the genotype. More than half of the individual differences in aerobic fitness could be explained by heritability [12,13]. Studying the independent associations of both physical activity and aerobic fitness on grey matter volumes, in the same individuals, will critically extend our understanding of the specific aspects of these measures.

In recent neuroimaging literature, aerobic fitness has been associated with grey matter properties in several brain regions. Surprisingly, only one group has investigated this association in adolescents. Herting et al. studied male adolescents (15–18 years old) and found that the level of aerobic fitness correlated with left hippocampus volume [14] and with right rostral middle frontal cortical volume [15]. In children, aerobic fitness has been demonstrated to associate with several subcortical regions, such as the hippocampus and basal ganglia [16–20]. In addition, exploratory analysis by Chaddock-Heyman et al. [21] proposes that more highly fit children may have decreased cortical thickness in superior frontal cortex, superior temporal areas, and lateral occipital cortex. To the best of our knowledge, only one study investigated the relationship between physical activity and grey matter

volumes in youth. Based on self-reported, but not objectively measured physical activity, Herting et al. [15] found that male adolescents in high physical activity group demonstrated larger right medial pericalcarine and left precuneus surface areas than in low activity group.

Given these findings, it is problematic to determine whether it is the regular physical activity or the aerobic fitness level that is more important for brain structures in youth. Animal models using rats bred for their response to exercise training (high vs. low induced change in running capacity) have shed some light on this issue. Nokia et al. [22] observed that high-response rats exhibited larger increases in hippocampal neurogenesis than low-response rats after physical training. However, high-response rats also ran more, so the amount of physical training was not equal between groups. When controlling for amount of physical activity, female high-response rats still showed a higher rate of neurogenesis in hippocampus [23]. Moreover, the training at same intensity levels induced different responses in brain-derived neurotrophic factor (BDNF) in low- and high-response rats [24]. Thus, in animal models, inherent running capacity seemed to affect brain responses even though the exercise was similar between groups. Taken together, these results suggest that neither the amount of physical exercise nor the running capacity can independently explain differential responses to exercise.

Age has been suggested to influence the physiological responses to exercise. Several studies have reported age-related differences in exercise responses [25–28]. Supporting these human studies, animal models also indicate that age-related responses to exercise can be seen in cognition [29,30], in BDNF [30], and in microvascular function [31]. Although the main focus has been on the differences between young and old individuals, distinct responses to exercise have been also observed between adolescents and adults. Interestingly, Hopkins et al. [30] found that exercise enhanced relative levels of BDNF across brain regions and object recognition memory in adolescent rats 2–4 weeks after training intervention, but not in adult rats. Thus, brain responses to exercise can differ depending on age.

Despite the considerable number of studies concerning the effects of aerobic fitness or physical activity on grey matter volume in older adults, the evidence for this relationship in adolescence is inconclusive. To the best of our knowledge, no studies to date have compared objectively measured physical activity and fitness with grey matter volumes in adolescents. Therefore, we investigated how both physical activity and aerobic fitness associates with grey matter volumes in the adolescent brain. We measured physical activity objectively with accelerometers and aerobic fitness with a 20-m shuttle run. Magnetic resonance imaging (MRI) was applied to measure grey matter volumes. Based on the previous literature we hypothesized that both physical activity and aerobic fitness would show positive association with grey matter volumes in the basal ganglia and hippocampus.

2 Methods

2.1 Participants

Participants for this study were recruited from three schools in Central and Southern Finland. Potential participants were selected among volunteers participating in a larger longitudinal study (for more details see [32]). Cross-sectional data, collected at the end of the longitudinal study, was used in the current study. Potential participants were screened for exclusion criteria, which were: MRI contraindications, neurological disorders, use of medication that influences the central nervous system, major medical condition and left-handedness, which was assessed by the Edinburgh Handedness Inventory during the first research visit. Furthermore, in order to evaluate pubertal development, participants self-reported their stage of puberty by using the Tanner scale [33,34]. Sixty-one right-handed participants were scanned. One participant was removed from the analysis owing to excessive motion artefacts in the MRI. Thus, 60 adolescents were included in the analysis. The Central Finland Healthcare District Ethical Committee accepted the study. The study was conducted according to the ethical principles stated in the Declaration of Helsinki and all participants and their legal guardian provided written informed consent. The participants were compensated with a 30-euro gift card for the participation in the brain scans.

