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Exercise-induced neuroplasticity: Balance training increases cortical thickness in visual and vestibular cortical regions

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Abstract

Physical exercise has been shown to induce structural plasticity in the human brain and to enhance cognitive functions. While previous studies focused on aerobic exercise, suggesting a link between increased cardiorespiratory fitness and exercise-induced neuroplasticity, recent findings have suggested that whole-body exercise with minor metabolic demands elicit beneficial effects on brain structure as well. In the present study, we tested if balance training, challenging the sensory-motor system and vestibular self-motion perception, induces structural plasticity. Thirty-seven healthy adults aged 19-65 years were randomly assigned to either a balance training or a relaxation training group. All participants exercised twice a week for 12 weeks. Assessments before and after the training included a balance test and the acquisition of high-resolution T1-weighted images to analyze morphological brain changes. Only the balance group significantly improved balance performance after training. Cortical thickness was increased in the superior temporal cortex, in visual association cortices, in the posterior cingulate cortex, in the superior frontal sulcus, and in the precentral gyrus in the balance group, compared to the relaxation group. Moreover, there was evidence that the balance training resulted in decreased putamen volume. Improved balance performance correlated with the increase of precentral cortical thickness and the decrease in putamen volume. The results suggest that balance training elicits neuroplasticity in brain regions associated with visual and vestibular self-motion perception. As these regions are known for their role in spatial orienting and memory, stimulating visual-vestibular pathways during self-motion might mediate beneficial effects of physical exercise on cognition.
1 Introduction

Physical activity has been discussed as a promising means to enhance the life-long ability of the human brain to adapt to environmental demands. For instance, regular aerobic training has been shown to increase memory and executive functions (Cassilhas, Tufik, & Mello, 2016; Hötting & Röder, 2013; Stimpson, Davison, & Javadi, 2018). Accordingly, brain imaging studies have observed structural plasticity in the hippocampus to be associated with increased cardiorespiratory capacity (Erickson et al., 2011; Kleemeyer et al., 2015; Maass et al., 2015). Aerobic training has been reported to increase hippocampal volume in young and in older adults (Erickson et al., 2011; Thomas et al., 2016). However, these results have not always been replicated (Jonasson et al., 2017; Ruscheweyh et al., 2011) and meta-analytic evidence for a direct link between aerobic training and hippocampal structural plasticity in adults is still lacking (Firth et al., 2017). Moreover, exercise studies have observed structural changes beyond the hippocampus. For example, aerobic training has been reported to increase gray and white matter volume in prefrontal and temporal brain areas (Colcombe et al., 2006), and improved aerobic fitness was associated with increased regional white matter integrity in prefrontal and temporal cortices (Voss et al., 2013).

Similar as aerobic exercise, practicing motor coordination and balance seem to elicit structural brain changes too. For instance, motor coordination training has been related to hippocampal volume increase (Niemann, Godde, & Voelcker-Rehage, 2014). Short-term balance training resulted in gray matter increases in premotor, frontal, and parietal cortices (Taubert et al., 2010). The frontal cortex volume increase correlated with the improvement of balance performance, suggesting learning-related structural adaptations. Structural changes were not limited to cortical structures but included the putamen, for which a volume reduction was observed after the 6 weeks training period (Taubert et al., 2010). Moreover, decreased functional connectivity between the basal ganglia, in particular the caudate nucleus and the
putamen, and cortical areas after balance training have been observed in participants who improved in postural stability compared to a passive control group (Magon et al., 2016). (Niemann, Godde, Staudinger, & Voelcker-Rehage, 2014), in contrast, reported a volume increase of the putamen and the globus pallidus after 12 months of motor coordination training.

In rodents, running is known to induce hippocampal neurogenesis and to improve spatial learning (Kempermann, 2012; van Praag, Christie, Sejnowski, & Gage, 1999). Structural plasticity in the rodent hippocampus has been reported after motor skill learning on a rotarod, which involved balancing on a rotating cylinder (Curlik, Maeng, Agarwal, & Shors, 2013). Rotarod-training with strong balance requirements was associated with a volume increase in the prefrontal cortex, the thalamus and the amygdala, whereas volume decreases were found in the retrosplenial cortex, the cerebellum and the vestibular nuclei of the brainstem (Scholz, Niibori, Frankland, & Lerch, 2015). Moreover, behavioral improvements after the training were associated with higher fractional anisotropy (FA) in several areas including the hippocampus and the striatum, but lower FA in the primary visual cortex and the entorhinal cortex. In mice, functional reorganization of the dorsomedial and dorsolateral striatum during motor learning and consolidation on a rotarod was observed using in vivo striatal neural recordings (Yin et al., 2009).

