Aus dem Deutschen Zentrum für Neurodegenerative Erkrankungen (DZNE)

Hippocampal and cortical neuroplasticity and functional changes induced by vestibular system stimulation through various methods of balance training

Dissertation

zur Erlangung des Doktorgrades

Dr. rer. medic.

(doctor rerum medicarum)

an der Medizinischen Fakultät

der Otto-von-Guericke-Universität Magdeburg

vorgelegt von	M.Sc. Miloš Đorđević
aus	Belgrad
Magdeburg	2018

Bibliographical Description

Đorđević, Miloš:

Hippocampal and cortical neuroplasticity and functional changes induced by vestibular system stimulation through various methods of balance training.- 2018.-

134 Bl., 25 Abb., 4 Anl.

Summary

The aim of this thesis was to investigate whether different types of vestibular system stimulation through balance training can have beneficial effects on vestibular-dependent path integration and balance abilities as well as on neuroplasticity in corresponding gray matter regions, especially in the hippocampal formation. For this reason, three separate studies were organized: 1) 3-month longitudinal slacklining training study with young subjects (18-35 years), b) cross-sectional study on professional ballet dancers (18-35 years) and c) 18-month longitudinal sportive dancing study with older subjects (60-85 years). The results showed significant improvements in vestibular-dependent path integration and balance abilities, along with the changes in gray matter in the hippocampal formation (hippocampus, parahipocampus) and other cortical regions in groups where a voxel-based morphometric (VBM) analysis was performed (ballet dancers and sportive dancers). These results show that strong stimulation of the vestibular system by behavioral interventions can lead to structural (neuroanatomical) and functional benefits. Possible implications of the findings are in the prevention of dementia and in the fall prevention.

Keywords

Neuroplasticity, Hippocampus, Path-integration, Balance, Dementia prevention, Fall prevention

Content

Abbreviations

AD	Alzheimer's disease
BVL	Bilateral vestibular loss
CBT	Clinical balance test
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
DZNE	Deutsches Zentrum für Neurodegenerative Erkrankungen
EC	Eyes closed
EO	Eyes open
FA	Fractional anisotropy
FWE	Family-wise error
GM	Grey matter
MCI	Mild cognitive impairment
MEC	Medial entorhinal cortex
MRI	Magnetic resonance imaging
PET	Positron-Emission-Tomography
PI	Path integration
ROI	Region of interest
SOT	Sensory organisation test
SPM 12	Statistical parametric mapping 12
TCT	Triangle completion task

VE	Virtual environment
VBM	Voxel-based Morphometry
VOR	Vestibulo-occular reflex
WM	White matter

List of referenced publications

Publication 1

Dordevic M, Schrader R, Taubert M, Müller P, Hökelmann A & Müller NG (2018). Vestibulo-hippocampal function is enhanced and brain structure altered in professional ballet dancers. *Frontiers in Integrative Neuroscience 12*.

Publication 2

Dordevic M, Hökelmann A, Müller P, Rehfeld K, Müller NG (2017). Improvements in orientation and balancing abilities in response to one month of intensive slackline-training. A randomized controlled feasibility study. *Frontiers in Human Neuroscience,* 11(26). DOI: 10.3389/fnhum.2017.00055

Publication 3

Müller P, Rehfeld K, Schmicker M, Hökelmann A, Dordevic M, Lessmann V, Brigadski T, Kaufmann J & Müller NG (2017). Evolution of neuroplasticity in response to physical activity in old age: the case for dancing. *Frontiers in Aging Neuroscience.* 9:56. DOI: 10.3389/fnagi.2017.00056

Publication 4

Rehfeld K, Müller P, Aye N, Schmicker M, Dordevic M, Kaufmann J, Hökelmann A & Müller NG (2017). Dancing or fitness sport? The effects of two training programs on hippocampal plasticity and balance abilities in healthy seniors. *Frontiers in Human Neuroscience*. DOI: 10.3389/fnhum.2017.00305

1 Introduction

Within the last four decades, our view of the mature vertebrate brain has changed significantly. Today it is generally accepted that the adult brain is far from being fixed and that neuroplastic adaptations to environmental challenges can even occur in the aging brain. A number of factors such as neurotransmitters, growth factors, environmental stimulation, learning and aging change neuronal structures and functions. The processes that these factors may induce are morphological alterations in brain areas, including changes in neuronal connectivity and the generation of new neurons in certain brain areas including the hippocampus. Physical activity is beneficial for these processes (1,2).

Since the early 1960s, researchers have speculated that the vestibular system contributes to spatial information processing and the development of spatial memory in the hippocampus. The structure of the hippocampal formation is altered in persons with bilateral vestibular loss (3,4). Navigational cues to the hippocampal formation can be provided by both the visual system (e.g., landmarks, optic flow) and the vestibular system (e.g., estimation of direction during path integration). It is thought that vestibular input is primarily processed in the anterior part of the hippocampal formation, whereas visual cues are primarily integrated in the posterior part. However, in cases of reduced vestibular or visual input or excessive sensory stimulation, this hippocampal navigational network is reorganized (5).

The spatial navigation strategy called path integration has been shown to involve a network of brain structures, with the entorhinal cortex (EC) playing a pivotal role in path integration. Some neurons in the medial EC display multiple firing fields producing a regular grid-like pattern across the environment. Lack of vestibular system’s input leads to disruption of theta rhythm in the medial EC induces and a disorganization of grid cell firing, which is essential to form a spatial representation of the environment (6).

An intact vestibular system is also crucial for the successful maintenance of an equilibrium. When this is not the case, falls become frequent, especially in the elderly. This affects mortality, morbidity, loss of functional capacity and institutionalization. Balance exercises, including slacklining, are recommended for fall prevention and improving balancing abilities (7,8).

Aging is a natural process associated with cognitive decline, functional and social impairments. The hippocampus is of particular interest when considering aging and cognitive decline, since it is known to play an important role in learning and memory consolidation. In the hippocampus, both functional and structural plasticity (e.g., neurogenesis) occur well into adulthood. Non-invasive strategies such as physical exercise and environmental enrichment have been shown to counteract many of the age-induced alterations in hippocampal signaling, structure, and function. Thus, such approaches may have therapeutic value in counteracting the deleterious effects of aging and protecting the brain against age-associated neurodegenerative processes (9–11).

This dissertation investigates whether various modes of balance training (ballet dance, choreography dance, slacklining) can have beneficial effects on vestibular-dependent path integration and balancing abilities, as well as on neuroplasticity in corresponding gray matter regions (primarily in the hippocampal formation) of young adults and elderly persons.

2 Theoretical Background

The theoretical background of the dissertation contains information necessary for understanding the basic concepts related to neuroplasticity (induced by motor skill learning), hippocampal formation and its connectivity with the vestibular system, as well as the systems underlying path integration and maintenance of equilibrium and their deterioration with healthy aging and dementia.

2.1. Neuroplasticity in response to motor skill learning

The ability of the human nervous system to adapt to new experiences by altering its connectivity and creating new neurons (in areas such as the hippocampus), a process termed neuroplasticity, has been confirmed by numerous previous studies that used movement interventions as stimulus (12,13). Such structural brain changes can often be detected using voxel-based morphometry (VBM) (14–16).

One of the initial studies on this topic was published in the Nature journal by Draganski and colleagues in 2004 (17). This discovery contradicted the traditionally held view that cortical plasticity is associated with functional rather than anatomical changes. They used voxel-based morphometry to show significant regional differences in grey matter in the mid-temporal area and left posterior intraparietal sulcus bilaterally between the group who learned how to juggle and the matched control group who did not (Figure 1). These differences were partially retained even at 3-month follow-up without any practice.

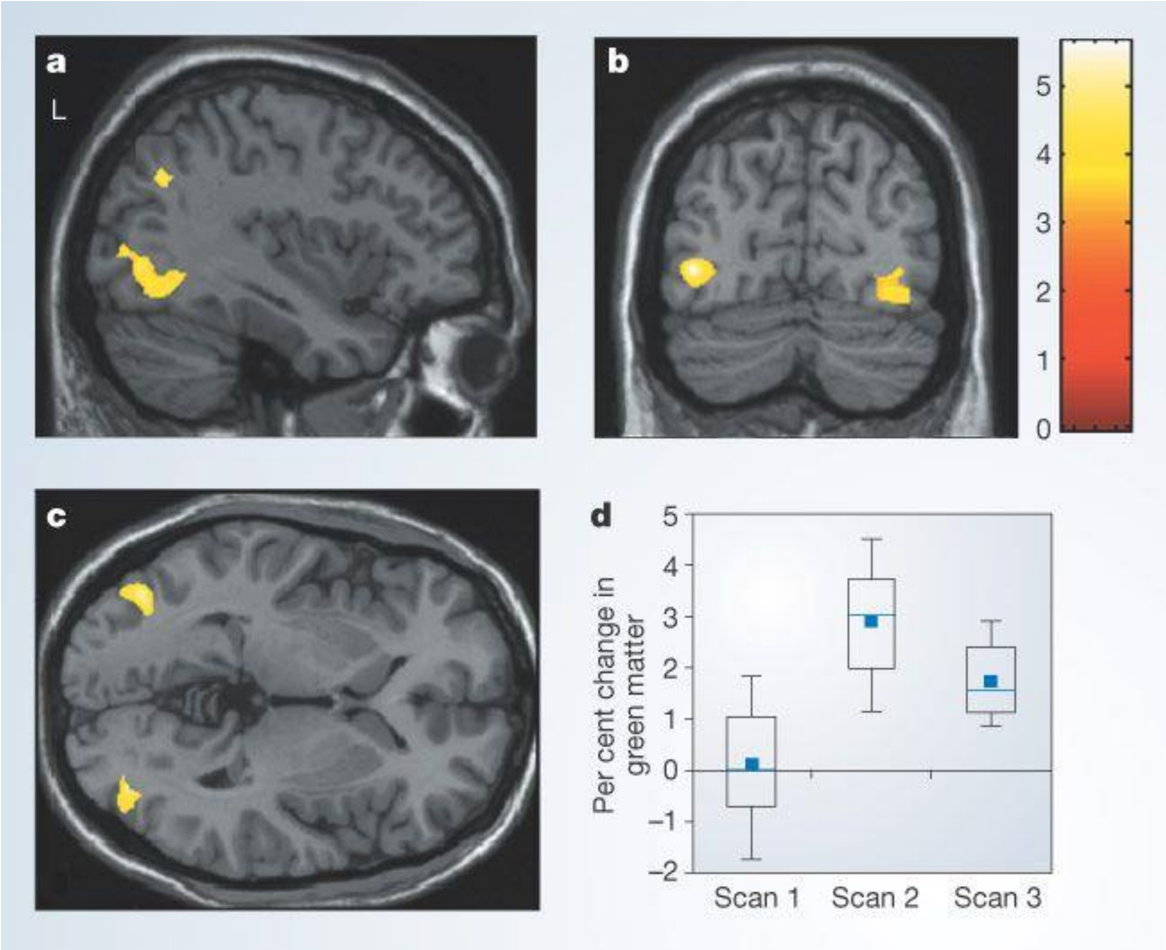


Figure 1 - A significant expansion in grey matter between the first and second scans in the mid-temporal area (hMT/V5) bilaterally and in the left posterior intraparietal sulcus of jugglers vs. controls.

A large number of studies on the topic followed, investigating localization of structural brain alterations in response to motor and non-motor learning, as well as the properties of these alterations, such as temporal dynamics. It has been shown that learning effects on the brain structure could be seen after only 2 hours of learning or training (18).

Many ensuing experimental studies were also designed using balance trainings as interventions. A balancing board was applied in a series of studies by Taubert and colleagues, on both healthy participants and those suffering from Parkinson’s disease (15,16,19). They demonstrated substantial GM volume expansion in frontal and parietal brain areas after only two practice sessions in a complex whole-body balancing task (Figure 2).

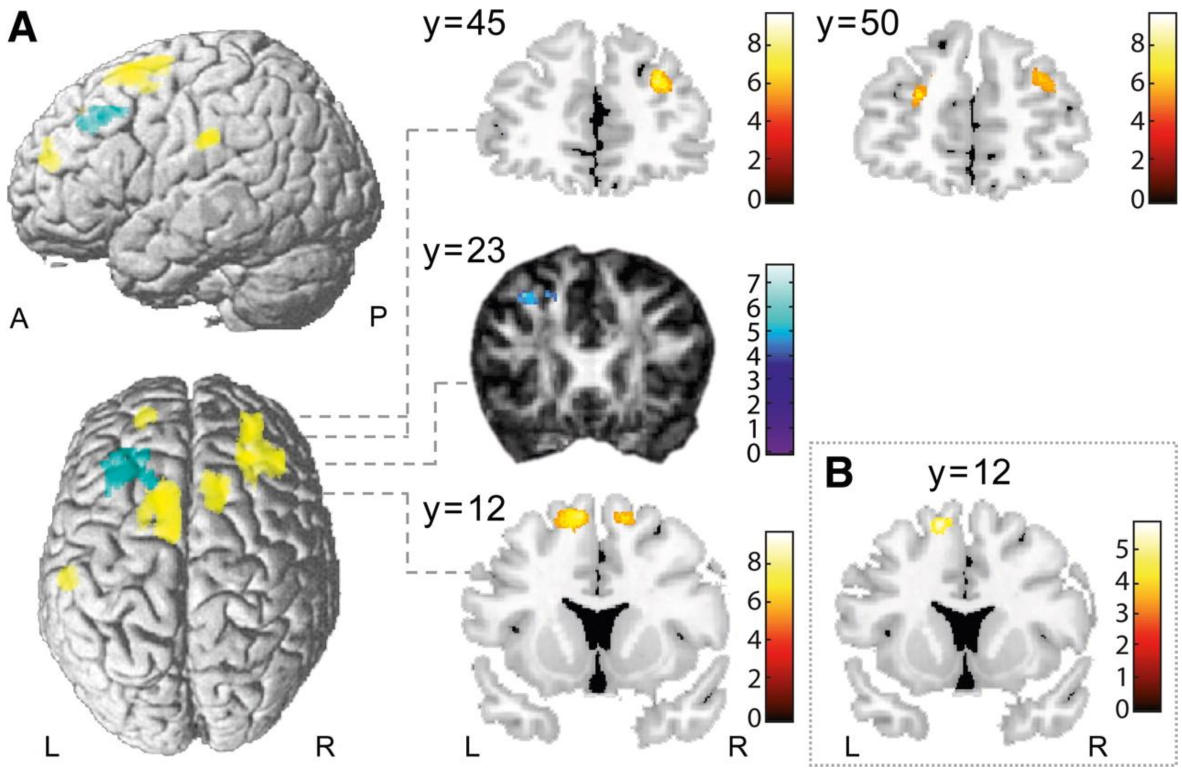


Figure 2 – ***A***, GM expansion (yellow) and FA decrease (cyan) after 2 sessions of training. Top image shows left side of the brain. Upper two coronal sections show GM expansion in bilateral lateral prefrontal cortex. Middle coronal section shows FA decrease in left prefrontal WM. Bottom left section indicates GM expansion in bilateral supplementary motor areas. ***B***, Positive linear correlation between GM expansion in left supplementary motor areas and individual adaptations in muscular imbalances across the whole learning period

Subsequently, an interest gathered around investigating professionals who intensively use their vestibular system on a daily basis, such as ballet dancers and slackliners (20,21). Hüfner and colleagues showed smaller volumes in the anterior hippocampal formation and in parts of the parieto-insular vestibular cortex in professional dancers (ballet dance and ice dance) and slackliners compared to non-professionals, but larger volumes in the posterior hippocampal formation and the lingual and fusiform gyri bilaterally (Figure 3) (21). The main reason for designing such a study stems from their previous research, where they wanted to find out more about the connectivity of the vestibular system to the hippocampal region (4), which revealed that bilateral vestibular loss may lead to a loss in hippocampal volumes (see figure 7, section 2.3). The hippocampus is considered to be the only neocortical region able to produce new neurons throughout lifetime (22), a finding that attracted a relatively large amount of attention from scientific community on this structure in recent years.

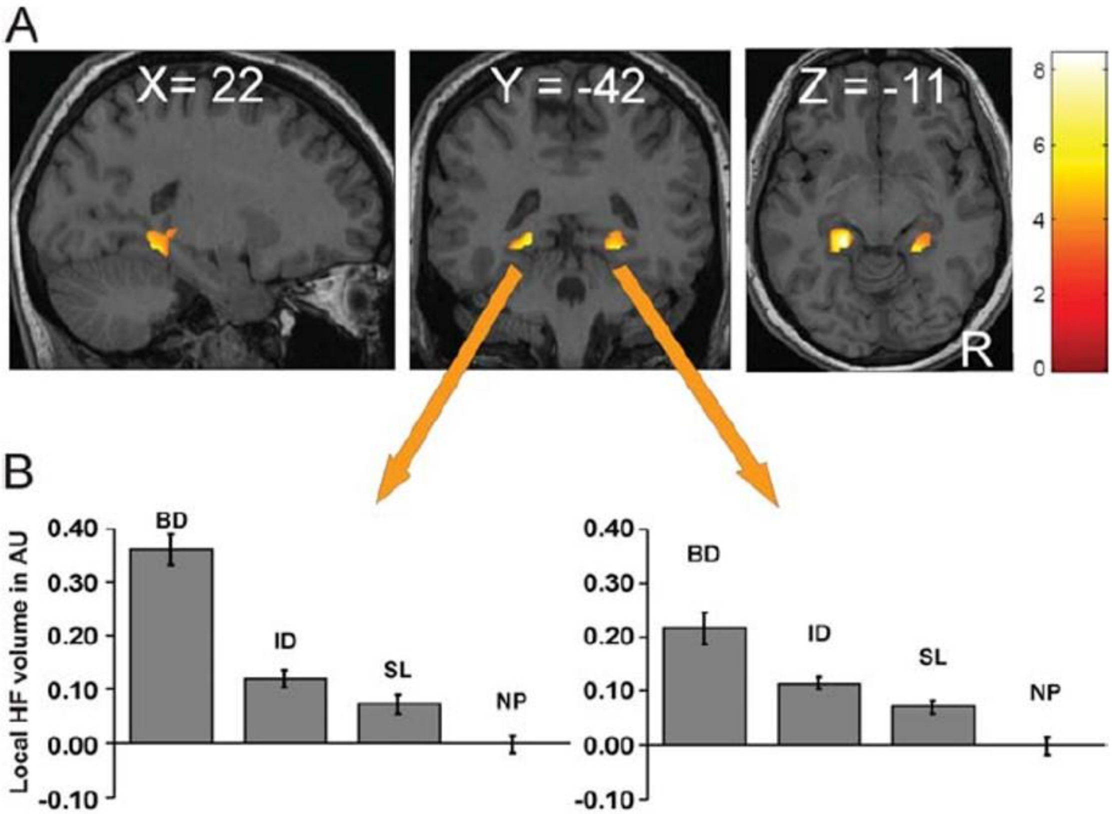


Figure 3 – Larger grey matter volumes in in posterior hippocampus of ballet dancers (BD), ice dancers (ID) and slackliners (SL) compared to the non-professionals (NP).

2.2. Hippocampal formation and spatial representation

The medial entorhinal cortex (MEC) and the hippocampus (Figure 4) are part of the brain's neural map of external space; this is based on successful functioning of place and grid cells that contribute to this representation (Figure 5). Place cells are hippocampal cells that fire selectively when animals are at certain locations in the environment. Grid cells are MEC place-selective cells that fire at regularly spaced locations that form a hexagonal pattern tiling the entire space that is available to the animal (23).