2.2 Physical activity and aerobic fitness

Physical activity was objectively measured using a triaxial ActiGraph (Pensacola, FL, USA) GT3X+ and wGT3X+ accelerometers (for full details see Joensuu et al. [32]). Subjects were instructed to wear accelerometers on their right hip during waking hours for seven consecutive days, except during water-related activities. A valid measurement day consisted of at least 10 hours of data. Subjects who had at least two valid weekdays and one valid weekend day, were included in analysis as such. For those subjects, that did not meet this criterion, a multiple imputation method (explained in more detail later) was employed to fill in the missing data. Activity counts were collected in 15 s epochs. If there was a period of at least 30 minutes of consecutive zero counts, it was considered as a non-wear time. A customized Visual Basic macro for Excel software was used for data reduction. MVPA was converted into a weighted-mean value of MVPA per day ([average MVPA min/day of weekdays × 5 + average MVPA min/day of weekend × 2] / 7). Data was collected at a sampling frequency of 60 Hz and standardly filtered. The cut points of Evenson et al. were utilised in the analysis [35,36].

Aerobic fitness was assessed with the maximal 20-m shuttle run test. The test was performed as described by Nupponen et al. [37]. Each participant ran between two lines 20-m apart at accelerating pace, which was indicated with an audio signal. The time participants ran until they failed to reach the end lines in two consecutive tones indicated the level of aerobic fitness. The speed in the first and second levels were 8.0 and 9.0 km/h, respectively. After the second level, speed was increased 0.5 km/h per level. The duration of each level was one minute. Participants were encouraged to keep running throughout the test.

In addition to physical activity and aerobic fitness tests, participants completed sets of tests on muscular fitness (push-up and curl-up), flexibility (four different measurements), and fundamental movement skills (5-leap test, throwing-catching combination test), which are not included in the current study article [32]. Of these tests, only the results of the throw-catch combination were used in multiple imputations, as explained in more detail later.

2.3 Magnetic resonance imaging acquisition

Images were acquired on a 3T MAGNETOM Skyra whole-body scanner (Siemens Healthcare, Erlangen, Germany) using a 32-channel head coil at the Aalto Neuroimaging unit, Aalto University, Espoo, Finland. Total scanning time was approximately 45 minutes and it included structural, diffusion, functional, field mapping, and perfusion MRI scans. All scans except perfusion MRI were acquired using "Auto Align" to minimize variation in slice positioning [38]. Prior to imaging, participants were familiarized with the measurement protocol. All participants were instructed to keep their head still during the scanning and pads were used to minimize head motion. In addition, the participants wore earplugs to compensate for the noisy environment.

T1-weighted structural images were acquired in the sagittal plane using the MPRAGE pulse sequence. The protocol included 176 sagittal slices and the scanning time was 6:02 minutes. The acquisition parameters were set as follows: TI = 1100 ms, TR = 2530 ms, TE = 3.3 ms, voxel size = 1.0 x 1.0 x 1.0 mm^3, flip angle = 7°, slice thickness = 1 mm, FOV = 256 x 256 x 176 mm^3, bandwidth = 200 Hz/Px, and using the GRAPPA parallel imaging technique with an acceleration factor R = 2 and with 32 reference lines.

2.4 Image analysis

Images were visually inspected for motion artefacts during scanning. Six participants had large motion artefacts, warranting a second T1-weighted scan immediately after the first T1-weighted scan. Image analysis suite, FreeSurfer 5.3.0 (http://surfer.nmr.mgh.harvard.edu), was used for cortical surface reconstruction and volumetric segmentation [39]. Briefly, this analysis includes processes such as removal of non-brain tissue, Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures [40,41], intensity normalization, tessellation of the grey matter white matter boundary, and automated topology correction. Following the automated FreeSurfer pipeline, all reconstructed volumes were visually inspected. First, all slices were manually inspected for errors in skullstripping, if errors occurred either watershed threshold was changed or manual edits were carried out as part of the recommended workflow for FreeSurfer. Then white matter segmentation and pial surface were inspected. Errors were manually corrected following FreeSurfer guidelines. Estimated intracranial volume (eTIV) was calculated based on the method described by Buckner et al. [42]. Subcortical region volumes were calculated using FreeSurfer's automatic whole brain segmentation [40]. Following subcortical volumes were chosen for regions of interests (ROIs): putamen, pallidum, caudate, nucleus accumbens, thalamus and hippocampus. Cortical regions were labelled according to the Desikan-Killiany cortical atlas [43]. The volumes of the following cortical structures were chosen as ROIs: paracentral lobule, postcentral gyrus, posterior