Thus, whole-body exercise challenging balance abilities in the absence of high metabolic demands seems to elicit distinct structural and functional changes in the brain. It has been hypothesized that vestibular input during self-motion, inevitable during any type of physical activity, may be an essential mediator of exercise-induced neuroplasticity in general (Smith, 2017). The vestibular system assesses self-motion to quickly adjust eyes and other body parts for balance control. Balance control requires the integration of visual, proprioceptive, as well as motor-related multisensory cues, which takes place as early as in
the first stage of central vestibular processing, that is, in the vestibular nuclei of the brain stem (Cullen, 2012). Pathways between the vestibular nuclei and the hippocampus, prefrontal and parietal areas provide information relevant for memory and spatial functions (Hitier, Besnard, & Smith, 2014), suggesting an essential contribution of the vestibular system to higher cognitive functions. For instance, vestibular deafferentation has been reported to result in hippocampal atrophy and deficits in spatial memory (Brandt, 2005). By contrast, caloric and galvanic stimulations of the peripheral vestibular system has been found to improve verbal memory and spatial cognition (Bigelow & Agrawal, 2015). Cross-sectional studies on balance experts with several years of practice in ballet dancing or slacklining reported superior performance in a hippocampus-dependent learning task and a larger posterior hippocampal volume, but smaller anterior hippocampal (Hüfner et al., 2011) and putamen volumes (Hänggi, Koeneke, Bezzola, & Jäncke, 2010) compared to control participants with no experience in balance training. In addition, occipital gray matter volume was higher in the balance experts while less gray matter was found in the insular cortex in this group (Hüfner et al., 2011).

Hence, whole-body exercise relying strongly on the vestibular system seems to result in specific structural adaptation of this system. We have recently reported that 12 weeks of balance training in healthy adults improved not only balance performance, but additionally associative memory and spatial cognition, compared to a control group who had participated in a relaxation training (Rogge et al., 2017). It might be hypothesized that these behavioral improvements resulted from changes in brain structures which are both part of the vestibular network and the neural network mediating memory and spatial cognition.

The goal of the present longitudinal study was to assess balance training-induced structural changes underlying cognitive benefits. To this end, healthy adults were randomly assigned to either a balance training or a relaxation training. All participants were scanned
with structural magnet resonance imaging (MRI) before and after the 12-week training period. The balance training was designed to cover a broad spectrum of postural control demands which involve the integration of vestibular, visual and proprioceptive information. The relaxation training served as an active control condition. In the balance group we predicted changes in cortical thickness and gray matter volume in brain structures of the sensory-motor and vestibular system, including the hippocampus and the basal ganglia.
2 Materials and Methods

2.1 Participants

A detailed description of the participant flow, behavioral assessments, and training protocol has been published before (Rogge et al., 2017). Briefly, participants were recruited via public advertisements in the city of Hamburg (Germany). Healthy adults between 19-65 years of age who reported no regular physical exercise (no more than five exercise sessions a month during the last five years) and no extensive experience in balance training and relaxation techniques were eligible for the study. Exclusion criteria were untreated heart diseases, untreated respiratory diseases, musculoskeletal illnesses, past lower extremity trauma, past traumatic brain injury and neurological or psychiatric diseases. After a prescreening by phone, seventy participants underwent an extensive sport medical examination to ensure that they were in appropriate constitution to take part. Two participants did not get medical approval for a physical exercise training and nine participants withdrew their willingness to take part after pretesting. Thus, n = 59 were randomized and allocated to the training groups. Forty participants successfully completed the training program including post-tests. Due to scanner incompatibilities (metallic implants), n = 3 were excluded from MRI imaging, leaving n = 37 for the final analysis (aged 19-65 years; mean ± SD, 45.00 ± 14.86, 23 females). Of the final sample, n = 2 (one in the balance group, one in the relaxation group) were on stable hypertension medication and n = 2 (one in the balance group, one in the relaxation group) on stable thyroid hypofunction medication at pre- and posttest.

The study was approved by the ethical board of the German Psychological Society (DGPs) and was conducted in accordance with the principles laid down in the Declaration of Helsinki. All participants gave written informed consent.
Based on a meta-analysis of previous balance interventions (Lesinski, Hortobágyi, Muehlbauer, Gollhofer, & Granacher, 2015), we expected a medium effect size for balance improvements in untrained adults. Such an effect size can be statistically detected in a Time × Group design with a total sample size of 34 participants (power = 0.80, alpha = 0.05).

2.2 Experimental design

Participants were grouped into matched pairs based on age, gender, and years of education. Individuals of each pair were randomly assigned to the balance or the relaxation group, using a random number generator. Randomization occurred after the pretesting. The characteristics of the participants are presented in Table 1.

Individuals were assessed with a balance test and structural MRI before and after the training period. The MRI and balance assessments were scheduled on different days, the order was counterbalanced across participants. The test assessors were blinded to the participants’ group assignment.