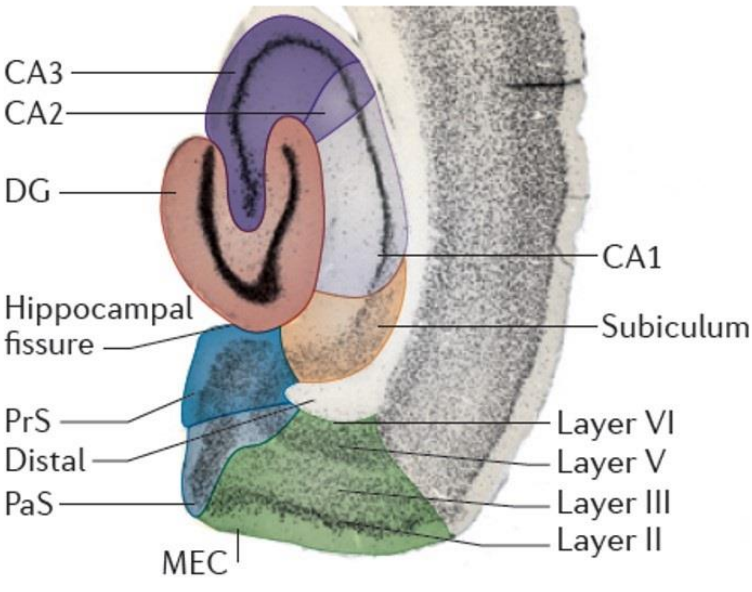


Figure 4 – Frontal view of the hippocampus and the medial entorhinal cortex (24)

Latest studies have shown that place cells are actually a part of a broader circuit for dynamic representation of self-location. A key component of this network is the entorhinal grid cells, which, by virtue of their tessellating firing fields, may provide the elements of a path integration–based neural map (25).

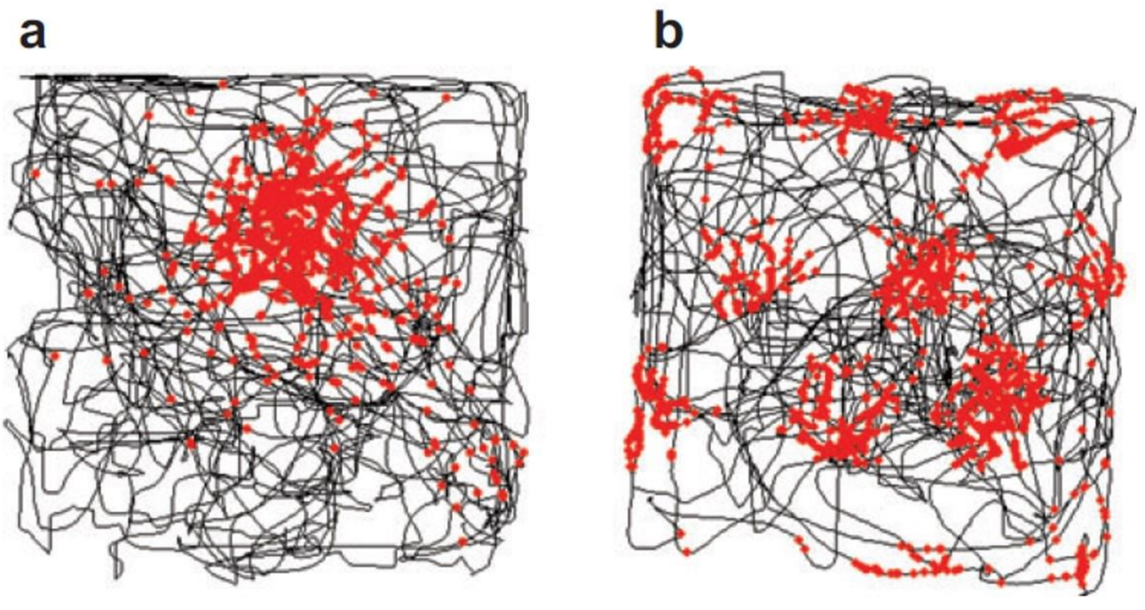


Figure 5 – Spike locations (red) superimposed on animals trajectory (black) for hippocampal place cells (a) and medial entorhinal cortex grid cells (b) (25)

One of the first insights into hippocampal functions in spatial memory, together with dissociation of the functions of the right and left hippocampus, was gained owing to the case of the famous patient H.M. This patient underwent surgical removal of large parts of both mesial temporal lobes in an attempt to cure severe epilepsy. He was compared to other patients with left or right temporal lobectomies and normal control subjects on the incidental recall of objects and their location, both immediately and after a delay. Whereas impairment was seen for both temporal-lobe groups in the delayed recall of objects, the behavioural effects in immediate and delayed recall of location could be observed only within the right temporal-lobe group. These deficits after right temporal lobectomy were contingent upon radical excision of the hippocampal region. In addition, in both object-recall and location-recall, H. M. was inferior to the most impaired patients with unilateral temporal lobectomies (26).

It has also been shown recently that activation of the right hippocampus predicts the use of an allocentric spatial representation, and activation of the left hippocampus predicts the use of a sequential egocentric representation. These results suggested that, rather than providing a single common function, the two hippocampi provide complementary representations for navigation, concerning places on the right and temporal sequences on the left (27).

Furthermore, when local grey matter (GM) volume was compared between a group of good and bad navigators, good male navigators showed significantly higher local GM volume in the right hippocampus than bad male navigators (28).

It has been suggested that the hippocampus receives two main types of input: theta rhythm from ascending brain stem– diencephaloseptal systems and information bearing mainly from thalamocortical/cortical systems. Some studies proposed that the fundamental function of grid cells is to provide a coordinate system for producing mind-travel in the hippocampus, a process that accesses associations with upcoming positions, and occurs during the second half of each theta cycle. By contrast, the first half of each theta cycle is devoted to computing the current position using sensory information from the lateral entorhinal cortex (LEC) and path integration information from the medial entorhinal cortex (MEC) (29).

The posterior hippocampi of London taxi drivers, who have an extensive navigation experience, are significantly larger relative to those of control subjects. A more anterior hippocampal region was larger in control subjects than in taxi drivers. These findings support the idea that the posterior hippocampus stores a spatial representation of the environment and demonstrates a capacity for local plastic change in the hippocampus in response to environmental demands (30).

2.3. Vestibular system connectivity with the hippocampal formation

The assumption of a vestibulo-hippocampal dependency is supported by previous research on the structural and functional connectivity between the vestibular and the medial temporal lobe orientation systems, which revealed multiple pathways that exist between the two as summarized by Hitier and colleagues (31) (Figure 6). Disruption in function of the latter when the input from the former ceases could be demonstrated in animals (32,33).

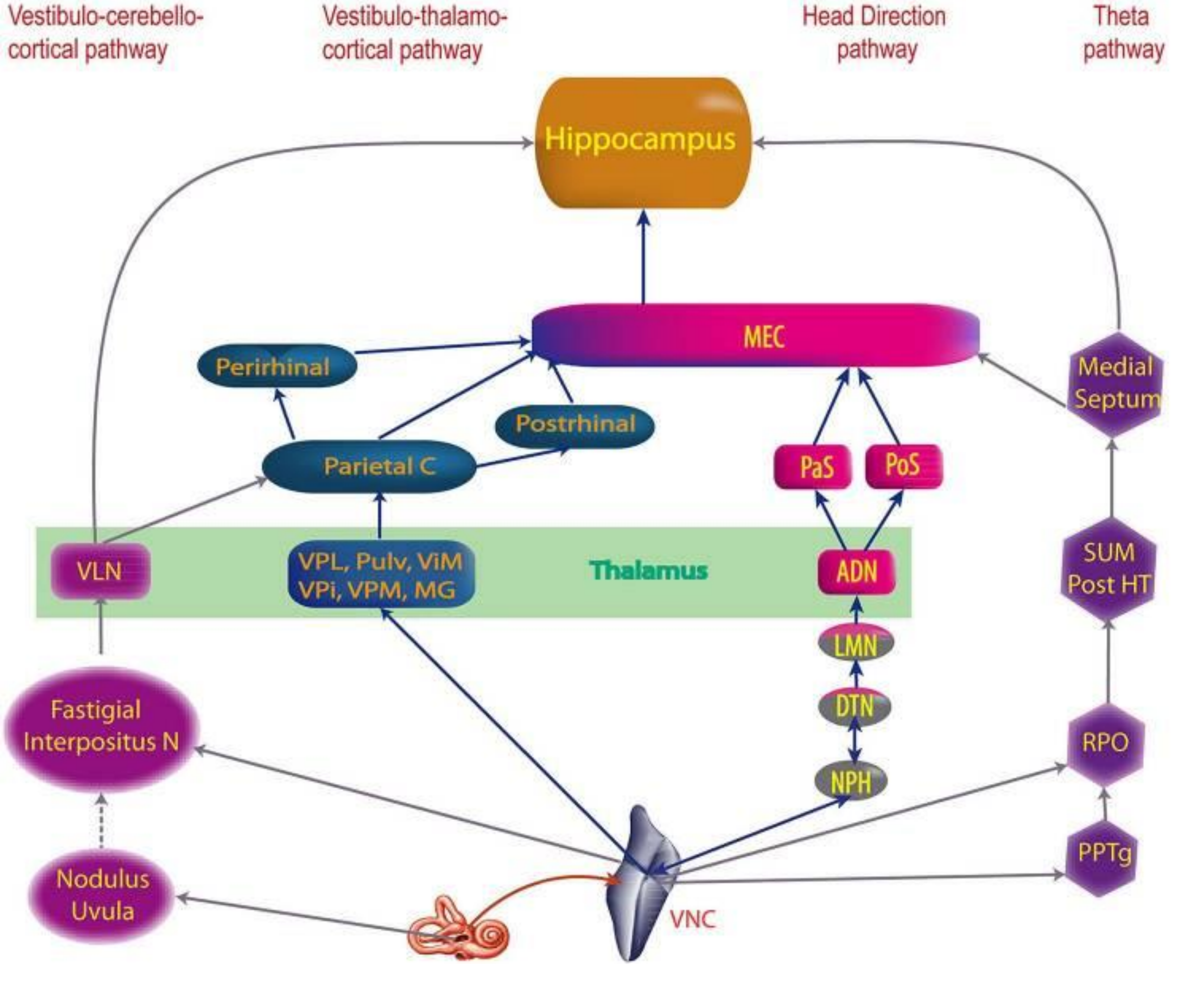


Figure 6 - **The four main vestibular pathways to hippocampus**. ADN, anterodorsal nucleus of the thalamus; DTN, dorsal tegmental nucleus; Interpositus N, anterior and posterior interposed nuclei; LMN, lateral mammillary nuclei; MEC, medial entorhinal cortex; MG, medial geniculate nucleus; NPH, nucleus prepositus hypoglossi; Parietal C, Parietal cortex; PaS, parasubiculum; Perirhinal, Perirhinal cortex; PoS, posterior subiculum (i.e dorsal part of the

presubiculum); Post HT, posterior hypothalamus; Postrhinal, postrhinal cortex; PPTg, pedunculopontine tegmental nucleus; Pulv, pulvinar; RPO, reticularis pontis oralis; SUM, supramammillary nucleus; ViM, ventralis intermedius nuclei of the thalamus; VLN, ventral lateral nucleus of the thalamus; VNC, vestibular nucleus complex; VPi, ventral posterior inferior nucleus of the thalamus; VPL, ventral posterior lateral nucleus of the thalamus; VPM, ventral posterior medial nuclei of the thalamus.

Human studies have also pointed towards a strong link between the vestibular system and orientation centres of the brain, considered to be located in the hippocampus and neighbouring regions (Brandt et al., 2005; Jahn et al., 2009). Besides serious deficits in the orientation function of the temporal lobe as a result of disturbed or lost vestibular input, Brandt and colleagues also found that complete abolishment of vestibular input, due to vestibulectomy, leads to atrophy in distinct medial temporal lobe areas (4) (Figure 7).

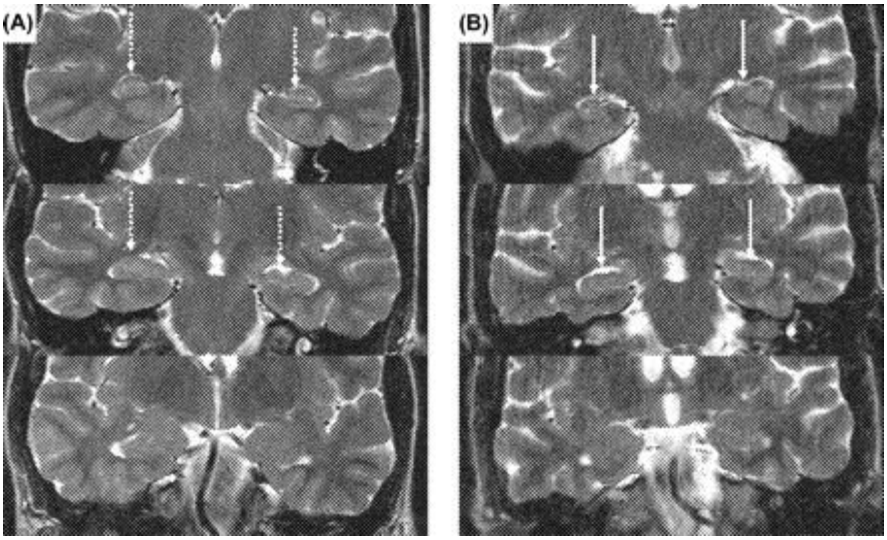


Figure 7 - In BVL patients, a 16.91% volume loss in the hippocampus (arrows) was observed in comparison to age- and sex-matched controls (normal hippocampus: dotted arrows). Volume loss was similar for the left and right hippocampus. (**A**) 39-year-old female volunteer. (**B**) 40-year-old female BVL patient

A review by Hüfner and colleagues revealed that vestibular input is primarily processed in the anterior part of the hippocampal formation, whereas visual cues are primarily integrated in the posterior part. In cases of reduced vestibular or visual input or excessive sensory stimulation, this hippocampal navigational network can be reorganized. Such separation of vestibular and visual information in the hippocampal formation has a twofold functional consequence: missing input from either system may be partially substituted for, and the task-dependent sensorial weight can be shifted to the more reliable modality for navigation (5).

Both vestibular and visual inputs to the hippocampal formation and other brain regions can be used for successful execution of path integration during spatial orientation tasks, as described in the next section.

2.4. Path integration

Path integration refers to the updating of positions on the basis of velocity and acceleration information. Accurate path integration (PI) requires the integration of visual, proprioceptive, and vestibular self-motion cues. To generalize, path integration is the process of navigation by which the traveller’s local translations and rotations are integrated to provide a current estimate of one’s own position and orientation within a larger spatial framework. There are several important functions of path integration. First, path integration allows one to enter unfamiliar territory and seek a destination. Second, as one explores an unfamiliar space, it is path integration that provides the traveller with an ongoing estimate of the current position, thus allowing the traveller to gradually develop an internal representation (cognitive map). In most of the studies on path integration subjects are guided by the experimenter over the outbound route (either walking or riding in a wheelchair) while being deprived of visual and auditory cues about their position and orientation (35–37).

Allen and colleagues used a triangle completion task (Figure 8) to assess path integration skills of younger and older adults (38). They found no difference between young and older participants when led, while blindfolded, along the route segments on foot, which provided both kinaesthetic and vestibular information. In contrast, older adults' performance was impaired, relative to that of younger adults, after they were pushed along the route segments in a wheelchair, which limited their sensory input principally to vestibular information. This implied a decline in hippocampal processing of vestibular input with age.

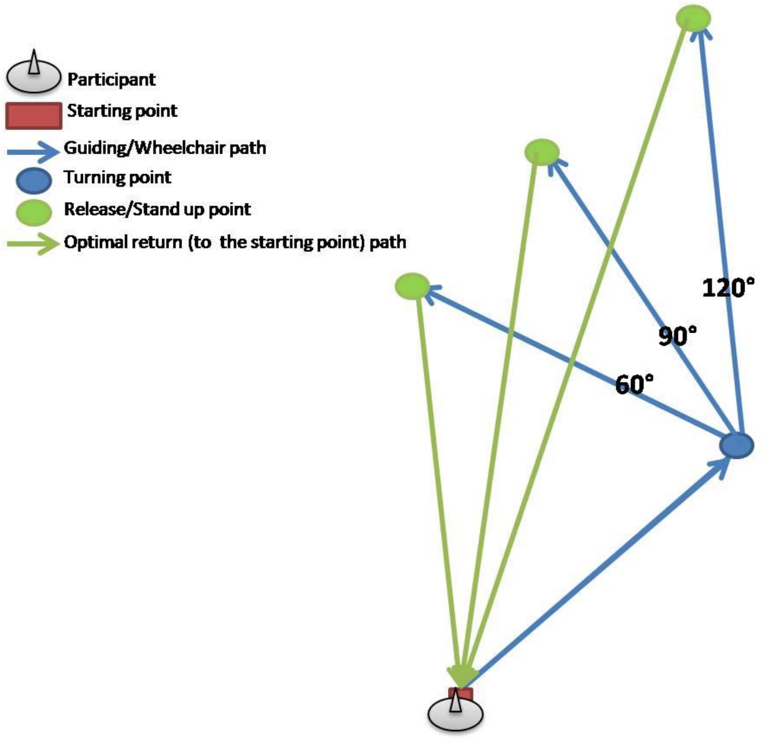


Figure 8 – An example of a triangle completion task (39)

Another study investigated age-related differences in PI using triangle completion tasks (TCTs) performed in the same two "real world" conditions - guided walking and wheelchair propulsion – plus in a virtual environment (VE). For walking and wheelchair propulsion conditions, participants wore a blindfold and wore noise-blocking headphones whereas in the VE condition, participants viewed self-motion information on a computer monitor and used a joystick to navigate through the environment. For TCTs, older compared to younger individuals showed greater errors in rotation estimations performed in the wheelchair condition, and for rotation and distance estimations in the VE condition. These findings demonstrated again that age differences in PI vary as a function of the available sources of information (36). However, mental spatial ability test scores correlated positively with homing performance on a triangle completion tasks in a simulated 3D environment, especially for the more complex conditions, suggesting that mental spatial abilities might be a determining factor for navigation performance (40).

Loomis and colleagues (37) found that subjects who are passively guided over the outbound path without vision exhibit significant errors when attempting to return to the origin but are nevertheless sensitive to turns and segment lengths in the stimulus path. They also found no major differences in path integration ability among blind and sighted populations.

To test whether human path integration recruits a cortical system similar to that of rodents and nonhuman primates (place cells, grid cells, and head direction cells) Wolbers and colleagues used functional magnetic resonance imaging and a virtual rendition of a triangle completion paradigm. Participants travelled along two legs of a triangle before pointing toward the starting location. In accordance with animal models, stronger right hippocampal activation predicted more accurate updating of the starting location on a trial-by-trial basis (41). The same group demonstrated different path integration strategies in a triangle completion paradigm. Namely, participants were instructed either to continuously update the start position during locomotion (continuous strategy) or to remember the shape of the outbound path and to calculate home vectors on basis of this representation (configural strategy). While overall homing accuracy was superior in the configural condition, participants were quicker to respond during continuous updating (42).

2.5. Maintenance of balance

In addition to their important functions in path integration, both the vestibular and the visual system, together with somatosensation, are playing a crucial role in the maintenance of equilibrium or balance. Interactions of the three systems for this purpose will be presented here briefly.