cingulate cortex, precentral gyrus, superior frontal gyrus, and lateral orbitofrontal cortex. In addition, the following three regions were calculated as a sum of two separate regions: anterior cingulate cortex (rostral anterior and caudal anterior division), middle frontal gyrus (rostral and caudal divisions), and medial orbitofrontal cortex (medial orbitofrontal cortex and frontal pole) (Fig. 1). The frontal pole was not treated as a single area as the reliability of the area was not measured in Desikan et al. [43]. We used following rationale to choose ROIs for the analysis. Those subcortical areas that are known to be involved in motor behaviour (such as putamen and thalamus) were selected. In addition, hippocampus was selected as a ROI, since it has been shown to relate to aerobic fitness by several animal and human studies [14,17,44]. Several studies suggest that either aerobic fitness or physical activity are related to cognitive skills and especially to executive functions in youth [e.g. 45–47]. Frontal brain areas have a critical contribution especially in executive functions [e.g. 48]. Therefore, we included those frontal brain areas that have been shown to relate to either physical activity or aerobic fitness.

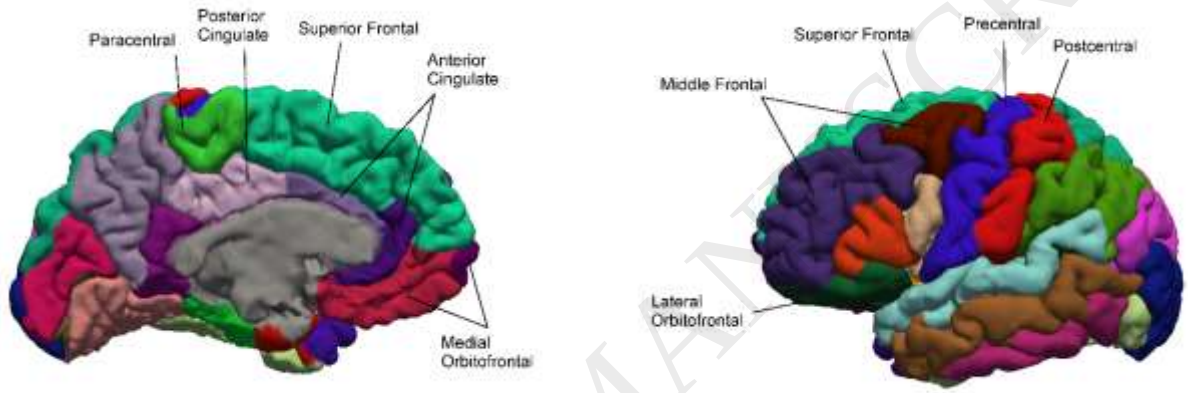

Fig. 1. Cortical ROIs. The medial (left) and lateral (right) surfaces of the human cerebral cortex parcellated with FreeSurfer.

2.5 Statistical analyses

We assessed the associations between volumetric measures of brain structure and physical activity as well as aerobic fitness with multiple regression model:

$$GM_i = \beta_0 + \beta_1 X_i + \beta_2 Age_i + \beta_3 Gender_i + \beta_4 PS_i + \beta_5 eTIV_i + \epsilon_i,$$

where GM_i is the grey matter volume of a ROI for subject i, X_i is physical activity or aerobic fitness, Age_i, $Gender_i$, PS_i and $eTIV_i$ are age, gender, pubertal stage and estimated intracranial volume, respectively and error terms ϵ_i are independent and identically distributed normal random variables with zero mean and same standard deviation. Body mass index (BMI) was not included as a covariate as there was no associations between BMI and any of the ROIs. All predictors were entered simultaneously into the model. Residual plots and Q-Q plots were used to check the assumptions of linearity as well as normality and homoscedasticity of the residuals. The means of the residuals in all models were close to zero. The highest correlations were between gender and eTIV r = 0.59 and between puberty and age r = 0.56 indicating that multicollinearity was not a problem. All variance inflation factors were below 2. False discovery rate (FDR) [49] was used to handle multiple

comparisons (separately for MVPA and 20-m shuttle run). The relationship between either physical activity or aerobic fitness and gray matter volumes has not been previously studied in this age group. Therefore, we chose to include a more comprehensive set of brain regions in the analysis. We acknowledge that with this sample size, a strict correction for multiple comparisons may increase the number of false negative findings. To balance between false positives and false negatives we chose to use FDR threshold of 0.1. Results reported according to this criterion were considered noteworthy. FDR-adjusted p-values were calculated according to Yekutieli and Benjamini [50].