Participants underwent 12 weeks of training in their assigned training group with two training sessions per week, each lasting 50 minutes. The training took place in groups of 10-12 individuals, separately for the balance group and the relaxation group. The same coaches supervised both groups. The primary coach is holding a PhD in sport science and licenses in movement therapy and relaxation techniques. A second coach is holding a bachelor’s degree in sport pedagogy and a trainer license supervised less than 12% of the training sessions. Moreover, the balance training and the relaxation training were comparable with regard to group size, training and session duration, number of social contacts, time of the day and training facility, yet only the experimental group received active physical training. All participants were briefed to not change their level of leisure physical activity throughout the training period.
2.2.1 Balance training

The balance training was designed as a circuit training with eight different stations per session. At different stations, each lasting five minutes, participants trained on various surfaces, such as wobble boards, wobble cushions, foam, and perturbation platforms to challenge functional stability limits and induce reactive stabilization. The difficulty of the exercises was varied by using the following conditions: static vs. dynamic stability, eyes open vs. closed, narrowed base of support (bipedal vs. tandem vs. one-leg stance), and stable vs. unstable undergrounds (Horak, 1997; Sibley, Beauchamp, van Ooteghem, Straus, & Jaglal, 2015). Thus, an individually adapted exercise progression was possible which is in line with recent recommendation for balance trainings (Lesinski et al., 2015). Upon decision of the experienced coaches, exercising difficulty was increased. No strategies were taught. Exercise stations were replaced with new ones after six weeks of training to keep the training challenging. Balance exercises were selected based on existing literature and own experience (Hrysomallis, 2011; Lesinski et al., 2015; Zech et al., 2010). The number of training sessions and the training period of 12 weeks was chosen according to the recommendations for balance training in healthy participants (Gebel, Lesinski, Behm, & Granacher, 2018; Lesinski et al., 2015).

2.2.2 Relaxation training

The relaxation group practiced progressive muscle relaxation (Jacobson, 1987) and autogenic training (Stetter & Kupper, 2002), while laying or sitting on mats. During the first six weeks, the short form of progressive muscle relaxation according to the manual of (Bernstein, Borkovec, & Ullmann, 1975) was instructed. Single muscle groups (hands, arms, shoulder, back, legs, feet and face) were tensed for 5-7 sec, followed by 45 sec of relaxation, with two repetitions each per session. After the first six weeks, autogenic training was introduced, whereby participants were instructed to concentrate on the breathing rhythm as well as on heartbeat. Participants were asked to imagine body parts as warm or cold, or as
heavy. Imaginations were repeated in blocks with 3-4 repetitions per session. During week 11 and 12, both relaxation methods were combined.

2.3 Balance assessment

Dynamic balance was assessed with a stability platform with digital control (Stability Platform, Modell 16030, Lafayette Instrument, USA). The testing protocol required participants to stand barefoot on an unstable platform with a maximum deviation of ± 15° to each side of its horizontal alignment. Participants were asked to place the hands on their hips, direct their gaze to a fixation cross straight-ahead at approx. 3 m distance, and to keep the platform horizontally as long as possible during a trial without leaving the testing position. A handrail was available to prevent falls. After a 60 sec practice-trial, six trials with a length of 30 sec each were run. Half of the trials were conducted with eyes open and eyes closed, respectively. Between trials, participants rested for 30 sec. The beginning and ending of the trials were automatically signalized by a short tone. During the eyes closed conditions, participants were asked to close their eyes, but to keep the head straight-ahead. The condition to start with (eyes open / eyes closed) was counterbalanced across participants. Participants were able to rest for 2 min between conditions to prevent fatigue. The testing procedure took 15 min on average. Whenever a participant left the testing position (i.e. lifted the hands from his/her hips, opened the eyes in eyes-closed conditions or grasped the handrail), the trial was restarted. If three unsuccessful attempts within a condition occurred, the respective test condition would have been marked as missing data. No missing data occurred in the present sample. A built-in digital encoder recorded the time (in sec) per trial the platform was in the horizontal position (±3 degrees deviation tolerance). The horizontal position was automatically referenced to 0 degrees when the platform was switched on before every testing session. For the final test score, the average over six trials (three with eyes open, three with eyes closed) was calculated.
2.4 MRI acquisition and preprocessing

Scanning was performed on a 3 Tesla MR system (Magnetom Trio, Siemens, Germany) using a padded standard head coil. Participants were told to lie as motionless as possible during the scan duration of 8 min. T1-weighted images were acquired using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2300 ms, TE = 2.98 ms, flip angle = 9°, FOV = 256 x 256, 240 coronal slices, voxel size = 1 mm³).

2.4.1 Cortical thickness

Processing of the images and surface-based morphometry were performed using the neuroimaging package FreeSurfer, version 6.0, [http://surfer.nmr.mgh.harvard.edu/](http://surfer.nmr.mgh.harvard.edu/). The procedure of the automated image processing has been described elsewhere (Fischl & Dale, 2000). Briefly, the image reconstruction includes removal of non-brain tissue (Ségonne et al., 2004), Talairach transformation, volumetric segmentation of subcortical white and gray matter structures (Fischl et al., 2002), intensity normalization, tessellation of white and gray matter boundaries, and topology correction (Fischl, Liu, & Dale, 2001). Further, surface inflation and spherical atlas registration using individual folding patterns to match cortical geometry (Fischl, Sereno, & Dale, 1999; Fischl, Sereno, Tootell, & Dale, 1999), and gyrus based cortical parcellation (Desikan et al., 2006) were performed. With this method, cortical thickness is calculated as the closest distance between the gray/white matter boundary and the gray/pial boundary at each vertex on the tessellated surface (Fischl & Dale, 2000).