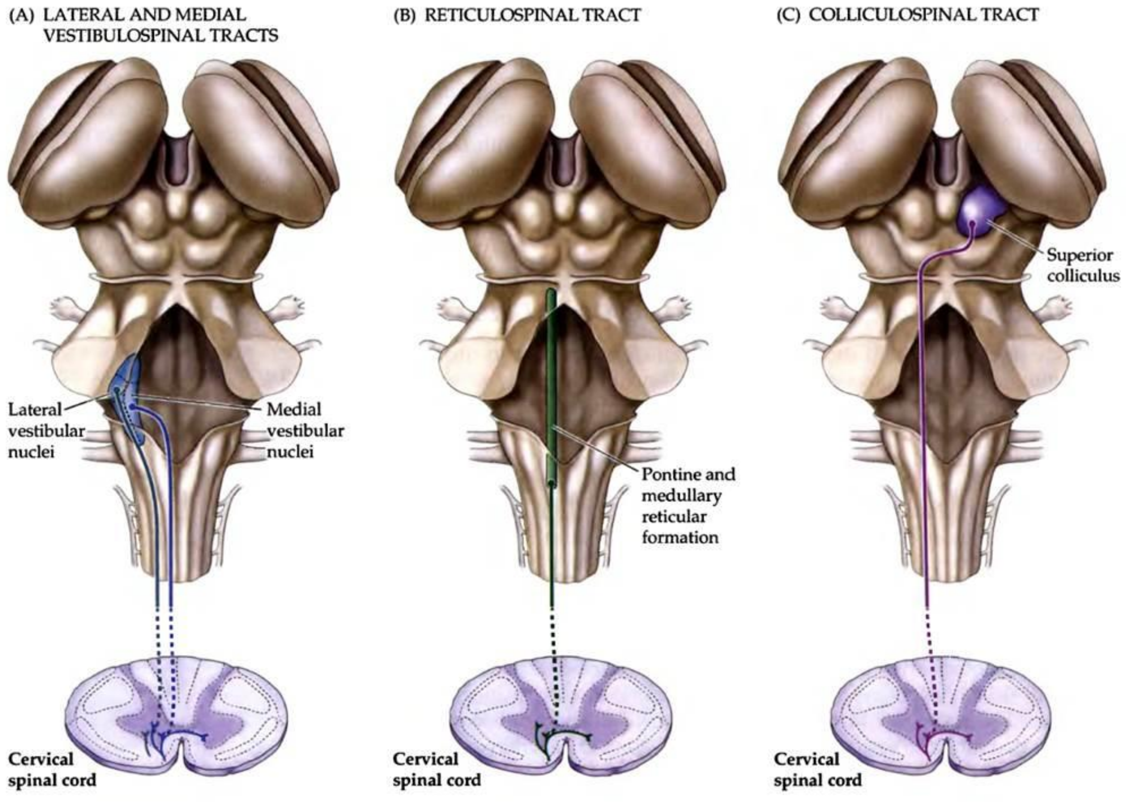


Figure 9 – Descending projections from the brainstem to the spinal cord. Pathways that influence motor neurons in the medial part of the ventral horn originate in the vestibular nuclei (A), reticular formation (B) and superior colliculus (C) (43).

Movements of the axial musculature and proximal limbs for the maintenance of balance, the regulation of posture and the orienting of visual gaze are governed by the following structures: a) upper motor nuclei of the vestibular complex, b) the reticular formation and c) the superior colliculus (Figure 9). These brainstem circuits are competent to direct motor behavior without supervision by higher motor centers in the cerebral cortex, but they usually work in concert with divisions of the motor cortex that organize volitional movements (43–45).

The vestibular nuclei receive sensory information from the semicircular canals and the otolith organs (through the eighthcranial nerve) that specifies the position and the angular and linear acceleration of the head. Neurons in the medial vestibular nucleus then give rise to a medial vestibulospinal tract that terminates bilaterally in in the medial ventral horn of the cervical cord, where it regulates head position by reflex activation of neck muscles in response to the stimulation of the semicircular canals resulting from rotational accelerations of the head.

Neurons in the lateral vestibular nucleus are the source of the lateral vestibulospinal tract, which courses through the anterior white matter of the spinal cord in a slightly more lateral position, and terminates among medial lower motor neuronal pools that govern proximal muscles of the limbs. This tract facilitates the activation of limb extensor (antigravity) muscles when the otolith organs signal deviations from stable balance and upright posture. Other upper motor neurons in the vestibular nuclei project to lower motor neurons in the cranial nerve nuclei that control eye movements (the third, fourth and sixth cranial nerve nuclei). This pathway produces the eye movements that maintain fixation while the head is moving (the VOR) (43–47).

The reticular formation is a complicated network of circuits in the core of the brainstem that extends from the rostral midbrain to the caudal medulla. The neurons within the reticular formation have a variety of movement-related functions, including those in sensory-motor reflexes, coordination of eye movements and the temporal and spatial coordination of limb and trunk movements. Both the vestibular nuclei and the reticular formation provide information to the spinal cord that maintains posture in response to both environmental and self-induced disturbances of body position and stability. Direct projections from the vestibular nuclei to the spinal cord ensure a rapid compensatory response to any postural instability detected by the vestibular labyrinth. In contrast, the motor centres of the reticular formation are controlled largely by other motor centres in the cerebral cortex, hypothalamus or brainstem. The relevant neurons in the reticular formation initiate adjustments that stabilize posture during ongoing movements (43,45,46,48,49).

Another brainstem structure, the superior colliculus, also contributes to the upper motor neuron pathway to the spinal cord. The axons arising from neurons in deep layers of the superior colliculus project via the colliculospinal tract to medial cell groups in the cervical cord, where they influence the lower motor neuron circuits that control axial musculature in the neck. These projections are particularly important in generating orienting movements of the head and eyes. Activations of a particular site in the superior colliculus or in the frontal eye field produce saccadic eye movements in a specified direction and for a specific distance (43,50–52).

2.6. Prevention of dementia and falls through motor training

The entorhinal cortex is particularly vulnerable to neurodegenerative processes during aging and Alzheimer's disease and deficits in grid cell function could be a key mechanism to explain age-related navigational decline. There is a significantly reduced grid-cell-like representation in entorhinal cortex of older adults, coupled with deficits in computations of self-position during path integration based on body-based or visual self-motion cues. Thus, impaired grid cell function may play a key role in the age-related decline of specific higher-order cognitive functions, such as spatial navigation (53). Even very early AD patients may become disoriented in their environment, a phenomenon that is more colloquially referred to as 'getting lost' or 'wandering'. The early emergence of topographical disorientation in AD would be expected from the overlap of neural mechanisms of spatial computation with AD pathology. Changes in the navigation circuit may be a predominant and early consequence of AD and manifest as behavioural deficiencies in spatial navigation (54). Since professional ballet dancers and slackliners (21), but also vestibulopathy patients (4), have differentially structured hippocampal formations compared non-dancers; learning such complex balancing skills could have a potential in the prevention of dementia.

Balance training leads to improved postural control in young persons (55). Specifically targeted functional balance training is effective in frail nursing home residents, as far as functional activities are concerned (56). It has also been shown that Tai Chi program and a balance training program lead to similar benefits with regards to static postural control and walking ability (57). Dance training is superior to repetitive physical exercise in inducing brain plasticity in the elderly, suggesting that dancing can be an effective dementia prevention strategy, too (58). Besides these effects in healthy populations, motor performance is affected already at mild stages of Alzheimer's disease, and functional performance other than gait may also be impaired (59). Balance training programmes are a feasible method that leads to decreased fear of falling, decreased time for step execution during dual-task performance and increased velocity during fast walking (60). Even a simple exercise program that involves balance training leads to a significantly slower decline in activity of daily life (ADL) scores in patients with AD living in a nursing home (61). Moreover, exercise improves functional performance in subjects with dementia but regular exercise may slow the rate of functional deterioration in mild AD and reduce falls in patients suffering from advanced AD (62). Thus, being physically active, through balance and motor training, represents a general recommendation for both young and old populations, but also for subjects suffering from various stages of AD.

3 Problem and Aim

Despite the fact that earlier studies revealed that professionals who intensively make use of their vestibular system during their daily artistic performances, such as ballet dancers and slackliners, have differently structured temporal brain regions, including the hippocampus, compared to non-professionals (21), studies using homogeneous groups of participants, i.e. only ballet dancers of both genders, are still lacking.

Although the vestibulo-hippocampal dependency is strongly supported by previous research on animals (31–33), this is not the case for humans. Functional links between the two systems remain unclear and our understanding about how they depend on each other is lacking. Remaining questions on this matter primarily relate to the specific influence of learning a highly complex skill that stimulates vestibular centres (e.g. slacklining or ballet dancing) on behavioural improvements in path integration (i.e. performance on triangle completion task) and balancing. It is reasonable to expect that behavioural improvements in vestibular-dependent path-integration or balancing arise in concert with associated neuroanatomical alterations, but this has not been proven yet.

The eventual findings in this respect could enhance our understanding of neuroplasticity processes and functional dependencies on a systems level. This may have further implications on both healthy aging and dementia prevention, since the decline in path-integration abilities has been linked to the degeneration of spatial navigation centres, located in the hippocampus and surrounding temporal brain areas (38,53). Also, loss of balancing abilities is a major burden for the health system since approximately 1.5% of healthcare expenditures in European countries are caused by falls, which mainly occur because of impaired balance, aging and cognitive decline (63–66). Prevention in the earliest stages, already at young age, is hence justified.

Thus, this dissertation attempted to answer whether stimulation of the vestibular system through various modes of balance training (ballet dancing, sportive dancing, slacklining) leads to neuroanatomical (grey matter) effects in any of the associated regions, with a primary focus on hippocampal areas, and functional transfer effects on hippocampal-dependent path-integration and balancing abilities. Improvements in balancing and path integration coupled with related neuroanatomical changes were hypothesized.

4 Publications and Results

Publication 1 (Dordevic et al, 2018) contains results of the research performed on nineteen professional ballet dancers of both genders – this study was cross sectional in nature and ballet dancers were matched to controls by main demographic characteristics, including age, gender, height, ethnicity etc.. The study goal was to investigate differences in brain grey matter between the two groups, as well as in their overall balancing and path-integration abilities, primarily those dependent on the vestibular system's function. Participants included in the study were young, aged from 18 to 35 years (detailed description in Dordevic et al, 2018). The tests used in this study were the same ones as those used in the Publication 2.

Publication 2 (Dordevic et al, 2017) was part of a larger longitudinal study where slacklining training was used to stimulate vestibular and related systems responsible for the maintenance of balance. At the moment of writing the dissertation only this manuscript was published, with another two being in the review process. The results of the reviewed manuscripts will be briefly presented as a supplement to this dissertation. The goal of this study was to investigate if learning a very complex balancing task (slacklining) over one month (12 hours of training in total) will lead to significant improvements in vestibular-dependent balancing and path-integration abilities. Still unpublished results also contain information about hereby caused grey matter changes in response to the intervention, as well as the complete results from the 2-month follow up. The study flow-chart is shown in Figure 10. Findings presented in Publication 2 pertain to the changes over time, from baseline to post-test, in two groups – one training with open eyes (EO) and a control group (C). Study participants came from a population of young and healthy persons aged form 18 to 35 (for details please see Dordevic et al 2017).

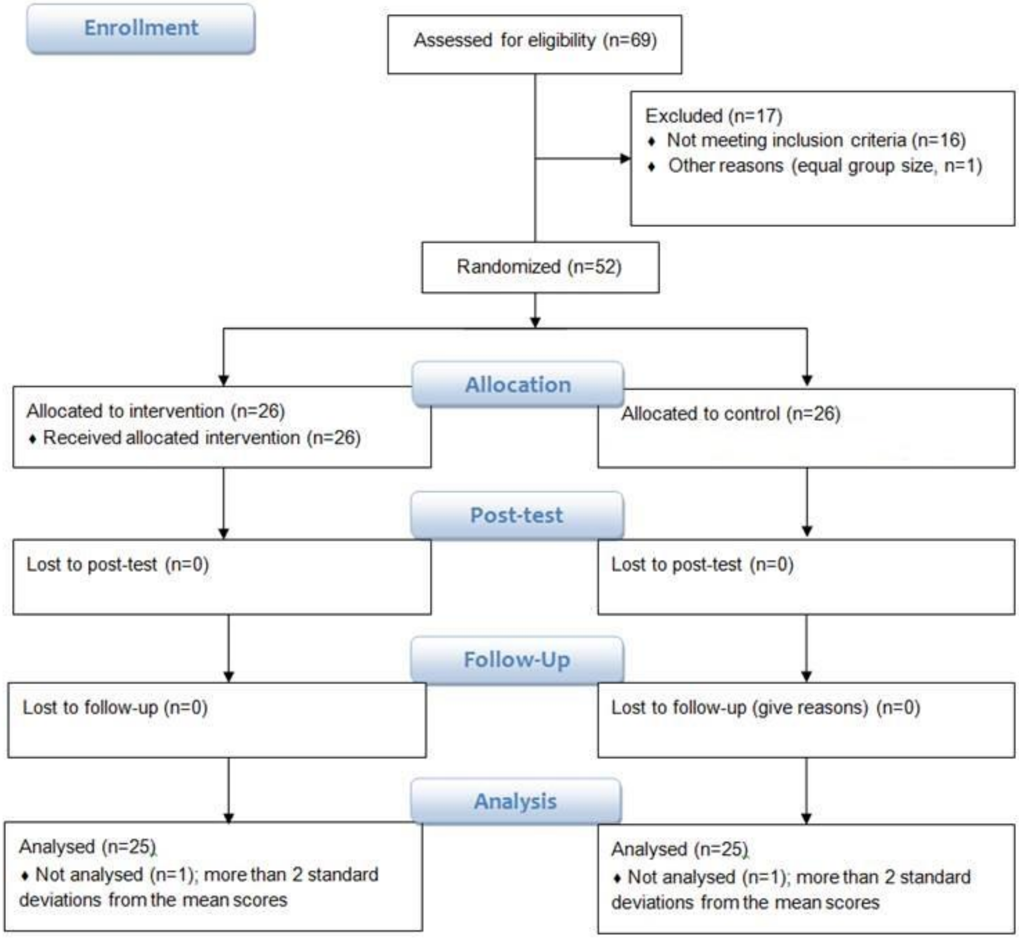


Figure 10 – Study flow-chart (Dordevic et al, 2017)

The methodology for behavioural testing in Publication 1 and Publication 2 was the same. The tests applied were the clinical balance test (CBT) and the triangle completion test (TCT). The conditions of the CBT consisted of standing on stable and unstable surfaces and walking conditions, all of which further contain sub-conditions with open and closed eyes. These are shown in the Figure 11.

No.	Condition	Task		Points (min = 0, max = 3)			
				0	1	2	3
1.	Static – stable surface (floor)	Stand with feet together – open eyes					
2.		Stand with feet together – closed eyes					
3.		One leg stance – left – open eyes					
4.		One leg stance – right – open eyes					
5.		One leg stance – left – closed eyes					
6.		One leg stance – right – closed eyes					
7.	Static – unstable surface (pad)	Stand normally (hip width stance) – open eyes					
8.		Stand with feet together – open eyes					
9.		Stand normally (hip width stance) – closed eyes					
10.		Stand with feet together – closed eyes					
11.		One leg stance – left – open eyes					
12.		One leg stance – right – open eyes					
13.		One leg stance – left – closed eyes					
14.		One leg stance – right – closed eyes					
15.	Dynamic	Walk inside the zone (4 m × 30 cm)	Forward				
16.			Turn (90°)				
17.			Backward				
18.		Walk on the line (4 m × 5 cm)	Forward				
19.			Turn (90°)				
20.			Backward				
21.		Walk on the line with feet one after the other (4 m × 5 cm)	Forward				
22.			Turn (90°)				
23.			Backward				
24.		Walk on the beam (4 m × 10 cm)	Forward				
25.			Turn (90°)				
26.			Backward				
27.		Walk on the beam sideways (4 m × 10 cm)	Rightward				
28.			Turn (90°)				
29.			Leftward				
30.		Walk on the line with closed eyes(4 m × 5 cm)	Forward				

Figure 11 – Conditions of the clinical balance test (CBT)

For the assessment of non-visual spatial orientation the triangle completion test (TCT) was used. In brief, six triangular paths were marked on the floor of a room (Figure 12), three in the left and three in the right direction, giving thus three pairs of triangular paths, with turning angles of 60, 90, and 120°. The test consisted of two conditions: active-walking and passive-wheelchair. In the active-walking condition, while being guided on foot, the participant's movement was controlled by leading him or her along two sides of the triangular path as he or she held onto a wooden bar. The passive-wheelchair condition included transport along the same routes with the use of a standard wheelchair. Each participant was walked (active) and pushed (passive) only once along each of the paths, giving thus 12 trials per participant in total (3 to the left and 3 to the right, times 2 conditions). Once the participant was walked/pushed in the wheelchair along two sides of each triangle, his or her task was to walk along the third one, back to the starting point. The outcome variables were error in centimetres (from participant's stopping point to the initial starting point) and error in degrees (angular deviation from the optimal direction).

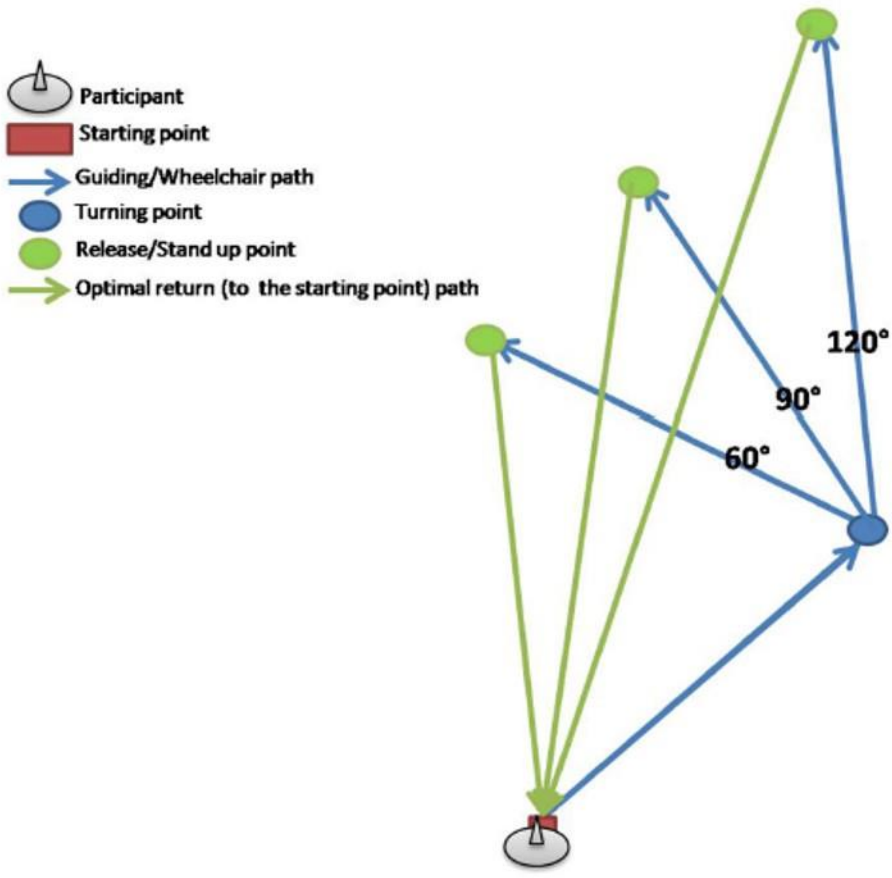


Figure 12 – Graphical representation of the triangle completion test (TCT) for right side direction

Publications 3 and 4 (Müller et al., 2017, Rehfeld et al., 2017) are co-authored publications and relate to a randomized 18-month intervention study on the effects of an especially developed sportive dance training versus classic fitness training on the neuroplasticity and balancing abilities of healthy seniors. The intervention consisted of two active groups, a dance and a fitness training group. Cognitively healthy seniors between the ages of 63 and 80 years were used as subject groups (detailed description of the sample in Müller et al., 2017). The participants continuously learned new, increasingly difficult (more complex, faster) choreographies similar to line dancing developed by sports scientists of the University of Magdeburg. Dancing choreographies required the timely retrieval of successive combinations of movements. The fitness training, on the other hand, was designed as cyclic-aerobic training, characterized by automated and alternating movements. The conditional load was controlled by documenting the pulse values during the training sessions and was based on the individually calculated training heart rate according to Karvonen (1957) with the factor 0.6 for extensive endurance training, so that the training conditions in the two groups with regard to intensity, duration and frequency were comparable. The study flow-chart is shown in Figure 13.