2.6 Multiple imputation

MRI volumetric measures, eTIV, age, and gender were available for all participants while missing data occurred in the pubertal stage and in both 20-m shuttle run and MVPA. The percentage of missing values per variable ranged between 10 % and 22 %.

Incomplete data for several variables (see Table 1 and Supplementary material) were imputed using multiple imputation under fully conditional specification (chained equations) [51]. The predominant cause of missing data entries was the subjects being absent from school during the measurement occasion because of sickness and insufficient number of valid measurement days (two weekdays and one weekend day) for physical activity. The analysis was performed under the assumption of data missing at random as the crucial predictors such as preceding measures (measured approximately six months before the current study) of puberty, shuttle-run tests (correlation with preceding 20-m shuttle run test = 0.57) and weekday measures of physical activity (correlation with total MVPA [also weekend days included] = 0.95) were available. These variables, as well as age, gender, height, BMI, results of throw and catch test, and grey matter volume in ROIs were used as predictors in the imputation model. As advised (Van Buuren, 2012 chapter 2.3.3 [52]), 50 imputed datasets were constructed and analyzed. Each data set was constructed using 50 iterations of the algorithm to ensure the convergence of the iterative process. Calculations were performed in R 3.4.0 (R Core Team, 2017) using the mice 2.3 package [53]. The model parameters and their standard errors were estimated for each imputed dataset and combined using Rubin's rules [47, p. 37-38] to obtain final estimates of parameters and their standard errors. More details about multiple imputation in Supplementary material.

Table 1. Missing data pattern for variables of interest. Available: number of individuals with data available; Missing: number of individuals with missing data, % missing: proportion of subjects with missing measure, Available from previous measurements: number of individuals with missing data on a variable that have data from previous occasions. Asterisk (*) indicates that seven subjects had measurement of weekday MVPA that could be utilized in imputations.

	Available	Missing	% missing	Available from previous measurements
Pubertal stage	54	6	10 %	4
Total MVPA	47	13	22 %	7*
20-m shuttle run	51	9	15 %	5

MVPA, moderate-to-vigorous intensity physical activity.

3 Results

Table 2 presents descriptive statistics for participant characteristics, physical activity, 20-m shuttle run, and brain volumes of the left superior frontal cortex and the left pallidum.

Table 2. Participant demographics. Data presented as mean ± SD.

	Mean	Range
Age (years)	14.3 ± 0.9	12.7 – 16.2
Weight (kg)	55.9 ± 9.9	35.7 – 94.3
Height (cm)	164.1 ± 9.6	147 – 194
BMI	20.7 ± 2.6	15.7 – 31.1
Pubertal stage	3.5 ± 0.9	1.5 – 5.0
20-m shuttle run (min)	5.8 ± 2.4	1.5 – 11.6
MVPA (min/day)	49.1 ± 20	18 – 105.9
Left SFC (mm^3)	28742 ± 3359	21669 – 35837
Left pallidum (mm^3)	1542 ± 287	977 – 2177
N=60 (female=40)		

BMI, body mass index; MVPA, moderate-to-vigorous intensity physical activity; SFC, superior frontal cortex

Multiple linear regression analysis was carried out to investigate the relationship between aerobic fitness (Table 3) and MVPA (Table 4) with brain volumes. After correcting for multiple comparisons there was a noteworthy negative (FDR-adjusted p = 0.020) relationship between 20-m shuttle run and superior frontal cortex volume after controlling for age, puberty status, gender and eTIV (Fig. 2A). For left superior frontal cortex volume, the regression model predicted a 500 mm^3 decrease in volume for each extra minute in 20-m shuttle run test. The left superior frontal cortex volume could be predicted by the following formula: 22.3 - 0.5 (shuttle run) - 0.83 (age) + 2.35 (gender) - 0.07 (pubertal stage) + 0.013 (eTIV). Here shuttle run is measured in minutes, age in years, pubertal stage as Tanner score (values between 1-5), eTIV as cm^3, and gender is coded as 0=female or 1=male. The adjusted R^2 value was 0.62. There was also a positive association between left pallidum volume and 20-m shuttle run test (FDR-adjusted p = 0.069) (Fig. 2B). For left pallidum there was a 48 mm^3 expected increase in volume for each extra minute in the shuttle run test. The left pallidum volume could be predicted by the following formula: 1.87 + 0.05 (shuttle run) - 0.11 (age) - 0.03 (gender) + 0.02 (pubertal stage) + 0.001 (eTIV). There were no noteworthy relationships between MVPA and any of the ROIs. In addition, there were no associations between BMI and any of the ROIs. The correlation between 20-m shuttle run and MVPA was r = 0.45 (p = 0.001).