To reduce within-subject noise, an unbiased, robust within-subject template (Reuter & Fischl, 2011) was created between the two time points of each participant, using the longitudinal stream of FreeSurfer (Reuter, Schmansk, Rosas, & Fischl, 2012). The preprocessing steps including motion correction, intensity normalization, Talairach registration, subcortical parcellation, surface reconstruction, cortical atlas registration and parcellations were performed based on the within-subject template information (Reuter et al.,
2012; Reuter, Rosas, & Fischl, 2010). All images were visually inspected for motion blurring and correct segmentation of the white and gray matter, and manually corrected in the event of tissue segmentation errors. The cortical thickness maps were smoothed with a 10 mm full-width half-maximum Gaussian kernel.

### 2.4.2 Subcortical gray matter volume

The hippocampus and basal ganglia were selected as regions of interest (ROIs) for subcortical gray matter volume analyses as their involvement in exercise-induced plasticity, motor coordination training, and balance has been reported previously (Boisgontier et al., 2017; Erickson, Leckie, & Weinstein, 2014; Hänggi et al., 2010; Magon et al., 2016; Niemann, Godde, Staudinger et al., 2014; Niemann, Godde, & Voelcker-Rehage, 2014; Taubert et al., 2010; van Praag, 2009). The hippocampus was automatically parcellated into subfields by FreeSurfer, based on a statistical atlas built upon ultra-high resolution ex vivo MRI data (Iglesias et al., 2015b). The basal ganglia comprised putamen, globus pallidus and caudate nucleus and were automatically labeled and parcellated by FreeSurfer, resulting in a mean gray matter volume for each structure, separately for the left and right hemisphere. A longitudinal processing stream based on subject-specific Bayesian atlases provided by FreeSurfer was used to increase power and robustness (Iglesias et al., 2015a; Iglesias et al., 2016).

### 2.5 Statistical analyses

#### 2.5.1 Balance performance

Data on balance performance were analyzed using R, version 3.5.0 (R Core Team, 2017). Intervention effects were compared by means of ANCOVA (Egbewale, Lewis, & Sim, 2014; Liu, Lebeau, & Tenenbaum, 2016): In the linear model, posttest scores were compared between groups by submitting pretest scores, group, and age as predictors. Age has been included as a covariate in the model as motor control and balance abilities were shown to be
affected by age (Seidler et al., 2010). The results are displayed as the pretest- and age-
adjusted group difference at posttest, along with the corresponding 95% confidence intervals
(CI) and Cohens $d$ as standardized effect size. The $\alpha$ level was set to $p < 0.05$, two-tailed. The
test-retest correlation coefficient for the balance score was $r = .62$, $p < .001$.

2.5.2 Brain imaging data

Cortical thickness data were analyzed in MATLAB (release 2016b, The MathWorks, Inc., USA) using linear mixed effects models (LME) for longitudinal mass-univariate data
with functions provided by FreeSurfer (Bernal-Rusiel, Greve, Reuter, Fischl, & Sabuncu,
2013). Equations included time (pretest and posttest), group, and the interaction between time
and group as fixed effects, and age (at baseline, centered to the mean) as covariate of no
interest, to adjust for age-related differences in cortical thickness (Barnes et al., 2010; Sowell
et al., 2003). The intercept was included as random effect.

The following random intercept model was applied to the data, were $Y_{ij}$ is the thickness
value of each vertex for the $i$th participant on the $j$th time point:

$$Y_{ij} = \beta_0 + \beta_1 \text{time}_{ij} + \beta_2 \text{group}_i + \beta_3 (\text{time}_{ij} \times \text{group}_i) + \beta_4 \text{age} + b_{0i} + \epsilon_{ij}$$

In the equation, $\beta$ denotes fixed-effect estimates, $b$ denotes subject-specific random-
effects estimates, and $\epsilon$ is the residual error. We chose a whole-brain approach for the cortical
thickness analysis. To control for multiple comparisons, a two-stage adaptive false discovery
rate (FDR) procedure with an array of q-values was used (Benjamini, Krieger, & Yekutieli,
2006). The results were thresholded at a corrected $p$-value of $p < 0.05$, two-sided. We further
analyzed the data at an explorative threshold of $p < .01$, uncorrected, together with a
minimum cluster extent threshold of $k = 50 \text{ mm}^2$ (Landin-Romero et al., 2017; Lieberman &
Cunningham, 2009). Significance maps for visualization were thresholded at $p < .01$,
uncorrected, and overlaid on a Freesurfer standard brain. Coordinates are reported in MNI space.