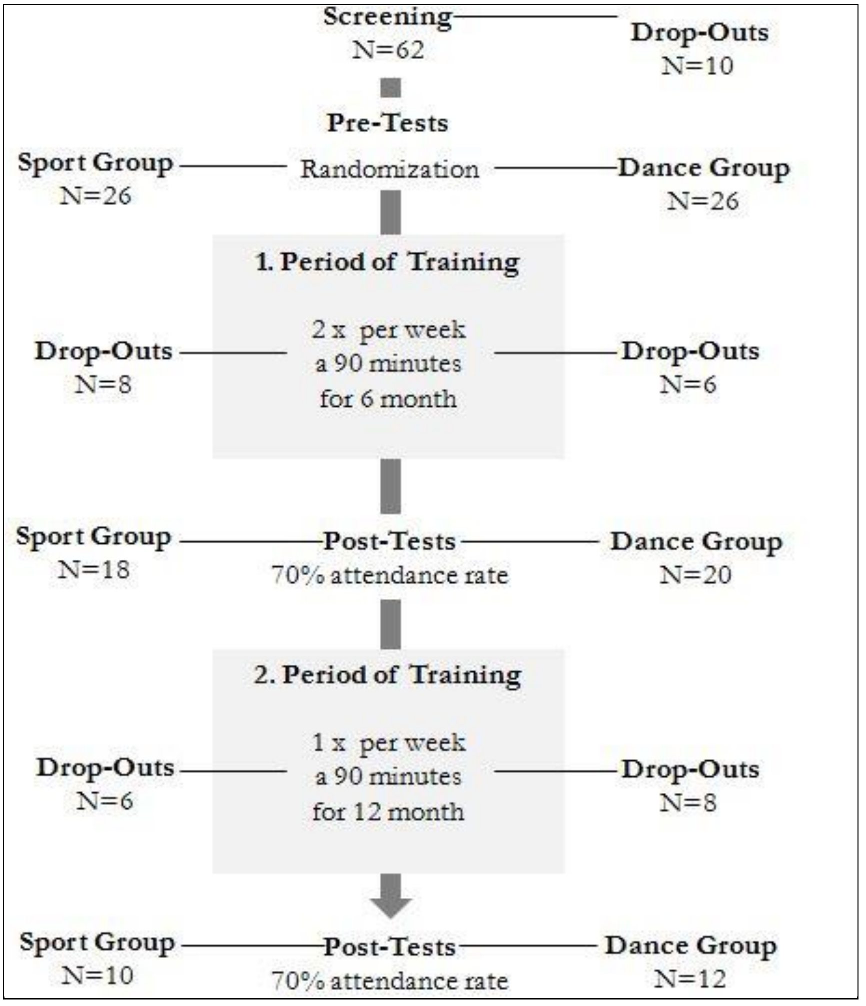


Figure 13 -:Study flow-chart (Müller et al., 2017, Rehfeld et al, 2017).

The results of the four reference publications are described below. All brain analyses were performed using statistical parametric mapping (SPM 8 and 12, UCL, UK) on T1-MRI images from MPRAGE sequences. Behavioural data were analysed with SPSS v.21.

4.1 Vestibulo-hippocampal function is enhanced and brain structure altered in professional ballet dancers [1]

The goal of this study was to clarify the differences between professional ballet dancers and the normal population with regards to brain structure, non-visual path integration and general balancing abilities.

The figure 5 shows characteristics of study participants.

Characteristic	Training (*n* = 19)	Control (*n* = 19)
Age (years)	27.5 ± 4.1	26.5 ± 2.1
Age when training begun (min–max)	8.0 ± 3.8 (3–16)	–
Sex (females)	10 (53%)	10 (53%)
Weight (kg)	59.4 ± 11.6	67.9 ± 10.4
Height (cm)	169.3 ± 10.1	172.5 ± 8.4
Hours of activity–per week	33.4 ± 13.5	3.3 ± 1.6
Handedness–right	18 (95%)	19 (100%)
Ethnic origin		
• European	16 (84%)	17 (90%)
• Asian (Indian)	0 (0%)	2 (10%)
• Asian (Japanese)	3 (16%)	0 (0%)

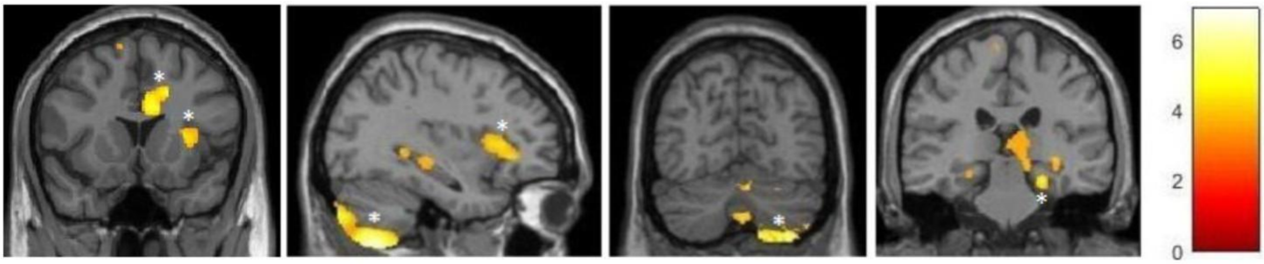
Figure 14 – Study participants

As mentioned earlier, grey matter differences between the two groups were analysed using SPM12 and VBM8 pipelines – these are based on T1-MRI images obtained with MPRAGE sequence. The behavioural measurements included the clinical balance test (CBT), conditions of which are listed in the Figure 11, and triangle completion test (TCT), depicted in the Figure 12.

For the ballet group, the VBM analysis revealed significantly larger cluster-based FWE-corrected grey matter volumes within the inferior and posterior areas of the right cerebellar

[1] Dordevic M, Schrader R, Taubert M, Müller P, Hökelmann A & Müller NG (2018). Vestibulo-hippocampal function is enhanced and brain structure altered in professional ballet dancers. *Frontiers in Integrative Neuroscience 12*

hemisphere, right parahippocampus, right cingulate motor cortex, and right insula (Figure 6). Additional tendencies at uncorrected level ($p < 0.001$) could be observed in the vermis, right posterior hippocampus, and right posterior thalamus. The respective MNI coordinates as well as the cluster sizes are listed in the Figure 15.



Brain region	Location	Direction of difference	MNI coordinates (*x*, *y*, and *z*)	Cluster size (in voxels)
Temporal	Right hippocampus	Larger	30, −26, −6	128
	Right parahippocampal gyrus	Larger	21, −32, −20	2,013
Temporo-parietal	Right insula	Larger	38, 24, 3	1,020
			27, 21, 2	
Cingulate cortex	Right hemisphere	Larger	6, 17, 24	800
			15, 17, 36	
Cerebellum	Right hemisphere	Larger	32, −68, −59	3,897
	Vermis	Larger	2, −68, −47	283
	Right hemisphere	Smaller	12, −65, −29	460
	Left hemisphere	Smaller	−12, −63, −29	30

Figure 15 – VBM-observed GM increments in the ballet group compared to the control group, with their locations, coordinates in MNI-space and sizes. *FWE-corrected at the cluster level.

As illustrated in the Figure 16, the ballet dancers performed significantly better on the CBT, which was true for all sub-conditions of the test except for the simplest task which involved standing on stable flat surface. The respective effect sizes were large to very large for all comparisons, including the condition where no significant difference was observed.

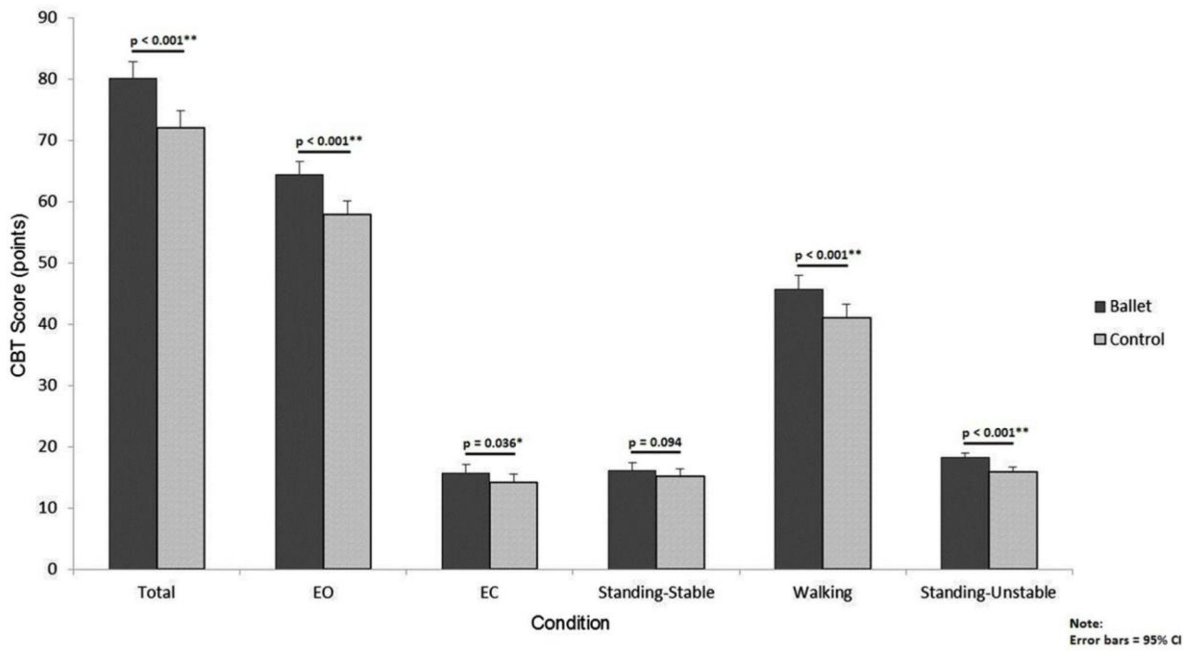


Figure 16 – Results from all conditions of the clinical balance test (CBT) for both ballet and control groups

Figure 17 illustrates the difference between the two groups on the TCT. The results demonstrated that ballet dancers performed significantly better on this test, by having smaller errors in both distance and angle, which was mainly attributable to their better performance in the wheelchair (vestibular) condition. Medium effect sizes were revealed for this condition.

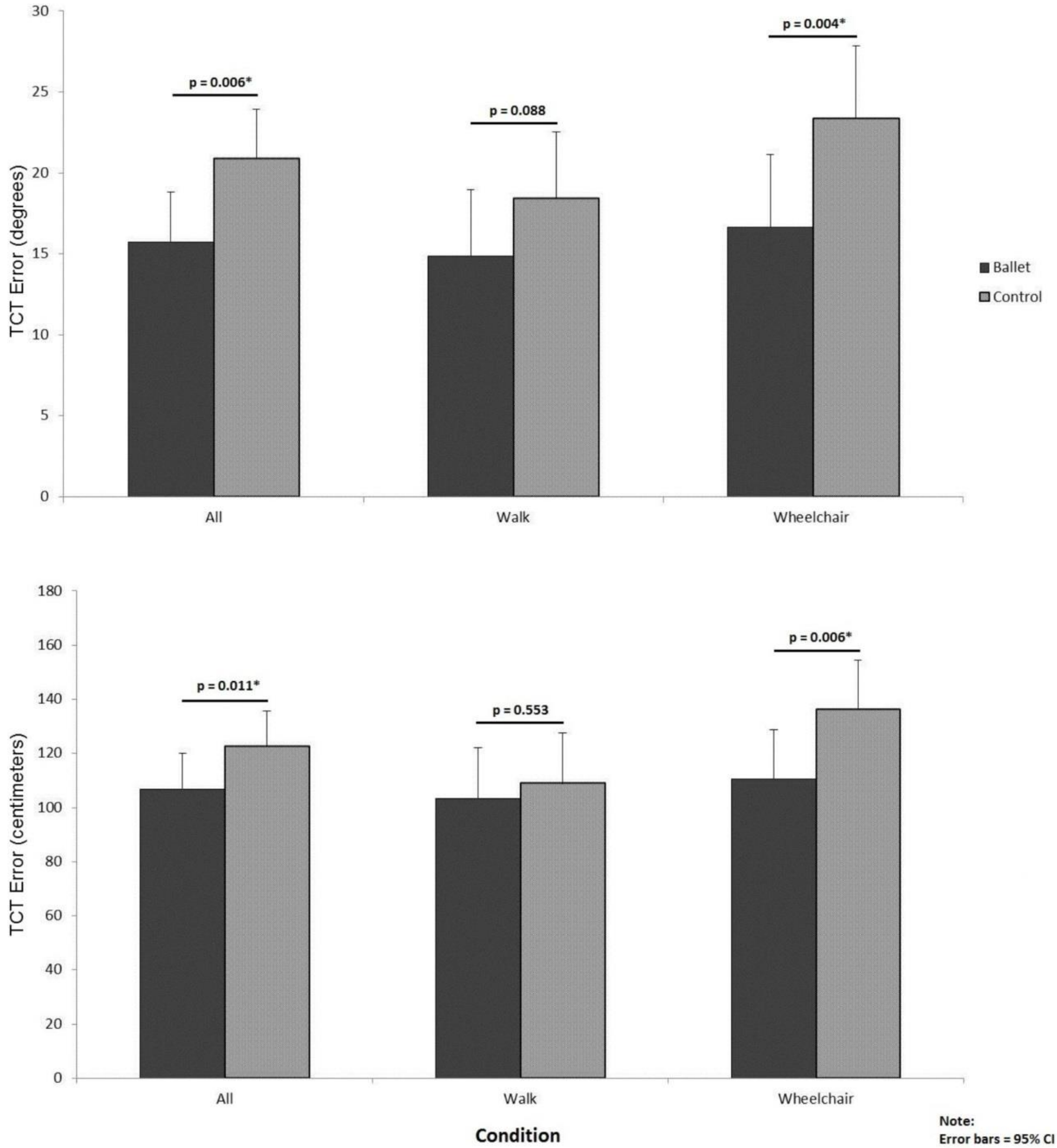


Figure 17 – Results from all conditions of the triangle completion test (TCT) for both ballet and control groups

4.2 Improvements in orientation and balancing abilities in response to one month of intensive slackline-training. A randomized controlled feasibility study [2]

Having in mind the close connection between the vestibular and orientation systems, we asked whether intensive slackline training can improve not only one's ability to maintain balance but also has transfer effects on the capability to successfully orientate in space. The goal of this study was to find out whether learning how to slackline over a period of one month can be of benefit for both stability and orientation skills.

Fifty healthy young subjects were recruited for this study and randomly assigned (without stratification) into two groups, control and training (Figure 18). The two groups did not significantly differ in any of the recorded demographic and other characteristics, including age, height, weight, years of education, handedness etc.

Characteristic	**Training (*n* = 25)**	**Control (*n* = 25)**
Age (years)	24.5 ± 2.7	23.2 ± 2.6
Sex (females)	11 (44%)	12 (48%)
Weight (kg)	69.1 ± 12.5	65.0 ± 10.0
Height (cm)	173.4 ± 9.2	170.3 ± 8.4
Hours of activity (per week)	3.0 ± 1.8	3.2 ± 2.5
Handedness (right)	24 (96%)	23 (92%)
Profession (student)	22 (88%)	23 (92%)
Suffered a small injury (e.g., ankle sprain)	5 (20%)	5 (20%)
Ethnic origin		
• European	20 (80%)	19 (76%)
• Asian (Indian)	5 (20%)	5 (20%)
• Arabic	0 (0%)	1 (4%)

Figure 18 – Characteristics of participants in the balance study

[2] Dordevic M, Hökelmann A, Müller P, Rehfeld K, Müller NG (2017). Improvements in orientation and balancing abilities in response to one month of intensive slackline-training. A randomized controlled feasibility study. *Frontiers in Human Neuroscience,* 11(26). DOI: 10.3389/fnhum.2017.00055

During this one month period the training group underwent intensive balance training consisting of 12 trainings (three trainings/week with each training lasting 1 h; max. 2 consecutive non-training days) on a 3-m long slackline, whilst the control group was instructed to abstain from any type of similar activity.

In contrast to the overall CBT results, when only those conditions were analysed in which the participants had their eyes closed, a significant interaction effect with medium to large effect size was observed (p = 0.011, η^2_p = 0.128), as can be seen from Figure 19**.** In these conditions the training group improved while the control group performed slightly worse on the post-test.

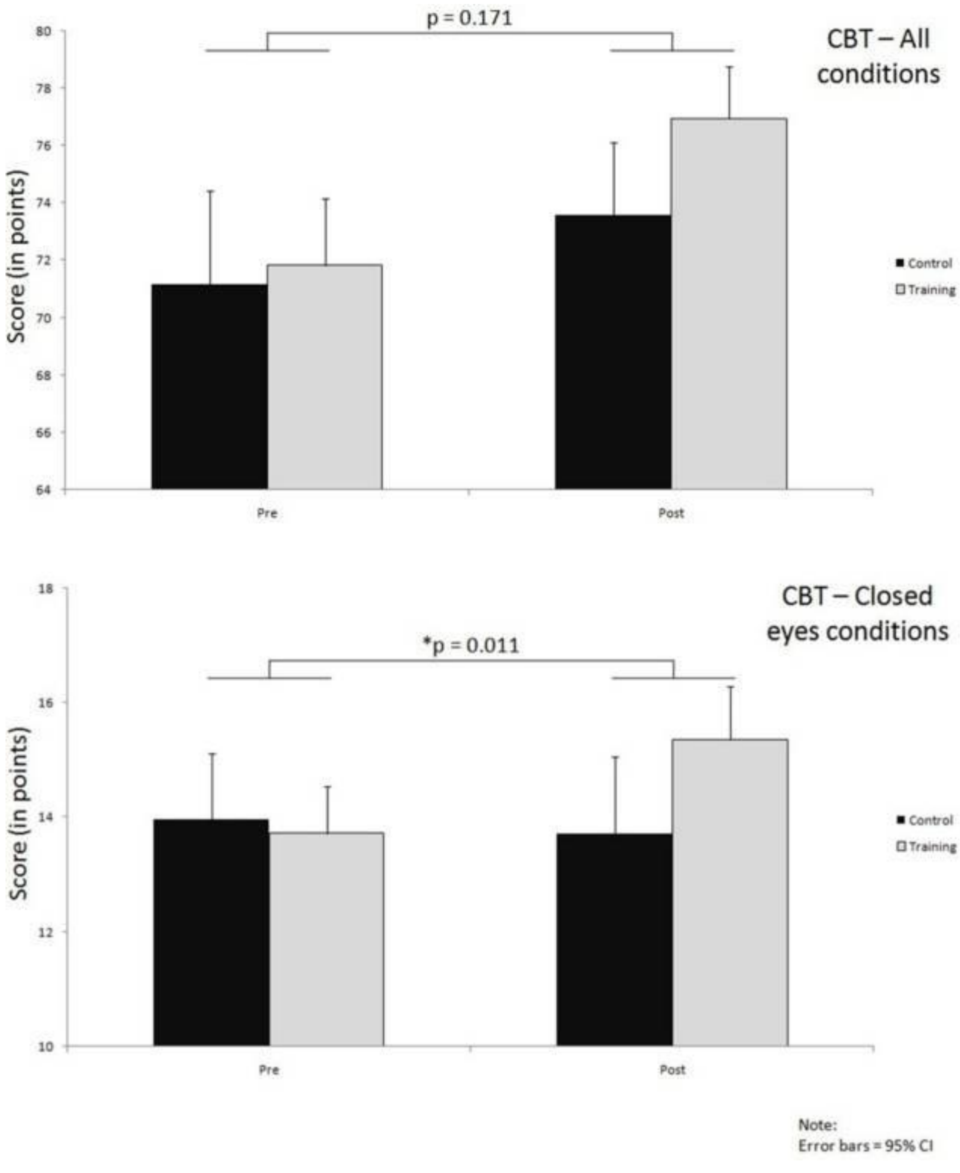


Figure 19 - Improvements over time in both groups on CBT, for all conditions together and closed eyes conditions only; significance levels (***p***) indicate time*group interaction effects

Overall TCT results gave a non-significant interaction effect with very small effect size (p = 0.063, η^2_p = 0.006) (Figure 20). Further analysis of the wheelchair condition results, however, revealed a much larger improvement in the training group compared to the control group; the training group improved by about 21 cm in comparison to a very small 1 cm improvement in the control group. This difference in improvements between the two groups that occurred over time led also to a significant interaction effect with a small effect size (p = 0.049, η^2_p = 0.013).