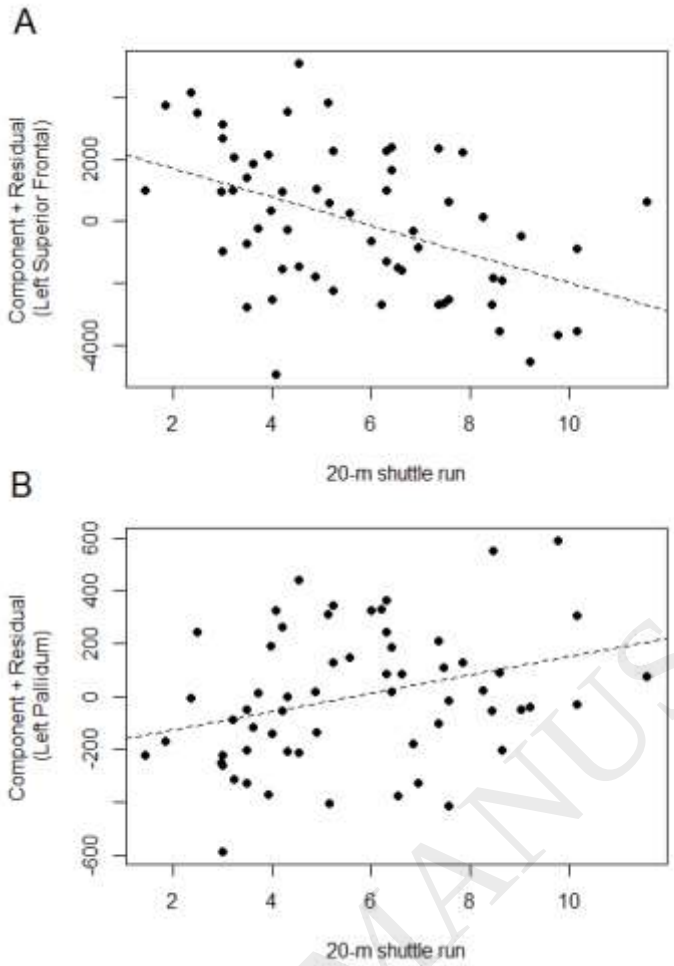

Fig 2. Partial residual plot for the relationship between A) left superior frontal cortex volume and 20-m shuttle run and B) left pallidum volume and 20-m shuttle run.

Table 3. Multiple linear regression analysis of 20-m shuttle run (min) on brain volumes after adjustment for age, pubertal stage, gender and eTIV.

	20-m shuttle run				
ROIs	β	95% CI	p-value	FDR-adjusted p-value	Adjusted R2
Left thalamus proper	-0.047	-0.117,0.022	0.177	0.442	0.63
Left caudate	0.009	-0.060,0.078	0.790	0.878	0.20
Left putamen	0.088	0.000,0.176	**0.050**	0.302	0.33
Left pallidum	0.048	0.016,0.081	**0.005**	**0.069**	0.30
Left hippocampus	-0.001	-0.049,0.048	0.971	0.973	0.39
Left accumbens area	0.008	-0.007,0.024	0.297	0.594	0.22
Right thalamus proper	-0.031	-0.096,0.034	0.345	0.598	0.62
Right caudate	0.025	-0.045,0.096	0.475	0.620	0.30
Right putamen	0.030	-0.047,0.106	0.439	0.598	0.28
Right pallidum	0.027	0.005,0.049	**0.018**	0.180	0.38
Right hippocampus	-0.033	-0.078,0.013	0.156	0.425	0.46
Right accumbens area	0.005	-0.007,0.017	0.432	0.598	0.30
Left anterior cingulate cortex[b]	-0.002	-0.135,0.130	0.973	0.973	0.17
Left lateral orbitofrontal	-0.094	-0.209,0.022	0.109	0.388	0.52
Left medial orbitofrontal[a]	-0.077	-0.171,0.016	0.102	0.388	0.44
Left middle frontal gyrus[c]	-0.145	-0.487,0.197	0.399	0.598	0.52
Left paracentral	-0.003	-0.084,0.078	0.942	0.973	0.31
Left postcentral	-0.046	-0.233,0.142	0.627	0.784	0.49
Left posterior cingulate	-0.049	-0.128,0.029	0.214	0.495	0.28
Left precentral	-0.035	-0.239,0.170	0.735	0.848	0.51