The analysis of changes in hippocampal volume focused on the hippocampus proper and the dentate gyrus. To increase the reliability of the measurements, mean volumes of the CA subfields (CA1-4) and the dentate gyrus were added up into one hippocampal volume of interest, separately for the left and right hemisphere. Gray matter volumes of the basal ganglia were analyzed separately for putamen, pallidum and caudate nucleus. The extracted gray matter mean volumes for each ROI were analyzed in MATLAB (release 2016b, The MathWorks, Inc., USA), using univariate LME functions for longitudinal data provided by FreeSurfer (Bernal-Rusiel et al., 2013). The fixed effects in the models included time, group, and the interaction between time and group. Age (at baseline, centered to the mean) and estimated total intracranial volume were submitted as covariates of no interest; the latter to adjust for head size in volume analyses (Barnes et al., 2010; Malone et al., 2015). The intercept was included as subject specific random effect in the model. The p-values of the eight separate subcortical ROI analyses were adjusted with Bonferroni-correction for multiple comparisons using the stats package within R. Uncorrected and Bonferroni-corrected p-values are reported in the results section.

The test-retest correlation coefficients ranged from $r = .82$ to $r = .96$ for the cortical thickness clusters and from $r = .96$ to $r = .99$ for the subcortical ROIs, all $ps < .001$.

In order to explore the relationship between cortical thickness changes and changes in balance performance, individual mean cortical thickness values of the significant clusters at $p < .01$, cluster size $k > 50 \text{ mm}^2$ were extracted. The changes of cortical thickness per cluster and balance performance, respectively, were calculated by subtracting the pretest from the posttest values.
To assess a relationship between cortical thickness change and balance performance on a whole-brain level, a difference map between the cortical thickness at pre- and posttest per participant was calculated and used as input for multiple regression against changes in balance performance.
3 Results

The groups (balance group: n = 19, relaxation group: n = 18) did not differ with respect to participants’ age, sex, number of training sessions, and balance performance before training (all ps > .300, see Table 1).

Table 1. Characteristics of the participants (Mean, SD)

<table>
<thead>
<tr>
<th></th>
<th>Balance group (n=19)</th>
<th>Relaxation group (n=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43.9 (14.92)</td>
<td>46.11 (15.37)</td>
<td>.67</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>12/7</td>
<td>11/7</td>
<td>.89</td>
</tr>
<tr>
<td>Training sessions</td>
<td>20.63 (2.67)</td>
<td>21.00 (2.74)</td>
<td>.68</td>
</tr>
<tr>
<td>Balance performance</td>
<td>5.29 (1.71)</td>
<td>5.89 (1.84)</td>
<td>.30</td>
</tr>
<tr>
<td>at pretest (sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. *a* = independent t-test. *b* = Chi-square test.

3.1 Balance performance

The analysis of balance performance changes yielded a significant effect of group, $F (1, 33) = 7.78, p = .008$. After training, the adjusted mean of the balance group was higher than the mean of the relaxation group (group difference = 1.58, 95% CI = [0.43, 2.74], $d = 0.66$, see Figure 1).
Figure 1. Balance performance on the stability platform for the balance (yellow bars) and the relaxation group (blue bars) before and after the training. Error bars indicate standard deviations. Circles depict individual participant data.

3.2 Imaging results

3.2.1 Cortical Thickness

The whole-brain analysis of the Time x Group interaction revealed significant higher cortical thickness increases in the balance group, compared to the relaxation group, in a number of brain regions: the superior temporal gyrus extending into the circular insular sulcus, the superior transverse occipital sulcus, the superior frontal sulcus of the left hemisphere, and the posterior cingulate of the right hemisphere (p < .05, FDR-corrected, see Table 2 and Figure 2).

At a more liberal threshold of p < .01, uncorrected, with a cluster extension threshold of k > 50 mm², additional larger cortical thickness increases in the balance group compared to the relaxation group were observed in the precentral gyrus bilaterally and in the right
pericalcarine gyrus (see Table 2 and Figure 2). There were not any larger cortical thickness increases in the relaxation compared to the balance group, irrespectively of the applied thresholds. There were no significant main effects of Time and Group at $p < .05$ (FDR-corrected).

### Table 2. Cortical thickness and subcortical volume changes for the Time x Group interaction

<table>
<thead>
<tr>
<th>Hem.</th>
<th>Region</th>
<th>X, Y, Z</th>
<th>$\beta$</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Superior temporal gyrus / circular insular sulcus</td>
<td>-40 -25 5</td>
<td>9.70</td>
<td>22</td>
<td>0.09</td>
</tr>
<tr>
<td>LH</td>
<td>Superior transverse occipital sulcus</td>
<td>-19 -86 17</td>
<td>6.94</td>
<td>11</td>
<td>0.09</td>
</tr>
<tr>
<td>LH</td>
<td>Superior frontal sulcus</td>
<td>-20 26 42</td>
<td>2.19</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>RH</td>
<td>Posterior cingulate gyrus</td>
<td>8 -43 22</td>
<td>4.54</td>
<td>18</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Cortical thickness cluster ($p < .01$, $k > 50 \text{ mm}^2$*)

<table>
<thead>
<tr>
<th>Hem.</th>
<th>Region</th>
<th>X, Y, Z</th>
<th>$\beta$</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Precentral gyrus</td>
<td>-19 -24 64</td>
<td>75.38</td>
<td>155</td>
<td>0.14</td>
</tr>
<tr>
<td>RH</td>
<td>Pericalcarine gyrus</td>
<td>15 -77 10</td>
<td>98.79</td>
<td>160</td>
<td>0.10</td>
</tr>
<tr>
<td>RH</td>
<td>Precentral gyrus</td>
<td>33 -5 46</td>
<td>73.77</td>
<td>145</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Subcortical volume in $\text{mm}^3$ ($p < .05$)