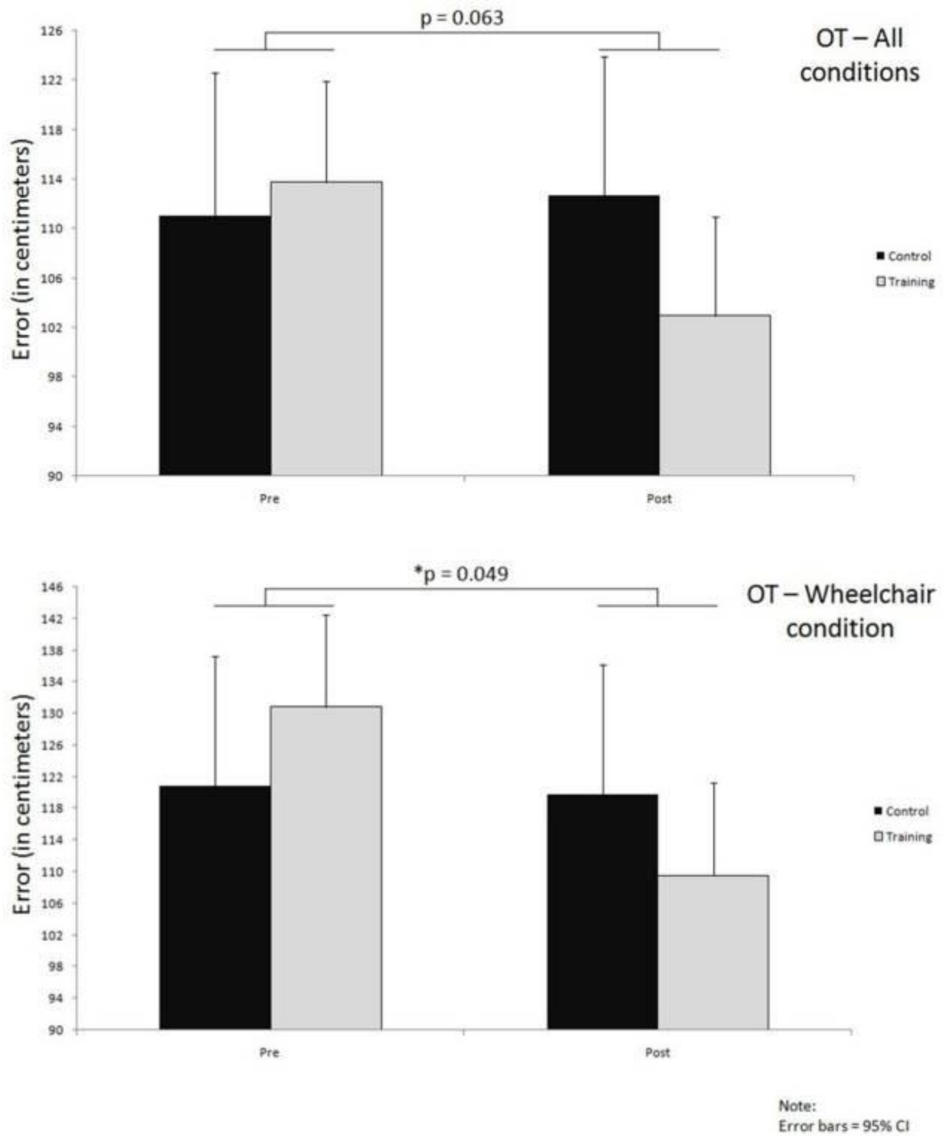


Figure 20 - Improvements over time in both groups on TCT, for all conditions together and wheelchair condition only; significance levels (***p***) indicate time*group interaction effects.

4.3 Evolution of Neuroplasticity in Response to Physical Activity in Old Age: The Case for Dancing[3]

In this 18-month study it has been assessed whether a newly designed dance training program that stresses the constant learning of new movement patterns is superior in terms of neuroplasticity to conventional fitness activities with repetitive exercises. The sample consisted of elderly subjects aged from 65 to 80 y, who were cognitively unimpaired (Figure 21).

Measure	Dancing group	Sport group
N	12	10
Age (years)	68.25 (3.91)	68.60 (2.79)
Gender (% female)	50%	40%
BMI	27.51 (3.87)	27.24 (2.94)
BDI-II	5.50 (2.94)	3.00 (3.77)
Education	15.50 (2.11)	16.40 (1.35)
MMSE	28.33 (1.07)	29.10 (0.57)

Figure 21 – Characteristics of participants at baseline

A significant group × time interaction was observed in the left precentral gyrus and the right parahippocampus (Figure 22). The volume increase in the precentral gyrus emerged after 6 months and remained stable over the remaining dance training interval, whereas the change in the parahippocampal gyrus occurred during the later training interval only.

[3] Müller P, Rehfeld K, Schmicker M, Hökelmann A, Dordevic M, Lessmann V, Brigadski T, Kaufmann J & Müller NG (2017). Evolution of neuroplasticity in response to physical activity in old age: the case for dancing. Front. Aging Neurosci. 9:56. doi: 10.3389/fnagi.2017.00056

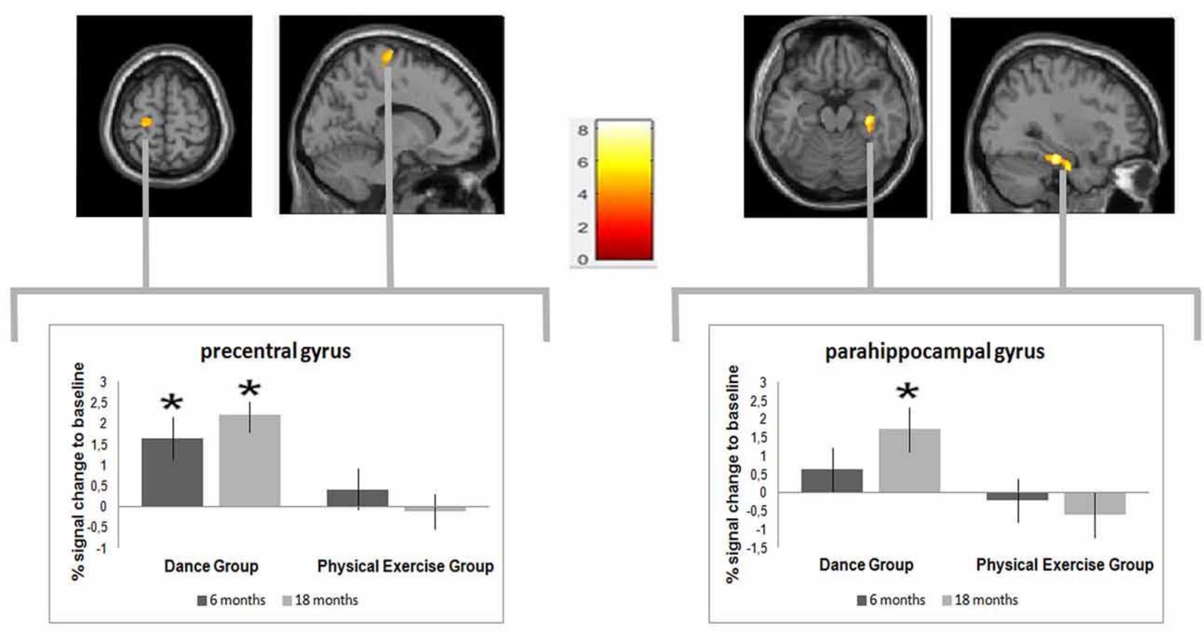


Figure 22 – Time x Group interaction analysis of gray matter. Significant volume increase was evident in the left precentral gyrus (MNI-Coordinates: x= -16; y= -18; z= 77) and right parahippocampal gyrus (MNI-coordinates: x= 34; y= -26; z= -20). The Box Plots show relative change in in gray matter ($p \leq 0.05$).

4.4 Dancing or Fitness Sport? The Effects of Two Training Programs on Hippocampal Plasticity and Balance Abilities in Healthy Seniors[4]

In Publication 4 the same intervention as in Publication 3 was used, on similar samples of participants in both the dance and fitness sport groups (Figure 23). Here in addition to neuroplasticity effects, it has been assessed whether the interventions have an effect on balancing capabilities. Postural control was tested using Sensory Organisation Test (SOT) implemented in the Balance Master System (Neurocom International, Inc. USA).

[4] Rehfeld K, Müller P, Aye N, Schmicker M, Dordevic M, Kaufmann J, Hökelmann A & Müller NG (2017). Dancing or fitness sport? The effects of two training programs on hippocampal plasticity and balance abilities in healthy seniors. *Frontiers in Human Neuroscience,* doi: 10.3389/fnhum.2017.00305

	Dance group [*N* = 14]		Sport group [*N* = 12]			
	M	*SD*	*M*	*SD*	*t*-value	*p*-value
Age [years]	67.21	3.78	68.67	2.57	−1.12	0.272
Sex [%]	50% male		58% male		0.154	0.695
MMSE [points]	28.07	0.92	29.17	0.58	−3.011	0.003
BDI-II [points]	4.5/5.54	3.37/2.97	3.75/3.39	3.79/3.77	0.53	0.598
Education [years]	15.40	2.05	16.10	1.45	−1.395	0.175
BMI [kg/m^2]	27.51	3.87	27.24	2.94	−0.275	0.786

BMI, Body-Mass-Index; BDI-II, Becks-Depressions-Inventar II; MMSE, Mini Mental State Examination; M, Mean; SD, Standard deviation; $p \leq 0.05$ = statistical significance.

Figure 23 – Dance study participants’ characteristics

To explore hippocampal grey matter volume changes during intervention we used repeated measurement ANOVA for comparison between baseline and post-test. There was a significant interaction effect in the right hippocampal region (Figure 24). *Post hoc* paired *t*-tests showed only in the dance group significant volume increases in this area.

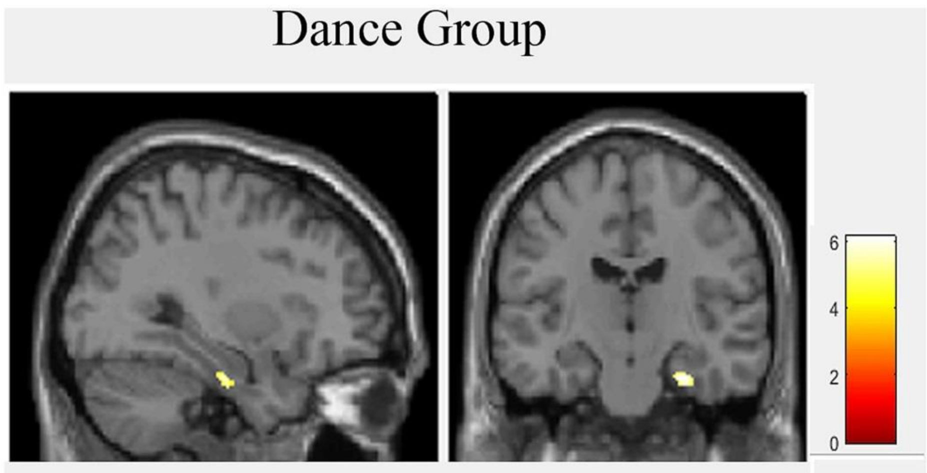
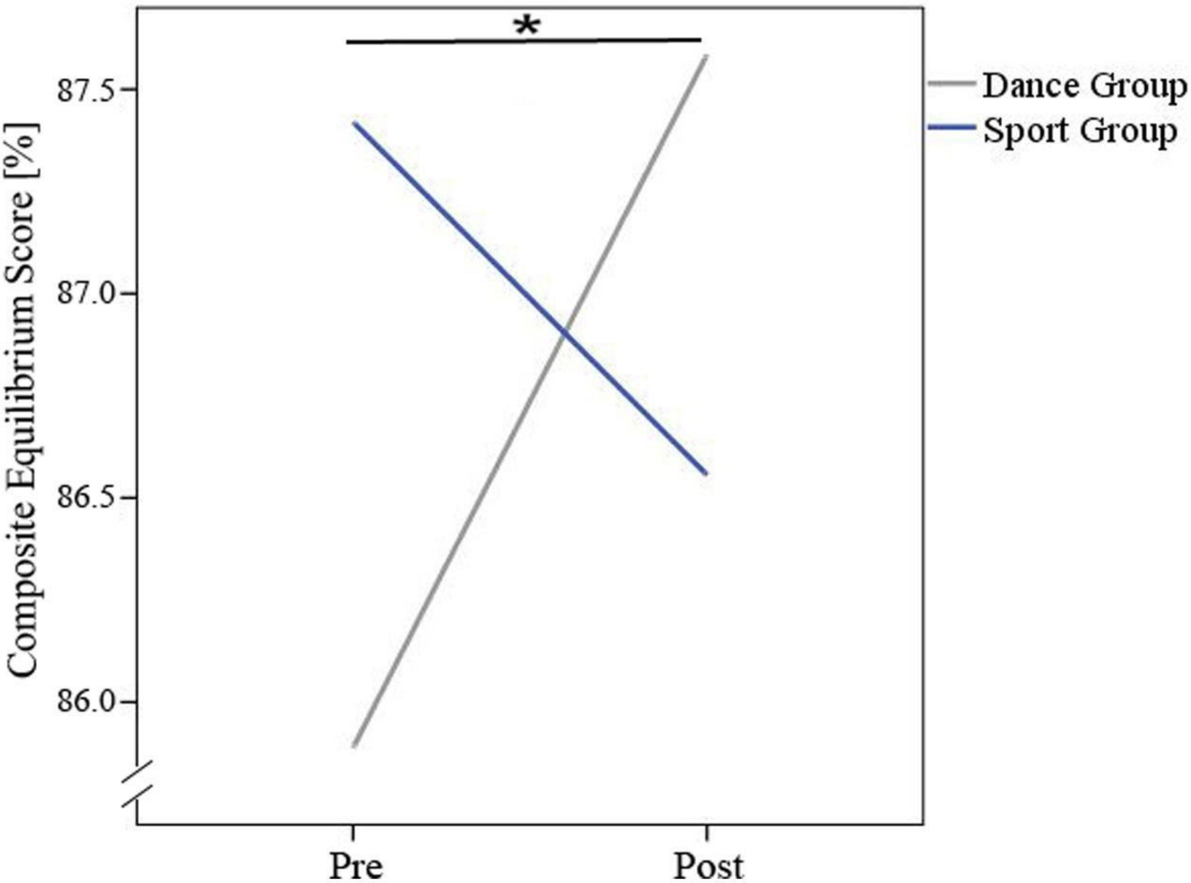


Figure 24 – VBM-observed volume increase in the dance group

Repeated measurement ANOVAs of the balance data showed an interaction effect with group for the composite equilibrium score (Figure 25). There was a main effect of time regarding the somatosensory and vestibular contribution but no significant time × group interaction effects after 18 months of training. *Post hoc* tests revealed that the dancers improved in the use of all three sensory systems (somatosensory, vestibular system and visual) to maintain balance. Members of the sports group improved in the use of the somatosensory system and the vestibular system but not in the visual system.



Sensory organization of balance [%]	Time				Group				Interaction (time × group)			
	F	df	*p*	η^2	*F*	df	*p*	η^2	*F*	df	*p*	η^2
Somatosensory system	30.340	1,25	**0.001*** **	0.591	1.208	1,25	0.284	0.054	0.692	1,25	0.415	0.032
Visual system	6.094	1,25	0.022*	0.225	0.363	1,25	0.553	0.017	0.296	1,25	0.592	0.014
Vestibular system	17.722	1,25	**0.001*** **	0.458	0.229	1,25	0.637	0.011	1.326	1,25	0.262	0.059
Composite equilibrium score	0.514	1,25	0.481	0.024	0.092	1,25	0.764	0.004	4.851	1,25	0.039*	0.188

*Level of significance: 0.01 ≤ α < 0.05: *"significant"; 0.001 ≤ α < 0.01: **"high significant"; α < 0.001: ***"highly significant".*

Figure 25 – Group*Time interaction effect for SOT

5 Discussion and Outlook

Following is the discussion of the results with reference to the problems and goals.

5.1. Hippocampal and cortical neuroplasticity – the cases of ballet dancing and sportive dancing

The results of the dissertation demonstrated that ballet dancers do have larger GM volumes in regions that contribute to balance and spatial orientation abilities, such as the posterior cerebellum and the vermis, insula, and hippocampal and parahippocampal regions, when compared to non-dancers; effects in the opposite direction, i.e., smaller GM volumes, were found in the cerebellar anterior lobes.

As a main finding in elderly dancers, we observed that after 18 months of sportive dance training, the volume in the right parahippocampal gyrus of the dancers had increased more than those in the control group. These were virtually in the same area as those seen in ballet dancers. Because the cardiovascular fitness levels over the course of the interventions remained constant in both groups, the observed effects could not be attributed to improvements in physical fitness but instead seemed to be related to the specific features of the dance program.

Increments in the posterior hippocampus have also been reported previously in several groups of professionals by Hüffner and colleagues (21) and were also observed after year-long experience in taxi-driving (30). In our study, we could also observe differences that were mainly localized on the right side of the brain. Our results indicated also that long-term training might be necessary for structural changes in posterior hippocampus to persist. The vestibular system does project to the hippocampus, it activates it functionally, and it is shown to have an important function for spatial orientation and learning (31,67–69). Humans can store spatio-temporal dynamic patterns of motion and retrieve it completely using vestibular and somatosensory cues (68,70). The effects in the right hippocampus are in accordance with several studies demonstrating that spatial orientation is mainly processed by the right hippocampus (26,27).

The differences in the right parahippocampal region for both the ballet dancers and choreography dancers were mainly located in the entorhinal cortex, particularly the medial entorhinal cortex. An investigation of the medial entorhinal cortex (MEC) in animals led to the discovery of grid cells that fire when the animal is in any of multiple locations that form a triangular grid (25,71,72). The parahippocampal gyrus constitutes the interface between memory and the experiential consciousness of the present, because it is interconnected by the perforant tract both to regions of the frontal lobe, which are associated with working memory, and to the hippocampus, the central structure in episodic memory encoding and spatial navigation.

For ballet dancers, we also observed highly significant differences in the caudal part of the cingulate cortex, area 24 of Broadman's classification. It is known from functional MRI studies that the cingulate motor cortex and the cerebellum are active during interlimb coordinative movements, together with primary and associative sensory and motor regions of the cortex (73). A somatotopy similar to that of the larger sensory-motor cortices can be found in those cingulate cortex regions where the changes were detected (74–78). Since such coordinative movements are a core element of ballet dancing, the finding of a larger volume of this brain region in dancers makes perfectly sense.

The changes we detected in the cerebellum were mainly in the expected direction, considering its close relationship with movement control and learning. It has been suggested that the learned programs are stored within the cerebellar cortex and that the memory capacity for storage is proportionate to the number of granule cells (79,80). Our study revealed a major GM expansion in the superficial layers of the cerebellum, which could be perhaps due to the increment in the capacity to store complex movement-related memories in ballet dancers. On the other hand, we observed significantly smaller GM volumes in slightly deeper cerebellar structures of the ballet dancers, parts that are known to be involved in the control of limb movements, which can be interpreted in terms of automaticity/stereotypy.