Left superior frontal	-0.504	-0.783,-0.226	**0.001**	**0.020**	0.62
Right anterior cingulate cortex[b]	-0.027	-0.168,0.114	0.698	0.838	0.13
Right lateral orbitofrontal	-0.086	-0.199,0.026	0.129	0.388	0.43
Right media lorbitofrontal[a]	-0.096	-0.187,-0.004	**0.042**	0.302	0.50
Right middle frontal gyrus[c]	-0.154	-0.481,0.173	0.349	0.598	0.55
Right paracentral	-0.045	-0.149,0.058	0.385	0.598	0.17
Right postcentral	-0.092	-0.259,0.074	0.270	0.579	0.39
Right posterior cingulate	-0.039	-0.127,0.048	0.369	0.598	0.17
Right precentral	-0.140	-0.317,0.038	0.120	0.388	0.52
Right superior frontal	-0.269	-0.576,0.039	0.085	0.388	0.53

β, regression coefficient; CI, confidence interval; FDR, false discovery rate; ROI, regions of interest; ROIs are measured as cm^3. Bolded values indicate p-values below 0.05 and FDR-adjusted p-values below 0.1. Adjusted R^2 denotes the adjusted proportion of the variance explained by the model.

[a] = sum of medial orbitofrontal cortex and frontal pole, [b] = sum of rostral anterior and caudal anterior division of anterior cingulate cortex, [c] = sum of rostral and caudal divisions of middle frontal gyrus

Table 4. Multiple linear regression analysis of MVPA (min/day) on brain volumes after adjustment for age, pubertal stage, gender and eTIV.

	MVPA				
ROIs	β	95% CI	p-value	FDR-adjusted p-value	Adjusted R2
Left thalamus proper	-0.001	-0.009,0.007	0.847	0.951	0.62
Left caudate	-0.004	-0.011,0.003	0.237	0.951	0.22
Left putamen	0.006	-0.005,0.016	0.274	0.951	0.29
Left pallidum	0.001	-0.003,0.005	0.579	0.951	0.18
Left hippocampus	-0.001	-0.006,0.005	0.706	0.951	0.39
Left accumbens area	0.000	-0.001,0.002	0.706	0.951	0.20
Right thalamus proper	-0.004	-0.011,0.003	0.259	0.951	0.62
Right caudate	-0.005	-0.012,0.003	0.197	0.951	0.31
Right putamen	0.004	-0.004,0.013	0.334	0.951	0.29
Right pallidum	0.002	-0.001,0.004	0.217	0.951	0.32
Right hippocampus	-0.002	-0.007,0.003	0.444	0.951	0.45
Right accumbens area	0.000	-0.001,0.001	0.866	0.951	0.29
Left anterior cingulate cortex[b]	-0.001	-0.016,0.015	0.919	0.951	0.17
Left lateral orbitofrontal	0.000	-0.013,0.013	0.959	0.959	0.49
Left medial orbitofrontal[a]	-0.001	-0.011,0.010	0.892	0.951	0.41
Left middle frontal gyrus[c]	-0.010	-0.049,0.029	0.596	0.951	0.51
Left paracentral	-0.002	-0.010,0.007	0.705	0.951	0.31
Left postcentral	-0.003	-0.023,0.017	0.754	0.951	0.49
Left posterior cingulate	0.005	-0.003,0.014	0.235	0.951	0.28
Left precentral	0.002	-0.020,0.025	0.828	0.951	0.50