<table>
<thead>
<tr>
<th>Hem.</th>
<th>Region</th>
<th>$\beta$</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Putamen</td>
<td>-61.27</td>
<td>5.99</td>
<td>0.019</td>
</tr>
<tr>
<td>RH</td>
<td>Putamen</td>
<td>-48.64</td>
<td>5.44</td>
<td>0.025</td>
</tr>
</tbody>
</table>

*Note.* LH = left hemisphere, RH = right hemisphere. * = only additional significant clusters are presented. Coordinates are in MNI space.
3.2.2 Subcortical gray matter volume

**Hippocampus.** Changes in hippocampal gray matter volume did not differ between groups; there was no Time x Group interaction for neither the left ($F(1, 35) = .11, p = .743, d = 0.11$) nor for the right hemisphere ($F(1, 35) = 1.52, p = .226, d = 0.41$). There were no main effects of Time (all $p$s $> .146$) and Group (all $p$s $> .082$).

**Basal ganglia.** Analyses of Time x Group interactions yielded a significant decrease of gray matter volume in the putamen bilaterally for the balance group compared to the relaxation group (left: $F(1, 35) = 5.99, p = .019, d = 0.66$, right: $F(1, 35) = 5.44, p = .025, d = 0.59$), but no Time x Group interaction for the pallidum and caudate nucleus (all $p$s $> .243$). The significant decrease of the putamen, however, did not survive Bonferroni-correction.

**Figure 2.** MRI results. Cortical thickness significance maps of the Time x Group interaction, depicting higher cortical thickness increases for the balance group, compared to the relaxation group. Maps are superimposed on the standard FreeSurfer brain and thresholded at $p < .01$, uncorrected. Color scale indicates $-\log_{10}$-values. LH = left hemisphere, RH = right hemisphere. Top: lateral view, bottom: medial view.
(correcting for the number of subcortical ROIs, all \( ps > .13 \)). Analyses of the substructures of the basal ganglia revealed no significant main effects of Time (all \( ps > .083 \)). There was a significant main effect of Group in the left pallidum (\( F(1, 35) = 4.54, p = .040, d = 0.66 \)) with a smaller volume in the balance group, compared to the relaxation group. This group difference, however, did not survive Bonferroni-correction (\( p = .322 \)). There was no main effect of Group for the putamen and the caudate nucleus (all \( ps < .398 \)).

### 3.2.3 Correlations with balance performance

#### 3.2.3.1 Cortical thickness

Improvements in balance performance significantly correlated with the cortical thickness changes in the left and right precentral gyrus across both groups (left: \( r = .58, p < .001 \); right: \( r = 39, p = .016 \), see Figure 3, left panel), indicating that a larger gain in balance performance was associated with a larger increase in precentral cortical thickness. Correlation analyses separately run for the training groups revealed significant positive correlations for the balance group in the left precentral gyrus (left: \( r = .66, p = .002 \); right: \( r = .19, p = .426 \)), but no significant associations for the relaxation group (left: \( r = .19, p = .437 \); right: \( r = .28, p = .270 \)). There were no additional significant correlations between balance performance and cortical thickness increase within the extracted clusters.

Moreover, a whole-brain regression of cortical thickness changes against behavioral change confirmed the positive correlation between the left precentral cortical thickness increase and the improved balance performance across both groups (see Table 3). The same relationship between changes in balance performance and cortical thickness was obtained for a second more inferior precentral cluster and two occipital clusters. However, none of the effects survived FDR-correction and are therefore reported at an uncorrected threshold of \( p < .001 \) with a cluster extent threshold of \( k > 30 \text{mm}^2 \).
**Table 3.** Correlations between cortical thickness change and balance performance change across both groups (whole-brain analysis)

<table>
<thead>
<tr>
<th>Hem.</th>
<th>Region</th>
<th>X, Y, Z mm</th>
<th>Number of vertices</th>
<th>β</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Precentral gyrus</td>
<td>-42 -10 58</td>
<td>69</td>
<td>.07</td>
<td>16.98</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>LH</td>
<td>Pericalcarine sulcus</td>
<td>-20 -73 2</td>
<td>65</td>
<td>.03</td>
<td>14.60</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>LH</td>
<td>Precentral gyrus</td>
<td>-20 -24 64</td>
<td>36</td>
<td>.05</td>
<td>14.57</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>RH</td>
<td>Lateraloccipital sulcus</td>
<td>23 -92 19</td>
<td>35</td>
<td>.03</td>
<td>14.54</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

*Note.* LH = left hemisphere, RH = right hemisphere. Cluster at $p < .001$, uncorrected, and $k > 30\text{mm}^2$. Coordinates are in MNI space.