Main between-group differences in the thalamus were observed for its posterior part, and only on the right side. Earlier studies have proposed a functional importance of the posterolateral thalamus as a unique relay station for vestibular input to the cortex, but also the dominance of the right hemisphere in right-handedness, and of ipsilateral ascending pathways (81). Multiple thalamic nuclei are involved in vestibular processing, as well as somatosensory and visual, and this may explain the enlarged thalamus in the dancers (67,82). Since the early 1960s, researchers have speculated that the vestibular system, the sensory system concerned with the perception of balance and self-motion, contributes to spatial information processing and the development of spatial memory in the hippocampus. Anatomical studies have suggested that various parts of the thalamus are likely to transmit vestibular information to the hippocampus, perhaps via the parietal cortex; however, more direct pathways are possible. Over the last 2-3 years there has been a number of direct electrophysiological demonstrations that vestibular stimulation affects head direction cells in the anterior thalamic nuclei and place cells in the hippocampus. These studies demonstrate the importance of vestibular-hippocampal interactions for hippocampal function (3).

Possible neurobiological mechanisms of the observed GM differences could be neurogenesis, synaptogenesis, hypertrophy of glia cells, and angiogenesis (83). The generation of new cells within the confines of our findings can only be expected for the hippocampus, but not for other areas of the ballet dancers' brains (22). Increments can also be based on the sensory experience which drives the formation and elimination of synapses and these changes might underlie adaptive remodelling of neural circuits (84).

The main limitation of the study on ballet dancers is its cross-sectional nature, whereby no causal relationship for the effects observed can be established. Previous studies have, however, shown that training-induced neuroplastic adaptations are actually sport-specific rather than just sport-general (85–89).

5.2. Improvements in path integration abilities in response to ballet dancing and slacklining

On a behavioural level, both ballet dancers and the group who learned how to slackline over one month, in comparison with controls, demonstrated an increased ability to orientate in space with closed eyes in the mere vestibular-dependent condition, in which they were pushed in a wheelchair. No previous studies investigated this possibility, making consequently our results novel in that sense.

The link between the vestibular system and its central vestibular-dependent spatial-orientation brain regions, primarily the hippocampal regions (90), can be affected by an adequately designed slackline-training. After learning how to slackline, our participants were able to return to the starting position more precisely after being taken away from it in a wheelchair along three different triangular paths, which was also true for professional ballet dancers.

The triangle completion task was already used by many previous studies, mainly to examine the difference between younger and older persons in their ability to navigate in space (91,92) or to investigate functions of the medial temporal lobe (42,93). Consequently, the design of these studies was cross-sectional and no particular treatment was used to improve this ability over time. Our study is the first one to our knowledge to show transfer effects of slackline-training on orientation abilities in young people assessed with this task.

Several authors studied rats to demonstrate the importance of the vestibular system for successful orientating in space (32,33,94). It has been shown that peripheral vestibular deficiency leads to impairments in the functioning of the medial temporal lobe in spatial orientation tasks as well as in spatial learning. These impairments are due to alterations in electrophysiological and neurochemical signalling between the two systems. Other previous studies went on further to investigate the importance of the vestibular system for orientation in humans (4,5,95), thereby confirming the findings of animal studies.

5.3. Improvements in balancing skills in response to ballet dancing, slacklining and sportive dancing

All three studied groups demonstrated an increased ability to maintain balance compared to controls. These findings are supportive of the a priori hypothesized improvements of vestibular system function in response to intensive balance training.

One month of intensive slackline training led to significantly better performance of our training group participants on the CBT compared to their control counterparts, but only on those measurements where their visual input was blocked, i.e., where they had to balance with eyes closed. In contrast, on tasks where visual input was not blocked, both groups improved about the same, thus revealing a potential practice effect which might have taken place between pre- and post-test. Considering that the input from three systems involved in balance maintenance is present normally in a moving person (visual, vestibular and somatosensory) (96), it appears from our test results that the vestibular and somatosensory systems were particularly affected by the slackline-training. Ballet dancers were, however, better than controls in all conditions of the CBT.

Many earlier studies used numerous diverse approaches to enhance balancing skills in various target groups (65,97). The majority of balance trainings were reported to be successful in improving outcome variables in healthy young (97) and elderly (65,98,99) participants, athletes (100,101), as well as patients suffering from Alzheimer's (102) and Parkinson's disease (16), post-stroke patients (103) and patients with vestibular disorders (104). A literature review pertained to our first finding (stability improvement in closed-eyes conditions of CBT) revealed that similar studies (involving slackline-training) published before suggested large task-specific improvements (standing on a slackline) in response to training but only small to moderate non-task specific improvements (for a meta-analytical review see (105)). However, these studies used different training and evaluation methodologies; that is, the only non-task specific transfer effects evaluated were postural sway displacement and velocity changes, while participants stood with open eyes on a firm or suddenly perturbed flat surface of a force platform, mostly in one-leg and tandem stance modes. In contrast to these studies, for our analysis outcome from a comprehensive clinical balance assessment was used, in which the standing conditions included standing on both and each leg separately (not only one by own choice) in open and closed eyes conditions, on a firm flat but also on a soft, unstable surface. In fact, our main finding here was related to the larger improvement in the closed eyes conditions, which was not even assessed by these studies. Also, in our intervention study a shorter slackline length (3 meters) was intentionally chosen for the purpose of stimulating semicircular canal function, in addition to that of otolith organs; this important input (106,107) might have been neglected in other training interventions and its effects could hence have been overlooked.

Choreography dancers' balancing abilities were not assessed using the CBT but a Balance Master device instead. The choreography dancers showed an increased balance composite score and they improved in all three involved sensory systems. This indicates that dancing drives all three senses and presumably also improves the integration of sensorimotor, visual and vestibular information.

5.4. Potential of findings for dementia prevention

The observations that engaging in sportive and ballet dance programs for a longer period can induce neuroplastic processes in brains crucial for memory and spatial orientation region (i.e. hippocampus and parahippocampus) is, therefore, particularly encouraging in terms of developing prevention strategies.

Also, although our sample of those who learned how to slackline consisted of young and healthy subjects, considering neuroplasticity principles in response to motor task learning over the entire lifespan (108), it is legitimate to hypothesize that similar results could be expected in older populations, particularly as a prevention strategy in those at early stages of dementia.

AD is expansive throughout our ageing society, and so even a small impact of nonpharmacological interventions, such as physical activity and exercise, may have a major impact on public health (109). A multimodal exercise intervention can also improve the frontal cognitive functions in patients with Alzheimer's disease (110).

It has been proposed that vestibular system degeneration might be a significant contributor to development of the Alzheimer’s disease (111). Many VBM studies have reported an age-related volume loss in parahippocampal regions (112). Furthermore, Echávarri et al have suggested that parahippocampal atrophy is an early biomarker of AD (113).

5.5. Potential of findings for fall prevention

Successful balancing requires complex and harmonical processing of many inputs simultaneously, only one of which is the vestibular system. Neural pathways of these two abilities communicate through large networks and units of both cortical and subcortical structures (67).

Intact balance control is required not only to maintain postural stability but also to assure safe mobility-related activities during daily life (114,115).

Falls are a major source of death and injury in elderly people. For example, they cause 90% of hip fractures and the current cost of hip fractures in the US is estimated to be about 10 billion dollars. Age-related changes in the physiological systems (somatosensory, vestibular and visual) which contribute to the maintenance of balance are well documented in older adults. These changes coupled with age-related changes in muscle and bone are likely to contribute to an increased risk of falls in this population. Exercise appears to be a useful tool in fall prevention in older adults, significantly reducing the incidence of falls compared with control groups, and it also enhances gait ability, balance and muscle strength. A multi-component exercise intervention composed by strength, endurance and balance training seems to be the best strategy to improve rate of falls, gait ability, balance, and strength performance in physically frail older adults (63,98).

It has been recommended that exercise for falls prevention should provide a moderate or high challenge to balance and be undertaken for at least 2 hours per week on an ongoing basis and to target both the general community and those at high risk for falls (116). This is also true for patients suffering from AD (117).

In our studies the reduction of risk of falling was not explicitly assessed, but since each of the studied groups has improved their balancing abilities, we could generally say that these types of interventions, and any combination of them, can be beneficial for the prevention of falls.

5.6. Future outlook

Larger studies with more representative samples are required in the future. They should include additional analysis of mediating factors and they should try to find ways to optimally adjust the training protocol to an individual's needs and preferences. Most of all, it needs to be investigated in longitudinal randomized clinical trials whether the proposed interventions indeed have the potential to reduce or postpone the risk of neurodegenerative diseases such as Alzheimer's, as well as in the prevention of falls.

Future studies are also necessary for clarifying the connectivity and inter-dependence between the vestibular system and hippocampal formation. These could include additional cohorts, such as persons suffering from vestibulopathy, temporal epilepsy and blind persons. This would lead to better understanding of which hippocampal and parahippocampal sub-regions are dependent on which input, including vestibular, visual and somatosensory. In addition, methodological improvements should also be considered in future studies. These pertain to novel technological advancements in MRI scanning, such as 7T scanners and better sequences, but also to novel approaches to data analyses, including tractography (e.g. DTI or DWI), resting-state analyses and region of interest (ROI) approaches to hippocampal volume analyses that were recently developed.

6 Summary

The goal of this dissertation was to investigate whether stimulation of the vestibular system through balance training (ballet dancing, sportive dancing, slacklining) leads to hippocampal and cortical grey matter changes or differences to controls, and to functional transfer effects on hippocampal-dependent path-integration and balancing.

The results revealed grey matter increases in the right hippocampal and/or parahippocampal areas for both groups in which VBM-analyses were undertaken, ballet dancers and sportive dancers. In addition, sportive dancers showed an increase in the left premotor cortex and ballet dancers in the right cerebellum, cingulate motor gyrus and thalamus. All of the findings were in accordance with the hypothesized localization of effects, which were primarily in the areas responsible for movement control, spatial orientation and memory.

These neuroanatomical findings were supported by simultaneous improvements on the behavioural level, related to path-integration abilities and balancing, predominantly in vestibular-dependent conditions. All studied groups scored better on the triangle completion task (i.e. path-integration task), except for the dance group which was not tested for this skill, and on respective balancing tasks (CBT or SOT) compared to their own baseline or the respective control group.

It can be concluded that balance trainings which stimulate the vestibular system's function lead to an enhancement in vestibular-dependent path-integration and balancing abilities, together with corresponding neuroanatomical changes in medial temporal lobe regions responsible for spatial orientation and memory and cortical regions responsible for motor control.

These findings are useful for better understanding of neuroplasticity, primarily in the hippocampus, and its relationship to behavioural performance on path-integration and balancing tasks. They can also be useful for designing programs for prevention of dementia and falls.

6 Zusammenfassung

Das Ziel dieser Dissertation war es zu untersuchen, ob die Stimulierung des vestibulären Systems durch Gleichgewichtstraining (Balletttanzen, sportliches Tanzen, Slacklining) zu volumetrischen Änderungenen der grauen Substanz im Hippocampus und-Kortex und zu funktionellen Übertragungseffekten auf Hippocampus-abhängigen Pfad- Integration- und Gleichgewichtsfähigkeiten.

Die Ergebnisse zeigten für beide Gruppen, in denen VBM-Analysen durchgeführt wurden, (Balletttänzer und Sporttänzer) eine Zunahme in der grauen Substanz im rechten Hippocampal- und / oder Parahippocampalbereich. Darüber hinaus zeigten sportliche Tänzer eine Zunahme in der linken Prämotor-Kortex- und Balletttänzer im rechten Kleinhirn, Cingula-Motor-Gyrus und Thalamus. Alle Befunde stimmten mit der hypothetisierten Lokalisierung von Effekten überein, die sich hauptsächlich in den Bereichen vorhanden waren, die für Bewegungssteuerung, räumliche Orientierung und Gedächtnis verantwortlich sind.

Diese neuroanatomischen Befunde wurden durch gleichzeitige Verbesserungen auf Verhaltensebene unterstützt, die sich auf Pfadintegrationsfähigkeiten und Gleichgewichtsfähigkeiten bezogen, primär bei vestibular-abhängigen Bedingungen. Alle untersuchten Gruppen erzielten bei der Dreieck-Test (d.h. bei der Pfadintegrationsaufgabe) einen besseren Punktestand (mit Ausnahme der Tanzgruppe, die nicht für diese Fähigkeit getestet wurde) und bei den jeweiligen Gleichgewichtsaufgaben (CBT oder SOT), im Vergleich zu ihrer eigenen Grundlinie oder der jeweiligen Kontrolle Gruppe.

Es kann gefolgert werden, dass Gleichgewichtstrainings, die die Funktion des vestibulären Systems stimulieren, zu einer Verbesserung der vestibular-abhängigen Pfadintegrations- und Gleichgewichtsfähigkeiten führen, zusammen mit entsprechenden neuroanatomischen Veränderungen - in den medialen Temporallappenbereichen (verantwortlich für räumliche Orientierung und Gedächtnis) und kortikalen Bereichen (für sensorisch-motorische Funktionen).

Diese Erkenntnisse sind hilfreich für ein besseres Verständnis der Neuroplastizität, hauptsächlich im Hippocampus, und ihrer Beziehung zur Verhaltensleistung bei Pfadintegrations- und Gleichgewichtsaufgaben. Sie können auch nützlich sein, um Stürz- und Demenz-prävention Programme zu weiterzuentwickeln.

7 References

1. Fuchs E, Flügge G. Adult neuroplasticity: More than 40 years of research. Vol. 2014, Neural Plasticity. 2014.
2. Hötting K, Röder B. Beneficial effects of physical exercise on neuroplasticity and cognition. Vol. 37, Neuroscience and Biobehavioral Reviews. 2013. p. 2243–57.
3. Smith PF. Vestibular-hippocampal interactions. Vol. 7, Hippocampus. 1997. p. 465–71.
4. Brandt T, Schautzer F, Hamilton DA, Br??ning R, Markowitsch HJ, Kalla R, et al. Vestibular loss causes hippocampal atrophy and impaired spatial memory in humans. Brain. 2005;128(11):2732–41.
5. Hüfner K, Strupp M, Smith P, Brandt T, Jahn K. Spatial separation of visual and vestibular processing in the human hippocampal formation. Ann N Y Acad Sci. 2011;1233(1):177–86.
6. Jacob P-Y, Poucet B, Liberge M, Save E, Sargolini F. Vestibular control of entorhinal cortex activity in spatial navigation. Front Integr Neurosci [Internet]. 2014;8. Available from: http://journal.frontiersin.org/article/10.3389/fnint.2014.00038/abstract
7. Ungar A, Rafanelli M, Iacomelli I, Brunetti MA, Ceccofiglio A, Tesi F, et al. Fall prevention in the elderly. Vol. 10, Clinical Cases in Mineral and Bone Metabolism. 2013. p. 91–5.
8. Granacher U, Iten N, Roth R, Gollhofer A. Slackline training for balance and strength promotion. Int J Sports Med. 2010;31(10):717–23.
9. Bettio LEB, Rajendran L, Gil-Mohapel J. The effects of aging in the hippocampus and cognitive decline. Vol. 79, Neuroscience and Biobehavioral Reviews. 2017. p. 66–86.
10. Cheng ST. Cognitive Reserve and the Prevention of Dementia: the Role of Physical and Cognitive Activities. Vol. 18, Current Psychiatry Reports. 2016.
11. Erickson KI, Weinstein AM, Lopez OL. Physical Activity, Brain Plasticity, and Alzheimer's Disease. Vol. 43, Archives of Medical Research. 2012. p. 615–21.
12. Seidel O, Carius D, Kenville R, Ragert P. Motor learning in a complex balance task and associated neuroplasticity: A comparison between endurance athletes and non-athletes. J Neurophysiol. 2017 Jun;jn.00419.2017.
13. Müller P, Rehfeld K, Schmicker M, Hökelmann A, Dordevic M, Lessmann V, et al. Evolution of neuroplasticity in response to physical activity in old age: The case for dancing. Front Aging Neurosci. 2017;9(MAR).

14. Taubert M, Draganski B, Anwander A, Müller K, Horstmann A, Villringer A, et al. Dynamic properties of human brain structure: learning-related changes in cortical areas and associated fiber connections. J Neurosci [Internet]. 2010;30(35):11670–7. Available from: http://www.jneurosci.org/content/30/35/11670%5Cnhttp://www.jneurosci.org/content/30/35/11670.full%5Cnhttp://www.jneurosci.org/content/30/35/11670.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/20810887

15. Taubert M, Mehnert J, Pleger B, Villringer A. Rapid and specific gray matter changes in M1 induced by balance training. Neuroimage. 2016;133:399–407.

16. Sehm B, Taubert M, Conde V, Weise D, Classen J, Dukart J, et al. Structural brain plasticity in parkinson's disease induced by balance training. Neurobiol Aging. 2014;35(1):232–9.

17. Draganski B, Gaser C, Busch V, Schuierer G, Bogdahn U, May A. Changes in grey, matter induced by training. Nature. 2004;427(6972):311–2.

18. Sagi Y, Tavor I, Hofstetter S, Tzur-Moryosef S, Blumenfeld-Katzir T, Assaf Y. Learning in the Fast Lane: New Insights into Neuroplasticity. Neuron. 2012;73(6):1195–203.

19. Taubert M, Draganski B, Anwander A, Muller K, Horstmann A, Villringer A, et al. Dynamic Properties of Human Brain Structure: Learning-Related Changes in Cortical Areas and Associated Fiber Connections. J Neurosci [Internet]. 2010;30(35):11670–7. Available from: http://www.jneurosci.org/cgi/content/abstract/30/35/11670%5Cnhttp://www.jneurosci.org/cgi/content/full/30/35/11670%5Cnhttp://www.jneurosci.org/cgi/reprint/30/35/11670.pdf

20. Hänggi J, Koeneke S, Bezzola L, Jäncke L. Structural neuroplasticity in the sensorimotor network of professional female ballet dancers. Hum Brain Mapp. 2010;31(8):1196–206.

21. Hüfner K, Binetti C, Hamilton DA, Stephan T, Flanagin VL, Linn J, et al. Structural and functional plasticity of the hippocampal formation in professional dancers and slackliners. Hippocampus. 2011;21(8):855–65.

22. Bhardwaj RD, Curtis MA, Spalding KL, Buchholz BA, Fink D, Bjork-Eriksson T, et al. Neocortical neurogenesis in humans is restricted to development. Proc Natl Acad Sci [Internet]. 2006;103(33):12564–8. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0605177103

23. Moser EI, Roudi Y, Witter MP, Kentros C, Bonhoeffer T, Moser M-B. Grid cells and

cortical representation. Nat Rev Neurosci. 2014;15(7):466–81.

24. Moser EI, Roudi Y, Witter MP, Kentros C, Bonhoeffer T, Moser M-B. Grid cells and cortical representation. Nat Rev Neurosci [Internet]. 2014;15(7):466–81. Available from: http://www.nature.com/doifinder/10.1038/nrn3766

25. Moser EI, Kropff E, Moser M-B. Place cells, grid cells, and the brain's spatial representation system. Annu Rev Neurosci. 2008;31:69–89.

26. Smith M Lou, Milner B. The role of the right hippocampus in the recall of spatial location. Neuropsychologia. 1981;19(6):781–93.

27. Iglói K, Doeller CF, Berthoz A, Rondi-Reig L, Burgess N. Lateralized human hippocampal activity predicts navigation based on sequence or place memory. Proc Natl Acad Sci [Internet]. 2010;107(32):14466–71. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1004243107%5Cnfile:///Users/alison/Documents/Library.papers3/Articles/2010/Iglói/Proceedings of the National Academy of Sciences 2010 Iglói.pdf%5Cnpapers3://publication/doi/10.1073/pnas.1004243107

28. Wegman J, Fonteijn HM, van Ekert J, Tyborowska A, Jansen C, Janzen G. Gray and white matter correlates of navigational ability in humans. Hum Brain Mapp. 2014;35(6):2561–72.

29. Vertes RP. Major diencephalic inputs to the hippocampus: Supramammillary nucleus and nucleus reuniens. Circuitry and function. In: Progress in Brain Research. 2015. p. 121–44.

30. Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RSJ, et al. Navigation-related structural change in the hippocampi of taxi drivers. Proc Natl Acad Sci [Internet]. 2000;97(8):4398–403. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.070039597

31. Hitier M, Besnard S, Smith PF. Vestibular pathways involved in cognition. Front Integr Neurosci [Internet]. 2014;8(July):59. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4107830&tool=pmcentrez&rendertype=abstract

32. Stackman RW, Clark AS, Taube JS. Hippocampal spatial representations require vestibular input. Vol. 12, Hippocampus. 2002. p. 291–303.

33. Russell NA, Horii A, Smith PF, Darlington CL, Bilkey DK. Long-term effects of permanent vestibular lesions on hippocampal spatial firing. J Neurosci. 2003;23(16):6490–8.

34. Jahn K, Wagner J, Deutschl??nder A, Kalla R, H??fner K, Stephan T, et al. Human hippocampal activation during stance and locomotion: FMRI study on healthy, blind, and vestibular-loss subjects. In: Annals of the New York Academy of Sciences. 2009. p. 229–35.

35. Loomis JM, Klatzky RL, Golledge RG, Philbeck JW. Human navigation by path integration. In: Wayfinding Behavior: Cognitive Mapping and Other Satial Processes. 1999. p. 125–51.

36. Adamo DE, Brice??o EM, Sindone JA, Alexander NB, Moffat SD. Age differences in virtual environment and real world path integration. Front Aging Neurosci. 2012;4(SEP).

37. Loomis JM, Klatzky RL, Golledge RG. Navigating without vision: Basic and applied research. Optom Vis Sci. 2001;78(5):282–9.

38. Allen GL, Kirasic KC, Rashotte M a, Haun DBM. Aging and path integration skill: kinesthetic and vestibular contributions to wayfinding. Percept Psychophys. 2004;66(1):170–9.

39. Dordevic M, Hokelmann A, Muller P, Rehfeld K, Muller NG. Improvements in Orientation and Balancing Abilities in Response to One Month of Intensive Slackline-Training. A Randomized Controlled Feasibility Study. Front Hum Neurosci. 2017;11:55.

40. Riecke BE, Van Veen HAHC, Bülthoff HH. Visual homing is possible without landmarks: A path integration study in virtual reality. Presence Teleoperators Virtual Environ. 2002;11(5):443–73.

41. Wolbers T, Wiener JM, Mallot HA, Büchel C. Differential recruitment of the hippocampus, medial prefrontal cortex, and the human motion complex during path integration in humans. J Neurosci. 2007;27(35):9408–16.

42. Wiener JM, Berthoz A, Wolbers T. Dissociable cognitive mechanisms underlying human path integration. Exp Brain Res. 2011;208(1):61–71.

43. Purves D, Augustine GJ, Fitzpatrick D. Neuroscience, 4th Edition. Nature Reviews Neuroscience. 2008. 1-773 p.

44. Barmack NH. Central vestibular system: Vestibular nuclei and posterior cerebellum. Vol. 60, Brain Research Bulletin. 2003. p. 511–41.

45. Nathan PW, Smith M, Deacon P. Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. Brain [Internet]. 1996;119(6):1809–33. Available from: https://academic.oup.com/brain/article-lookup/doi/10.1093/brain/119.6.1809

46. Fukushima K. Corticovestibular interactions: Anatomy, electrophysiology, and functional considerations. Vol. 117, Experimental Brain Research. 1997. p. 1–16.

47. Barmack NH, Nastos MA, Pettorossii VE. The horizontal and vertical cervico-ocular reflexes of the rabbit. Brain Res. 1981;224(2):264–78.

48. Cohen B, Siegel JM, Mcginty DJ. Pontine reticular formation neurons and motor activity. Science (80-). 1978;199(4325):207–8.

49. Engberg I, Lundberg A, Ryall RW. Reticulospinal inhibition of transmission in reflex pathways. J Physiol. 1968;194(1):201–23.

50. Castiglioi AJ, Gallaway MC, Coulter JD. Spinal projections from the midbrain in monkey. J Comp Neurol. 1978;178(2):329–45.

51. Qu J, Zhou X, Zhu H, Cheng G, Ashwell KWS, Lu F. Development of the human superior colliculus and the retinocollicular projection. Exp Eye Res. 2006;82(2):300–10.

52. Meredith MA, Wallace MT, Stein BE. Visual, auditory and somatosensory convergence in output neurons of the cat superior colliculus: multisensory properties of the tecto-reticulo-spinal projection. Exp Brain Res. 1992;88(1):181–6.

53. Stangl M, Achtzehn J, Huber K, Dietrich C, Tempelmann C, Wolbers T. Compromised Grid-Cell-like Representations in Old Age as a Key Mechanism to Explain Age-Related Navigational Deficits. Curr Biol. 2018;28(7):1108–1115.e6.

54. Lester AW, Moffat SD, Wiener JM, Barnes CA, Wolbers T. The Aging Navigational System. Vol. 95, Neuron. 2017. p. 1019–35.

55. Strang AJ, Haworth J, Hieronymus M, Walsh M, Smart LJ. Structural changes in postural sway lend insight into effects of balance training, vision, and support surface on postural control in a healthy population. Eur J Appl Physiol. 2011;111(7):1485–95.

56. Rugelj D. The effect of functional balance training in frail nursing home residents. Arch Gerontol Geriatr. 2010;50(2):192–7.

57. Lelard T, Doutrellot PL, David P, Ahmaidi S. Effects of a 12-Week Tai Chi Chuan Program Versus a Balance Training Program on Postural Control and Walking Ability in Older People. Arch Phys Med Rehabil. 2010;91(1):9–14.

58. Rehfeld K, Lüders A, Hökelmann A, Lessmann V, Kaufmann J, Brigadski T, et al. Dance training is superior to repetitive physical exercise in inducing brain plasticity in the elderly. PLoS One. 2018;13(7).

59. Pettersson AF, Engardt M, Wahlund LO. Activity level and balance in subjects with mild Alzheimer's disease. Dement Geriatr Cogn Disord. 2002;13(4):213–6.

60. Halvarsson a., Olsson E, Faren E, Pettersson a., Stahle a. Effects of new, individually adjusted, progressive balance group training for elderly people with fear of falling and tend to fall: a randomized controlled trial. Clin Rehabil. 2011;25(11):1021–31.

61. Rolland Y, Pillard F, Klapouszczak A, Reynish E, Thomas D, Andrieu S, et al. Exercise program for nursing home residents with Alzheimer's disease: A 1-year randomized, controlled trial. J Am Geriatr Soc. 2007;55(2):158–65.

62. Ohman H, Savikko N, Strandberg T, Kautiainen H, Raivio M, Laakkonen M-L, et al. Effects of Exercise on Functional Performance and Fall Rate in Subjects with Mild or Advanced Alzheimer's Disease: Secondary Analyses of a Randomized Controlled Study. Dement Geriatr Cogn Disord. 2016;41(3–4):233–41.

63. Carter ND, Kannus P, Khan KM. Exercise in the Prevention of Falls in Older People and the Evidence. Sport Med. 2001;31(6):427–38.

64. Kannus P, Sievänen H, Palvanen M, Järvinen T, Parkkari J. Prevention of falls and consequent injuries in elderly people. Vol. 366, Lancet. 2005. p. 1885–93.

65. Sherrington C, Tiedemann A, Fairhall N, Close JCT, Lord SR. Exercise to prevent falls in older adults: an updated meta-analysis and best practice recommendations. N S W Public Health Bull [Internet]. 2011;22(3–4):78–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21632004

66. Ambrose AF, Paul G, Hausdorff JM. Risk factors for falls among older adults: A review of the literature. Vol. 75, Maturitas. 2013. p. 51–61.

67. Lopez C, Blanke O. The thalamocortical vestibular system in animals and humans. Vol. 67, Brain Research Reviews. 2011. p. 119–46.

68. Vitte E, Derosier C, Caritu Y, Berthoz A, Hasboun D, Soulié D. Activation of the hippocampal formation by vestibular stimulation: a functional magnetic resonance imaging study. Exp Brain Res. 1996;112(3):523–6.

69. Smith PF, Zheng Y. From ear to uncertainty: vestibular contributions to cognitive function. Front Integr Neurosci [Internet]. 2013;7(November):84. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3840327&tool=pmcentrez&rendertype=abstract

70. Berthoz a, Israël I, Georges-François P, Grasso R, Tsuzuku T. Spatial memory of body

linear displacement: what is being stored? Science. 1995;269(5220):95–8.

71. Moser EI, Roudi Y, Witter MP, Kentros C, Bonhoeffer T, Moser MB. Grid cells and cortical representation. Vol. 15, Nature Reviews Neuroscience. 2014. p. 466–81.

72. Sanders H, Rennó-Costa C, Idiart M, Lisman J. Grid Cells and Place Cells: An Integrated View of their Navigational and Memory Function. Vol. 38, Trends in Neurosciences. 2015. p. 763–75.

73. Debaere F, Swinnen SP, Beatse E, Sunaert S, Van Hecke P, Duysens J. Brain areas involved in interlimb coordination: a distributed network. Neuroimage [Internet]. 2001;14:947–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11697927

74. Vogt BA, Pandya DN, Rosene DL. Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents. J Comp Neurol. 1987;262(2):256–70.

75. Vogt BA, Finch DM, Olson CR. Functional heterogeneity in cingulate cortex: The anterior executive and posterior evaluative regions. Cereb Cortex. 1992;2(6):435–43.

76. Paus T, Petrides M, Evans a C, Meyer E. Role of the human anterior cingulate cortex in the control of oculomotor, manual, and speech responses: a positron emission tomography study. J Neurophysiol. 1993;70(2):453–69.

77. Petit L, Orssaud C, Tzourio N, Salamon G, Mazoyer B, Berthoz A. PET study of voluntary saccadic eye movements in humans: basal ganglia-thalamocortical system and cingulate cortex involvement. J Neurophysiol. 1993;69(4):1009–17.

78. Wu CWH, Bichot NP, Kaas JH. Converging evidence from microstimulation, architecture, and connections for multiple motor areas in the frontal and cingulate cortex of prosimian primates. J Comp Neurol. 2000;423(1):140–77.

79. Thach WT. A role for the cerebellum in learning movement coordination. Neurobiol Learn Mem [Internet]. 1998;70(1–2):177–88. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9753595

80. Boyden ES, Katoh A, Raymond JL. CEREBELLUM-DEPENDENT LEARNING: The Role of Multiple Plasticity Mechanisms. Annu Rev Neurosci [Internet]. 2004;27(1):581–609. Available from: http://www.annualreviews.org/doi/10.1146/annurev.neuro.27.070203.144238

81. Dieterich M, Bartenstein P, Spiegel S, Bense S, Schwaiger M, Brandt T. Thalamic infarctions cause side-specific suppression of vestibular cortex activations. Brain.

2005;128(9):2052–67.

82. Morel A, Magnin M, Jeanmonod D. Multiarchitectonic and stereotactic atlas f the human thalamus. J Comp Neurol. 1997;387(4):588–630.

83. Zatorre RJ, Fields RD, Johansen-Berg H. Plasticity in gray and white: neuroimaging changes in brain structure during learning. Nat Neurosci [Internet]. 2012;15(4):528–36. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3660656&tool=pmcentrez&rendertype=abstract

84. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature [Internet]. 2002;420(6917):788–94. Available from: http://www.nature.com/doifinder/10.1038/nature01273

85. Jäncke L, Koeneke S, Hoppe A, Rominger C, Hänggi J. The architecture of the golfer's brain. PLoS One. 2009;4(3).

86. Meier J, Topka MS, Hänggi J. Differences in Cortical Representation and Structural Connectivity of Hands and Feet between Professional Handball Players and Ballet Dancers. Neural Plast. 2016;2016.

87. Schlaffke L, Lissek S, Lenz M, Brüne M, Juckel G, Hinrichs T, et al. Sports and brain morphology - A voxel-based morphometry study with endurance athletes and martial artists. Neuroscience. 2014;259:35–42.

88. Huang R, Lu M, Song Z, Wang J. Long-term intensive training induced brain structural changes in world class gymnasts. Brain Struct Funct. 2015;220(2):625–44.

89. Wenzel U, Taubert M, Ragert P, Krug J, Villringer A. Functional and structural correlates of motor speed in the cerebellar anterior lobe. PLoS One. 2014;9(5).

90. Hitier M, Besnard S, Smith PF. Vestibular pathways involved in cognition. Front Integr Neurosci [Internet]. 2014;8(July):59. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4107830&tool=pmcentrez&rendertype=abstract

91. Allen GL, Kirasic KC, Rashotte MA, Haun DBM. Aging and path integration skill: Kinesthetic and vestibular contributions to wayfinding. Percept Psychophys. 2004;66(1):170–9.

92. Adamo DE, Briceño EM, Sindone JA, Alexander NB, Moffat SD. Age differences in

virtual environment and real world path integration. Front Aging Neurosci. 2012;4(SEP).

93. Wolbers T, Wiener JM, Mallot HA, Büchel C. Differential recruitment of the hippocampus, medial prefrontal cortex, and the human motion complex during path integration in humans. J Neurosci [Internet]. 2007;27(35):9408–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17728454

94. Smith PF, Horii A, Russell N, Bilkey DK, Zheng Y, Liu P, et al. The effects of vestibular lesions on hippocampal function in rats. Vol. 75, Progress in Neurobiology. 2005. p. 391–405.

95. Previc FH, Krueger WW, Ross R a, Roman M a, Siegel G. The relationship between vestibular function and topographical memory in older adults. Front Integr Neurosci. 2014;8(June):46.

96. Horak FB. Postural orientation and equilibriumL What do we need to know about neural control of balance to prevent falls? Age Ageing. 2006;35-S2(SUPPL.2):ii7-ii11.

97. Zech A, H??bscher M, Vogt L, Banzer W, H??nsel F, Pfeifer K. Balance training for neuromuscular control and performance enhancement: A systematic review. J Athl Train. 2010;45(4):392–403.

98. Cadore EL, Rodríguez-Mañas L, Sinclair A, Izquierdo M. Effects of different exercise interventions on risk of falls, gait ability, and balance in physically frail older adults: a systematic review. Rejuvenation Res. 2013;16(2):105–14.

99. El-Khoury F, Cassou B, Latouche A, Aegerter P, Charles M-A, Dargent-Molina P. Effectiveness of two year balance training programme on prevention of fall induced injuries in at risk women aged 75-85 living in community: Ossébo randomised controlled trial. BMJ. 2015;351:h3830.

100. Hubscher M, Zech A, Pfeifer K, Hansel F, Vogt L, Banzer W. Neuromuscular training for sports injury prevention: A systematic review. Med Sci Sports Exerc. 2010;42(3):413–21.

101. Boccolini G, Brazzit A, Bonfanti L, Alberti G. Using balance training to improve the performance of youth basketball players. Sport Sci Health. 2013;9(2):37–42.

102. Ries JD, Hutson J, Maralit LA, Brown MB. Group Balance Training Specifically Designed for Individuals With Alzheimer Disease : Impact on Berg Balance Scale, Timed Up and Go, Gait Speed, and Mini-Mental Status Examination. J Geriatr Phys Ther. 2015;37:1–11.

103. Lubetzky-Vilnai A, Kartin D. The effect of balance training on balance performance in

individuals poststroke: a systematic review. J Neurol Phys Ther. 2010;34(3):127–37.

104. Porciuncula F, Johnson CC, Glickman LB. The effect of vestibular rehabilitation on adults with bilateral vestibular hypofunction: A systematic review. Vol. 22, Journal of Vestibular Research: Equilibrium and Orientation. 2012. p. 283–98.

105. Donath L, Roth R, Zahner L, Faude O. Slackline Training (Balancing Over Narrow Nylon Ribbons) and Balance Performance: A Meta-Analytical Review. Sports Med. 2016 Oct;

106. Highstein SM. The central nervous system efferent control of the organs of balance and equilibrium. Vol. 12, Neuroscience Research. 1991. p. 13–30.

107. Cullen KE, Minor LB. Semicircular canal afferents similarly encode active and passive head-on-body rotations: implications for the role of vestibular efference. J Neurosci. 2002 Jun;22(11):RC226.

108. Dayan E, Cohen LG. Neuroplasticity subserving motor skill learning. Vol. 72, Neuron. 2011. p. 443–54.

109. Paillard T, Rolland Y, de Souto Barreto P. Protective Effects of Physical Exercise in Alzheimer’s Disease and Parkinson’s Disease: A Narrative Review. J Clin Neurol [Internet]. 2015;11(3):212–9. Available from: http://apps.webofknowledge.com.proxy1.lib.uwo.ca/full_record.do?product=UA&search_mode=GeneralSearch&qid=1&SID=3DEisdtiwykymieY6np&page=7&doc=67

110. De Andrade LP, Gobbi LTB, Coelho FGM, Christofoletti G, Riani Costa JL, Stella F. Benefits of multimodal exercise intervention for postural control and frontal cognitive functions in individuals with Alzheimer’s disease: A controlled trial. J Am Geriatr Soc. 2013;61(11):1919–26.

111. Previc FH. Vestibular loss as a contributor to Alzheimer’s disease. Med Hypotheses. 2013;80(4):360–7.

112. Tisserand DJ, Pruessner JC, Sanz Arigita EJ, Van Boxtel MPJ, Evans AC, Jolles J, et al. Regional frontal cortical volumes decrease differentially in aging: An MRI study to compare volumetric approaches and voxel-based morphometry. Neuroimage. 2002;17(2):657–69.

113. Echávarri C, Aalten P, Uylings HBM, Jacobs HIL, Visser PJ, Gronenschild EHBM, et al. Atrophy in the parahippocampal gyrus as an early biomarker of alzheimer’s disease. Brain Struct Funct. 2011;215(3–4):265–71.

114. Mancini M, Horak FB. The relevance of clinical balance assessment tools to differentiate

balance deficits. Eur J Phys Rehabil Med. 2010;46(2):239–48.

115. Karlsson MK, Vonschewelov T, Karlsson C, Cöster M, Rosengen BE. Prevention of falls in the elderly: a review. Scand J Public Health. 2013;41(5):442–54.

116. Sherrington C, Tiedemann A, Fairhall N, Close JCT, Lord SR. Exercise to prevent falls in older adults: an updated meta-analysis and best practice recommendations. N S W Public Health Bull. 2011;22(3–4):78–83.

117. Suttanon P, Hill KD, Said CM, LoGiudice D, Lautenschlager NT, Dodd KJ. Balance and mobility dysfunction and falls risk in older people with mild to moderate alzheimer disease. Am J Phys Med Rehabil. 2012;91(1):12–23.

Acknowledgement

I would like to express a special gratitude to my supervisor Prof. Dr. med. Notger Müller for his valuable and knowledgeable input and endless support with numerous projects we have been working on together.

I am also very grateful to Prof. Dr. phil. Anita Hökelmann for her support with starting the balance study project.

Also, I greatly appreciate the work of my colleagues Dr. rer. medic. Patrick Müller and Dr. phil. Kathrin Rehfeld during cooperation on several common projects. I would also like to say thanks to Dr. rer. nat. Marlen Schmicker for cooperating on several other projects and to numerous colleagues and students in DZNE without whom none of the projects could have been finished or running, without listing them here name by name for practical reasons. Many thanks also to all of my co-authors for their help in the publication process.

Last but not the least, I would like to thank a lot to my family for their endless support and trust in everything that I was working on during my career.

Selbstständigkeitserklärung

Ich erkläre, dass ich die an der Medizinischen Fakultät der Otto-von-Guericke-Universität zur Promotion eingereichte Dissertation mit dem Titel

Hippocampal and cortical neuroplasticity and functional changes induced by vestibular system stimulation through various methods of balance trainings

am Deutschen Zentrum für Neurodegenerative Erkrankungen (DZNE)

mit Unterstützung durch

Prof. Dr. med. Notger G. Müller

ohne sonstige Hilfe durchgeführt und bei der Abfassung der Dissertation keine anderen als die dort aufgeführten Hilfsmittel benutzt habe.