Left superior frontal	-0.022	-0.057,0.013	0.208	0.951	0.53
Right anterior cingulate cortex[b]	-0.011	-0.026,0.005	0.168	0.951	0.16
Right lateral orbitofrontal	-0.002	-0.015,0.011	0.731	0.951	0.41
Right medial orbitofrontal[a]	0.004	-0.006,0.014	0.443	0.951	0.46
Right middle frontal gyrus[c]	-0.003	-0.040,0.034	0.873	0.951	0.55
Right paracentral	-0.003	-0.015,0.009	0.610	0.951	0.16
Right postcentral	0.005	-0.013,0.023	0.604	0.951	0.38
Right posterior cingulate	-0.003	-0.013,0.007	0.511	0.951	0.17
Right precentral	0.003	-0.017,0.023	0.768	0.951	0.50
Right superior frontal	-0.016	-0.051,0.018	0.341	0.951	0.51

MVPA, moderate-to-vigorous intensity physical activity; β, regression coefficient; CI, confidence interval; FDR, false discovery rate; ROI, regions of interest; ROIs are measured as cm^3. Adjusted R^2 denotes the adjusted proportion of the variance explained by the model.

[a] = sum of medial orbitofrontal cortex and frontal pole, [b] = sum of rostral anterior and caudal anterior division of anterior cingulate cortex, [c] = sum of rostral and caudal divisions of middle frontal gyrus

4 Discussion

We investigated how both physical activity and aerobic fitness associate with frontal, motor and subcortical grey matter volumes in adolescents. Our findings suggest that aerobic fitness and MVPA relate to grey matter volumes differently in the studied age range between 12.7 and 16.2 years. Aerobic fitness was negatively associated with left superior frontal cortex and positively associated with left pallidum volume. Contrary to our expectations, we did not find clear associations between MVPA and any ROI. These results demonstrate unequal contribution of physical activity and aerobic fitness on brain volume, with inherent or achieved capacity (aerobic fitness) showing clearer associations than physical activity.

The negative association between aerobic fitness and left superior frontal cortex volume supports and extends the results of Chaddock-Heyman et al. [21] who demonstrated similar association between aerobic fitness and the thickness of this area in preadolescent children. When compared with the results of Herting et al. [15] with a slightly older age group (15-18 years), our results are partially contradictory. Herting et al. [15] observed that the only cortical area associated with aerobic fitness is the right rostral middle frontal cortex, located laterally to the superior frontal cortex. Taken together, the current and earlier results suggest that better aerobic fitness is associated with lower thickness and volume of superior frontal brain areas in youth.

Adolescence is characterized by substantial changes in brain function and structure. Neuroimaging studies show that cortical grey matter decreases and white matter increases during adolescence [54–56]. Among other brain regions, also frontal cortical areas seem to undergo large changes during adolescence [57,58]. Interestingly, a recent study by Teeuw et al. [59] indicated that the largest decrease in cortical thickness during adolescence (12-17 years) takes place in the superior frontal cortex. They also demonstrated that these changes are under strong genetic control [59]. Intriguingly, aerobic fitness in adolescents seems to be related to the brain area, which presumably exhibits the largest (genetically-driven) cortical thinning between the ages of 12 and 17 years. There are several possible underlying factors for this linkage. As demonstrated by earlier studies, both aerobic fitness and changes in superior frontal cortex thickness during adolescence depend largely on genetic factors [12,59]. Our results could thus reflect, at least partly, shared genetic factors underlying both aerobic fitness and superior frontal area development.

We also found a positive association between aerobic fitness and left pallidum volume. The pallidum is a structure within the basal ganglia that is involved in regulating motor activity. Previous results suggest that aerobic fitness is related to pallidum shape and volume in normal weight preadolescent children [18,20]. Our results demonstrate that this association also exist in adolescents.

The association between objectively measured physical activity and grey matter structures, to the best of our knowledge, has not been studied in either children or adolescents. In contrast to the results concerning older adults (e.g. [1,60,61]), the current results indicate that MVPA is not related to brain volumes in frontal, motor, or subcortical regions in this age group. Herting et al. [15] used questionnaires to evaluate the physical activity of 15–18-year-old males. Although measuring physical activity with accelerometers and questionnaires differs, these methods are related to each other. Our results support the findings of Herting et al. [15], where no differences were found in terms of the size

of the frontal and motor surface areas between high and low physical activity groups. However, Herting at al. [15] reported that males who were more physically active showed larger right medial pericalcarine, right cuneus, and left precuneus surface areas than males who were less active. Our results do not confirm or oppose this result, as our regions of interest did not include occipital, temporal or parietal brain areas (except for the post central gyrus).