### 3.2.3.2 Subcortical gray matter volume

The volume decrease of the left putamen was significantly correlated with the balance performance gain ($r = -.48$, $p = .002$, see **Figure 3**, right panel), that is, participants showing a larger increase in balance performance showed a larger decrease in gray matter volume in the left putamen. The negative relationship remained significant when only the balance group was considered ($r = -.47$, $p = .043$).
Figure 3. Correlation between pre-posttraining changes of the left precentral cortical thickness (left panel) and the left putamen volume (right panel) with pre-posttraining balance performance change.
4 Discussion

The goal of the present study was to identify the influence of balance training on cortical and subcortical gray matter structures. To this end, healthy participants were randomly assigned to either a balance training or a relaxation training. Both groups exercised twice a week for 12 weeks. Balance performance improved only in the balance group from pre- to posttest. At posttest, the balance group showed a larger increase in cortical thickness in the left superior temporal gyrus, superior transverse occipital, superior frontal sulci, and in the right posterior cingulate gyrus compared to the relaxation group. At a more liberal threshold, similar changes were observed for the left and right precentral gyrus and the right pericalcarine sulcus. By contrast, the volume of the putamen decreased in the balance group. Benefits in balance performance correlated with gray matter increases in the left precentral gyrus as well as with volume decreases in the left putamen. We did not find evidence for specific structural changes of the hippocampus associated with balance training.

4.1 Cortical thickness

Balance tasks require the integration of vestibular, visual, and proprioceptive self-motion signals (Cullen, 2012). In line with this challenge profile, we observed training-induced changes particularly in areas involved in the processing of self-motion. For instance, a larger cortical thickness increase in the balance group compared to the relaxation group was found in the left superior temporal gyrus extending into the circular insular sulcus. Though it is still a matter of debate whether a specific vestibular cortex exists and where it might be located (Eulenburg, Caspers, Roski, & Eickhoff, 2012), recent studies have localized the processing of vestibular signals predominantly in the parieto-insular vestibular cortex and in the posterior insular cortex in the Sylvian fissure (Frank, Wirth, & Greenlee, 2016; Lopez, Blanke, & Mast, 2012; Schlindwein et al., 2008; Wirth, Frank, Greenlee, & Beer, 2018).
Electrical stimulation of the superior temporal gyri in epilepsy patients evoked illusory body motion including perceived head tilts (Kahane, Hoffmann, Minotti, & Berthoz, 2003), indicating a contribution of the superior temporal gyrus to self-motion perception, a function specifically targeted with balance tasks.

Larger gray matter changes for the balance group were additionally observed in visual association areas in the superior transverse occipital sulcus. This region overlaps with area V3A, a motion-selective region involved in the processing of optic flow. It has been demonstrated that area V3A reliably responds to objective planar motion, suggesting an essential contribution to visual self-motion perception (Fischer, Bülthoff, Logothetis, & Bartels, 2012; Strong, Silson, Gouws, Morland, & McKeefry, 2017). Additionally, area V3A receives vestibular input, indicating an early visual-vestibular interaction (Roberts et al., 2017). The present findings are in line with results in professional ballet dancers and slackliners showing larger gray matter volumes in visual association areas compared to non-balance experts (Hüfner et al., 2011). While it was not possible to unequivocally associate balance experience and cortical gray matter changes due to the cross-sectional nature of this study, the present experiment provides strong evidence for a causal relation between balance exercise and changes in visual cortex. Since the implemented balance exercise tasks required planar motion detection to maintain an upright posture, the thickness increase of area V3A is likely related to visual-vestibular motion processing during the balance training.

Similar group differences were observed for the right posterior cingulate gyrus, a brain region proposed to be an anatomical interposition of the parieto-medial temporal pathway to the hippocampus (Kravitz, Saleem, Baker, & Mishkin, 2011). While receiving input from almost all sensory modalities, including the vestibular system (Hitier et al., 2014; Wirth et al., 2018), the posterior cingulate has been reported to be involved in the processing of depth motion cues (Kovács, Cziraki, & Greenlee, 2010) and the perception of self-location.
(Guterstam, Björnsdotter, Gentile, & Ehrsson, 2015). Noteworthy, the observed cluster coordinates are located at the borders of the retrosplenial cortex, being reciprocally connected with the subiculum, the parahippocampus and the entorhinal cortex (Vann, Aggleton, & Maguire, 2009). The retrosplenial cortex has been found to be functionally connected with area V3A during path integration (Sherrill et al., 2015). The posterior cingulate gyrus seems to contribute to the integration of egocentric and allocentric spatial representations and spatial navigation (Kravitz et al., 2011; Maguire, 2001). Additionally, the retrosplenial cortex has been found to be involved in verbal and visual memory (Maguire, 2001; Vann et al., 2009).

As memory and spatial cognition have been shown to benefit from physical exercise, in particular from balance trainings (Dordevic, Hokelmann, Muller, Rehfeld, & Muller, 2017; Hüfner et al., 2011; Rogge et al., 2017), it might be speculated that the posterior cingulate gyrus contributes to the beneficial effects of exercise trainings on these cognitive processes.