The differential association of brain volumes with aerobic fitness vs. MVPA in adolescents may reflect the role of genetic factors. As mentioned previously, shared genetic factors could explain the association between aerobic fitness and left superior frontal cortex volume. In addition, physical activity and exercise-related factors could be contributing to the observed association, although we want to highlight that the cross-sectional nature of the current results does not allow us to determine a causal relationship. It is well-established that responsiveness to exercise or physical activity differs between individuals. Even though the level of physical activity or the intensity and duration of an exercise is same for different individuals, physiological responses may differ quite dramatically [13,62–64]. Aerobic fitness is a measure that is considered to be at least partially the outcome of long-term physical activity or exercise, and therefore, it is influenced by individual responsiveness. On the other hand, physical activity is a measurement of an individual's behaviour and does not supply information about responsiveness. It is plausible that this individual responsiveness to exercise may also apply to brain responses to physical activity or exercise. Our results also raise the possibility that only physical activity or exercise, with high enough duration, intensity and frequency to improve aerobic fitness, beneficially impacts the adolescent brain. However, intervention studies are required to determine if this is the case.

Several earlier investigations suggest that physical activity is related to grey matter structures in older adults (e.g. [1,60,61,65]). Why were we not able to find this association in young subjects? One explanation for this might be that on average, older adults are much less active than adolescents [66]. There is hence a large difference in the average physical activity level between these age groups, and the proportion of less active individuals is much higher in older adults. Larger variances could lead to clearer associations, but also, factors related to capacity (aerobic fitness) may underlie the effects demonstrated in older adults. Perhaps the more active individuals in the older adults group also have proportionally higher fitness levels than those in adolescents. It remains to be shown if the association between grey matter structures and physical activity is still present if the activity levels of older adults and adolescents are matched.

Another possible reason for the differential results regarding the effect of physical activity on the brain between young and old individuals relates to cerebral blood flow, which has been demonstrated to decrease with age [67,68]. Contrary to the age-related decline in cerebral blood flow, physical activity and exercise have been shown to increase regional blood flow in older adults [69–71]. Thus, in older adults exercise may increase blood flow closer to the values observed in younger individuals. These different results might also reflect the age-dependent responses to exercise. Finally, if the associations in adolescence are just weaker than in older adults, the sample size may have been insufficient to detect the effect.

Few limitations need to be considered when interpreting the general effects reported in this study. First, the choice of the brain parcellation might have a minor influence on the results. We chose to use the Desikan-Killiany Atlas [43] to keep the regions of interest relatively small and sensitive to possible

changes. However, even smaller parcellations could have been more sensitive to, but would have required more subjects for analysis. Second, hip-worn accelerometers are commonly used in research to objectively measure physical activity. However, they are not able to detect all kinds of activity, such as those featured as water sports or bike riding. Third, in the multiple imputation, the data were assumed to be missing at random. There is no way to verify this assumption empirically but the availability of good predictors for pubertal age, physical activity and shuttle run in the imputation model reduces the impact of the potential violations of this assumption.

4.1 Conclusions

Herein, we provide new information about the association between aerobic fitness and physical activity and grey matter volume in 13–16-year-old adolescents. Our results show that that higher aerobic fitness is negatively associated with left superior frontal cortex volume, a brain area that exhibits large cortical thinning during adolescence, and positively associated with left pallidum volume. We also demonstrate that in this age group objectively measured physical activity does not relate with grey matter volume in frontal, motor and subcortical areas, which is in contrast to findings concerning older adults. The cortical thinning of the superior frontal cortex is proposed to be under strong genetic influence during adolescence. The current results together with previous results concerning brain development raise the possibility of the common genetic pathway between brain development and aerobic fitness in youth. Future work should determine if those common genetic pathways do exist. Finally, our results highlight the importance of measuring both aerobic fitness and physical activity in order to separate the effects of these distinct factors on the brain.

Acknowledgements

This work was supported by the Academy of Finland [grant numbers 273971, 274086 and 311877]. The authors declare no conflict of interest. We thank Marita Kattelus, Riikka Pasanen and Jenni Silvo for their valuable help in the data collection.

Appendix A. Supplementary material

The following is a Supplementary material for this article:

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