The balance training induced a greater cortical thickness increase in the left superior frontal sulcus. This area overlaps with those reported to have lower gray matter volumes in professional ballet dancers as compared to a control group with no balance training experience (Hänggi et al., 2010). The opposite effects might be related to the different study designs: while we investigated gray matter changes following short-term balance training, Hänggi et al. (2010) analyzed long-term effects of ballet expertise in a cross-sectional design. It is known that morphological adaptations after motor trainings depend on the stage of practicing (Wenger, Brozzoli, Lindenberger, & Lövdén, 2017). For instance, it has recently been demonstrated that tissue changes followed an inverse-quadratic shape during motor learning: an initial expansion of precentral gray matter partly renormalized to its baseline value despite continued practice and further increase in task proficiency (Taubert et al., 2010; Wenger, Kühn et al., 2017). These authors interpreted this pattern as an initial overproduction of synapses followed by a selective stabilization of relevant new connections. In the present study, exercising difficulty was progressively increased in order to promote continuous motor
learning. Thus, we speculate that the phase of selective stabilization was not yet reached at posttest. However, balance training studies with repeated measurements are necessary to prove this hypothesis.

Cortical thickness increased in the precentral gyri bilaterally after the balance training. However, the effects were significant only at an explorative threshold. The left precentral thickness change correlated significantly with the improved balance performance, providing evidence for an association with the balance training. This result is in line with previous exercise studies reporting balance training-related gray matter increase in the primary motor cortex (Taubert et al., 2010; Taubert, Mehnert, Pleger, & Villringer, 2016).

Changes in cortical thickness after the balance training were primarily left lateralized. A left hemispheric dominance for motor and coordination skills has been reported in recent training studies using balance (Taubert et al., 2010; Taubert et al., 2016) or dancing exercises (Müller et al., 2017) and might be related to hemispheric specialization and handedness (Serrien, Ivry, & Swinnen, 2006).

4.2 Subcortical gray matter volume

In contrast to the cortical results, the balance training induced a bilateral gray matter decrease in the putamen. The volume reduction of the left putamen significantly correlated with the benefit in balance performance, suggesting a link between these structural changes and balance performance. However, the change in putamen volume did not survive Bonferroni-correction for multiple comparisons. Nevertheless, this result is in line with reports of a previous balance training study (Taubert et al., 2010). Moreover, cross-sectional findings in balance experts revealed smaller putamen volumes compared to controls (Hänggi et al., 2010), and lower volume of basal ganglia predicted better postural performance in young and older adults (Boisgontier et al., 2017).
In contrast to previous exercise studies in humans and rodents, we did not find a significant group difference for gray matter volume changes of the hippocampus. Such hippocampal structural increases have, however, mostly been reported after aerobic exercise training (Erickson et al., 2011; Thomas et al., 2016) and related increases of cardiorespiratory fitness (Erickson et al., 2011; Kleemeyer et al., 2015; Maass et al., 2015). Motor coordination and dance interventions reporting changes in hippocampal volume lasted for 12 to 18 months (Niemann, Godde, & Voelcker-Rehage, 2014; Rehfeld et al., 2017). It might be speculated that hippocampal volume changes follow balance training too, provided they last for longer durations than implemented in the present study. Finally, we might speculate that dancing and coordination training involves higher memory demands than the balance tasks used in the present study, such as choreographies participants had to memorize accurately. This difference might have contributed to the beneficial effects of motor coordination and dancing on the hippocampus reported in previous studies (Niemann, Godde, & Voelcker-Rehage, 2014; Rehfeld et al., 2017).

4.3 Limitations

Some limitations of the study need to be mentioned. The effects in the precentral gyrus and the putamen did not survive correction for multiple comparisons and should be interpreted with caution. Physical exercise intervention studies are time consuming; the present findings should nevertheless be replicated with larger samples. Moreover, to trace the time course of structural changes, multiple assessments during the intervention and a follow-up after several months would be necessary.

In addition, the MRI scanning was performed with a standard T1 sequence resulting in a voxel size of 1mm$^3$. A higher resolution and complementing T2-weighted images would allow a more precise segmentation of hippocampal subfields (Iglesias et al., 2015a; Wisse et al., 2017) which in turn would have increased the sensitivity for detecting hippocampal
volume changes. Rodent studies have reported that stimulating the vestibular system seems to modulate hippocampal theta activity and the firing pattern of hippocampal place cells (Aitken, Zheng, & Smith, 2018). Thus, functional brainimaging studies might have a higher sensitivity to detect first changes in the medial temporal lobe than structural imaging studies.

4.4 Conclusion

The present results suggest that 12 weeks of balance training is capable of inducing structural plasticity in the superior temporal cortex, in the visual association cortex, in the posterior cingulate cortex and in the superior frontal sulcus. These regions are known to be involved in visual-vestibular self-motion processing and in the integration of spatial information from different sensory signals. Moreover, these areas contribute to higher cognitive functions such as memory and spatial cognition. Thus, physical exercise-induced neuroplasticity in these regions might mediate not only the increase in balance performance, but also the beneficial effects of physical exercise on cognition.
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Conflict of Interest Statement

The authors declare no competing financial interests.
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