Bei der Abfassung der Dissertation sind Rechte Dritter nicht verletzt worden.

Ich habe die Dissertation bisher an keiner in- oder ausländischen Hochschule zur Promotion eingereicht. Ich übertrage der Medizinischen Fakultät das recht, weitere Kopien einer Dissertation herzustellen und zu vertreiben.

Magdeburg, den 30.11.2018

Miloš Đorđević

Curriculum Vitae

Studies/Internships

from 07/2014	Doctoral studies (Dr. rer. medic) at the Otto-von-Guericke-University Magdeburg
from 02/2014	Research Assistant at the Deutschen Zentrum für Neurodegenerative Erkrankungen in the Neuroprotection Research Group (Prof. Dr. med. Notger Müller)
10/2011 - 09/2013	Master studies in Performance Analysis (Master of Science) at the Otto-von-Guericke-University, Nottingham Trent University, Valencia University and Basel University
08/2012 - 9/2013	Research Assistant at the Kantosspital Baselland - Bruderholz (PD. Dr. med. Michael Hirschmann)
01/2011 - 07/2011	Assistant at the Serbian Institute of Sports Medicine (PD Dr. med. Sanja Mazic)
09/2009 - 12/2010	Assistant at the Serbian Olympic Committee (Prof. Dr. phil. Branislav Jevtic)
09/2003 - 09/2009	Diploma Studies in Sports Science and Physical Education at the University of Belgrade

Scholarships/Rewards

07/2014	Sachsen-Anhalt Scholarship (30.000€)
2012/2013	Erasmus Scholarships (6.000€)
08/2012	Scholarship of Stifftung für Biomechanische Forschung - Basel (20.000€)

Magdeburg, den 30.11.2018

Miloš Đorđević

List of all publications

Papers assesed through a Peer-Review-Process

Dordevic M, Schrader R, Taubert M, Müller P, Hökelmann A, Müller NG (2018) Vestibulo-Hippocampal Function Is Enhanced and Brain Structure Altered in Professional Ballet Dancers . Front Integr Neurosci 12:50 Available at: https://www.frontiersin.org/article/10.3389/fnint.2018.00050.

Becke A, Müller P, Dordevic M, Lessmann V, Brigadski T, Müller NG (2018) Daily Intermittent Normobaric Hypoxia Over 2 Weeks Reduces BDNF Plasma Levels in Young Adults – A Randomized Controlled Feasibility Study. Front Physiol 9 Available at: https://www.frontiersin.org/article/10.3389/fphys.2018.01337/full.

Schmicker M, Kunz V, Müller P, Dordevic M, Müller N (2018) P73. A new cognitive diagnostic marker to distinguish between Subjective Cognitive Decline, mild cognitive impairment and healthy adults? Clin Neurophysiol 129:e97 Available at: http://www.sciencedirect.com/science/article/pii/S1388245718310162.

Rechsteiner J, Hirschmann MT, Dordevic M, Falkowski AL, Testa EA, Amsler F, Hirschmann A (2018) Meniscal pathologies on MRI correlate with increased bone tracer uptake in SPECT/CT. Eur Radiol 28:4696–4704.

Dordevic M, Müller-Fotti A, Müller P, Schmicker M, Kaufmann J, Müller NG (2017b) Optimal Cut-Off Value for Locus Coeruleus-to-Pons Intensity Ratio as Clinical Biomarker for Alzheimer’s Disease: A Pilot Study. J Alzheimer’s Dis Reports 1:159–167.

Rehfeld K, Müller P, Aye N, Schmicker M, Dordevic M, Kaufmann J, Hökelmann A, Müller NG (2017) Dancing or Fitness Sport? The Effects of Two Training Programs on Hippocampal Plasticity and Balance Abilities in Healthy Seniors. Front Hum Neurosci 11 Available at: http://journal.frontiersin.org/article/10.3389/fnhum.2017.00305/full.

Müller P, Rehfeld K, Schmicker M, Hökelmann A, Dordevic M, Lessmann V, Brigadski T, Kaufmann J, Müller NG (2017) Evolution of neuroplasticity in response to physical activity in old age: The case for dancing. Front Aging Neurosci 9.

Dordevic M, Hökelmann A, Müller P, Rehfeld K, Müller NG (2017a) Improvements in

Orientation and Balancing Abilities in Response to One Month of Intensive Slackline-Training. A Randomized Controlled Feasibility Study. Front Hum Neurosci 11:1–12.

Dordevic M, Hirschmann MT, Rechsteiner J, Falkowski A, Testa E, Hirschmann A (2016) Do Chondral Lesions of the Knee Correlate with Bone Tracer Uptake by Using SPECT/CT? Radiology 278:223–231.

Mucha A, Dordevic M, Hirschmann A, Rasch H, Amsler F, Arnold MP, Hirschmann MT (2015) Effect of high tibial osteotomy on joint loading in symptomatic patients with varus aligned knees: a study using SPECT/CT. Knee Surg Sports Traumatol Arthrosc 23:2315–2323

Mucha A, Dordevic M, Testa EA, Rasch H, Hirschmann MT (2013) Assessment of the loading history of patients after high tibial osteotomy using SPECT/CT--a new diagnostic tool and algorithm. J Orthop Surg Res 8:46 Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4029186&tool=pmcentrez&rendertype=abstract.

Book chapters

Dordevic M, Hirschmann MT (2014a) Injury mechanisms of ACL tear. In: Anterior Cruciate Ligament Reconstruction, pp 49–53 Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed9&AN=2009302001 http://dk-elib-srv-sfx02.elibdrift.dk/sfxvgr?sid=OVID:embase&id=pmid:&id=doi:10.1053/j.otsm.2009.02.003&issn=1060-1872&isbn=&volume=17&issue=1&spage=2&pages=2-10&date.

Dordevic M, Hirschmann MT (2014b) Biomechanics of the knee with intact anterior cruciate ligament. In: Anterior Cruciate Ligament Reconstruction, pp 39–48 Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed9&AN=2009302001 http://dk-elib-srv-sfx02.elibdrift.dk/sfxvgr?sid=OVID:embase&id=pmid:&id=doi:10.1053/j.otsm.2009.02.003&issn=1060-1872&isbn=&volume=17&issue=1&spage=2&pages=2-10&date.

Dordevic M, Hirschmann MT (2014c) Biomechanics of the Knee After Complete and Partial ACL Tear. In: Anterior Cruciate Ligament Reconstruction: A Practical Surgical Guide (Siebold R, Dejour D, Zaffagnini S, eds), pp 55–57. Berlin, Heidelberg: Springer Berlin Heidelberg. Available at: https://doi.org/10.1007/978-3-642-45349-6_8.

Enclosure 1

Publication 1

Dordevic M, Schrader R, Taubert M, Müller P, Hökelmann A & Müller NG (2018). Vestibulo-hippocampal function is enhanced and brain structure altered in professional ballet dancers. *Frontiers in Integrative Neuroscience 12*.

Enclosure 2

Publication 2

Dordevic M, Hökelmann A, Müller P, Rehfeld K, Müller NG (2017). Improvements in orientation and balancing abilities in response to one month of intensive slackline-training. A randomized controlled feasibility study. *Frontiers in Human Neuroscience,* 11(26). DOI: 10.3389/fnhum.2017.00055

Enclosure 3

Publication 3

Müller P, Rehfeld K, Schmicker M, Hökelmann A, Dordevic M, Lessmann V, Brigadski T, Kaufmann J & Müller NG (2017). Evolution of neuroplasticity in response to physical activity in old age: the case for dancing. *Frontiers in Aging Neuroscience.* 9:56. DOI: 10.3389/fnagi.2017.00056

Enclosure 4

Publication 4

Rehfeld K, Müller P, Aye N, Schmicker M, Dordevic M, Kaufmann J, Hökelmann A & Müller NG (2017). Dancing or fitness sport? The effects of two training programs on hippocampal plasticity and balance abilities in healthy seniors. *Frontiers in Human Neuroscience*. DOI: 10.3389/fnhum.2017.00305

frontiers
in Integrative Neuroscience

ORIGINAL RESEARCH
published: 18 October 2018
doi: 10.3389/fnint.2018.00050

Vestibulo-Hippocampal Function Is Enhanced and Brain Structure Altered in Professional Ballet Dancers

Milos Dordevic[1,2]*, Robert Schrader[1,3], Marco Taubert[3], Patrick Müller[1], Anita Hökelmann[3] and Notger G. Müller[1,2,4]

[1]German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany, [2] Neurology Clinic, Otto von Guericke University Magdeburg, Magdeburg, Germany, [3] Institute of Sports Science, Otto von Guericke University Magdeburg, Magdeburg, Germany, [4] Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany

OPEN ACCESS

Edited by:
Pierre Denise,
INSERM U1075 Université de Caen Normandie–2 Pôle des Formations et de Recherche en Santé, France

Reviewed by:
Paul Smith,
University of Otago, New Zealand
Philippe Perrin,
Université de Lorraine, France

****Correspondence:***
Milos Dordevic
milos.dordevic@dzne.de

Received: 19 March 2018
Accepted: 01 October 2018
Published: 18 October 2018

Citation:
Dordevic M, Schrader R, Taubert M, Müller P, Hökelmann A and Müller NG (2018) Vestibulo-Hippocampal Function Is Enhanced and Brain Structure Altered in Professional Ballet Dancers. Front. Integr. Neurosci. 12:50. doi: 10.3389/fnint.2018.00050

Background and Objective: Life-long balance training has been shown to affect brain structure, including the hippocampus. Data are missing in this respect on professional ballet dancers of both genders. It is also unknown whether transfer effects exist on general balancing as well as spatial orientation abilities, a function mainly supported by the hippocampus. We aimed to assess differences in gray matter (GM) structure, general balancing skills, and spatial orientation skills between professional ballet dancers and non-dancers.

Methods: Nineteen professional ballet dancers aged 18–35 (27.5 4.1 years; 10 females) and nineteen age-matched non-dancers (26.5 2.1 years; 10 females) were investigated. Main outcomes assessed were the score of a 30-item clinical balance test (CBT), the average error distance (in centimeters) on triangle completion task, and difference in GM density as seen by voxel-based morphometric analysis (VBM, SPM).

Results: Ballet group performed significantly better on all conditions of the CBT and in the wheelchair (vestibular-dependent) condition of the spatial orientation test. Larger GM volumes for ballet dancers were observed in the right hippocampus, parahippocampal gyrus, insula, and cingulate motor cortex, whereas both larger and smaller volumes were detected within cerebellum bilaterally in comparison to non-dancers.

Conclusion: Our results indicate that life-long ballet training could lead to better clinically relevant balancing abilities as well as vestibular-dependent spatial orientation capabilities; both of the benefits might be caused by positive influence of ballet training on the vestibular system function, and—possibly—its connectivity with temporal lobe regions responsible for vestibular-dependent orienting in space.

Keywords: balance, ballet, orientation, vestibular system, hippocampus, MRI, VBM

INTRODUCTION

Ballet dancing does not only require the coordination of complex movement patterns but also demanding in terms of the processing of vestibular input and maintaining balance. The assumption of a vestibulo-hippocampal dependency is supported by previous research in humans which showed that complete abolition of vestibular input, due to bilateral vestibulectomy, leads to atrophy in distinct medial temporal lobe areas (Brandt et al., 2005). Results of more recent studies also revealed hippocampal changes in patients suffering from various vestibular system disorders (Zu Eulenburg et al., 2010; Göttlich et al., 2016; Kremmyda et al., 2016). Multiple pathways that exist between the two have been discovered (Hitier et al., 2014), and there is a disruption in function of the latter when the input from the former ceases (Stackman et al., 2002; Russell et al., 2003). One study on professional ballet dancers, together with slackliners and ice-skaters, has demonstrated structural differences in temporal brain regions, particularly the hippocampus, compared to normal controls (Hüfner et al., 2011). This study could not, however, answer the question on neuroanatomical differences specifically related to ballet dancing, since it included other professionals in its cohort. Another study reported decrements in gray matter (GM) of several brain regions (Hänggi et al., 2010), including both cortical and subcortical structures, but the cohort consisted of only female ballet dancers, whose brain and development have already been shown to differ from that of males (Giedd et al., 1999; Good et al., 2001). Therefore, based on previous research it was not possible to determine which specific brain regions show significant differences in GM when comparing professional ballet dancers to non-dancers.

Another remaining question pertains to non-visual-dependent spatial orientation skills, as well as balancing skills, of ballet dancers. That is, it is unknown if these professionals have better developed non-visual-dependent abilities to orientate in space and balance when compared to persons not involved in such activities. Results of our own previously published research indicated that one-month of intensive slackline training can lead to significant improvements in these abilities (Dordevic et al., 2017b). The eventual findings in this respect could also be useful for a better understanding of both healthy aging and dementia prevention, as the decline in this ability has been linked to the degeneration of spatial navigation centers, located in the hippocampus and surrounding temporal brain areas (Allen et al., 2004). Additionally, loss of balance is an important cause of injuries, especially in old age, and it represents a major burden for the health system (Carter et al., 2001; Kannus et al., 2005; Sherrington et al., 2011).

Accordingly we hypothesized that: (1) brain regions, and particularly those responsible for balance and spatial orientation functions, of professional ballet dancers would show different structure compared to non-dancers and (2) ballet dancers would perform better in balancing and spatial orientation tasks, especially those that are vestibular-dependent (in which the vestibular input is the dominant one and thus governs the performance). Hence, the goal of our study was to clarify the differences between professional ballet dancers and the normal population with regards to brain structure, non-visual spatial orientation abilities, and general balancing abilities.

MATERIALS AND METHODS

Ethical Approval

This study was carried out in accordance with the recommendations of guidelines of Ethics Committee of the Medical Faculty at the Otto von Guericke University (approval number: 156/14) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Medical Faculty at the Otto von Guericke University.

Subjects

Twenty-six professional ballet dancers (18–35 years old) were initially recruited for this study. Since seven of them were not eligible for MRI scanning according to the safety procedures of our department (tooth braces and similar metal implants, tinnitus, etc.), they had to be excluded. The remaining nineteen ballet dancers were age- and gender-matched by the control participants (**Table 1**). Physical activity was assessed by asking subjects how many hours they spend on training weekly on average; all sports were taken into consideration, including jogging, various team sports, cycling, etc., but not walking. Participants of both groups were paid the same amount of money for their participation in the study. Sample size and characteristics, as well as the balance-training duration have been justified by our previous studies (Dordevic et al., 2017a,b).

Eligible control subjects for this study were all those aged from 18 to 35 years who had no previous experience in any ballet-related similar activity (i.e., highly demanding balancing activities, such as slacklining, rhythmic gymnastics, etc.) and had normal or corrected to normal vision. Exclusion criteria were injuries to the musculoskeletal system and systemic diseases (e.g., cardiovascular, metabolic, nervous system diseases, etc.) that might influence their performance. Participants were recruited through advertisement in the buildings of Otto von Guericke University in Magdeburg.

Study Design

This study was planned and organized as a cross-sectional one with one factor—namely group (control, ballet). The participants of the control group were age- and gender-matched to the participants of the ballet group. All the measurements took place in the movement lab of the German Center for Neurodegenerative Diseases from July 2015 to December 2016.

Behavioral Tests

Both tests have been described in our previously published work (Dordevic et al., 2017a). In brief, clinical balance test (CBT) consisted of standing on stable and unstable surfaces and walking

conditions, all of which further contain sub-conditions with open and closed eyes.
Standing conditions included:

Two- and one-leg stance on both stable (floor) and unstable (soft pad) surfaces, with both open and closed eyes.

Walking conditions included:

Walking forward, backward, and turning inside a 30 cm wide and 4 m long polygon with open eyes, followed by the same test on a 5 cm wide line as well as on 10 cm wide balance beam.
Walking forward on 5 cm line with closed eyes.

In total, there are 30 assessment items within this test, 14 of which assess standing; 16 walking; and 8 of all measurements are performed with closed eyes. The maximum amount of points that could be collected on the test was 90, with each condition carrying the minimum of 0 and the maximum of 3 points. In each of the standing conditions participants were instructed to maintain the required position for 15 s, whereas in walking conditions there was no time requirement and participants were asked to walk at their own pace. For detailed list of conditions see **Table 1**.
For assessment of non-visual spatial orientation the triangle completion test (TCT) was used. In brief, six triangular paths were marked on the floor of a room, three in the left and three in the right direction, giving thus three pairs of triangular paths, with turning angles of 60, 90, and 120 . The test consisted of two conditions: active-walking and passive-wheelchair. In the active-walking condition, while being guided on foot, the participant's movement was controlled by leading him or her along two sides of the triangular path as he or she held onto a wooden bar. The passive-wheelchair condition included transport along the same routes with the use of a standard wheelchair with attached footpads. Each participant was walked (active) and pushed (passive) only once along each of the paths, giving thus 12 trials per participant in total (3 to the left and 3 to the right, times 2 conditions). Once the participant was walked/pushed in the wheelchair along two sides of each triangle, his or her task was to walk along the third one, back to the starting point, using thus the shortest possible way back; that is, the participants were instructed not to walk back along the two sides that were used to

TABLE 1 | Test conditions of the Clinical balance test (CBT).

No.	Condition	Task		Points (min = 0, max = 3)		
			0	**1**	**2**	**3**
1	Static–stable surface (floor)	Stand with feet together–open eyes				
2		Stand with feet together–closed eyes				
3		One leg stance–left–open eyes				
4		One leg stance–right–open eyes				
5		One leg stance–left–closed eyes				
6		One leg stance–right–closed eyes				
7	Static–unstable surface (pad)	Stand normally (hip width stance)–open eyes				
8		Stand with feet together–open eyes				
9		Stand normally (hip width stance)–closed eyes				
10		Stand with feet together–closed eyes				
11		One leg stance–left–open eyes				
12		One leg stance–right–open eyes				
13		One leg stance–left–closed eyes				
14		One leg stance–right–closed eyes				
15	Dynamic	Walk inside the zone (4 m 30 cm)	Forward			
16			Turn (90)			
17			Backward			
18		Walk on the line (4 m 5 cm)	Forward			
19			Turn (90)			
20			Backward			
21		Walk on the line with feet one after the other (4 m 5 cm)	Forward			
22			Turn (90)			
23			Backward			
24		Walk on the beam (4 m 10 cm)	Forward			
25			Turn (90)			
26			Backward			
27		Walk on the beam sideways (4 m 10 cm)	Rightward			
28			Turn (90)			
29			Leftward			
30		Walk on the line with closed eyes (4 m 5 cm)	Forward			

bring them to the drop-off point, but to use the shortest possible way back to the starting point instead, which is actually always the third side of the respective triangle. The first main outcome variables were the distance error on each trial, which was assessed by marking the participant's stopping point with adhesive dots on the floor and later measuring the distance from that stopping point to the starting point, from which the respective movement was initiated. The second outcome variable was angular error, which was estimated as the angular deviation from the optimal direction (that would take directly to the start point). For the whole duration of the test participants were blindfolded in a quiet room and thereby could not use any visual or auditory cues that might help them in finding their way back to the starting point. It can thus be assumed that the only cues they could use were somatosensory and vestibular in the active-walking condition and vestibular only in the passive-wheelchair condition.

MRI

MR images were acquired on a 3 Tesla Siemens MAGNETOM Verio scanner (Syngo MR B17) using a 32-channel head coil. High-resolution T1-weighted MPRAGE sequences were acquired using a 3D magnetization-prepared rapid gradient echo imaging protocol (224 sagittal slices, voxel size: 0.8 mm 0.8 mm 0.8 mm, TR: 2,500 ms, TE: 3.47 ms, TI: 1,100 ms, and flip angle: 7).

Voxel-based morphometry (VBM) is a whole-brain unbiased technique for analysis of regional GM volume and tissue changes (Ashburner and Friston, 2000). Preprocessing involved gray-matter segmentation, template creation *via* DARTEL, spatial normalization to standardized Montreal Neurological Institute (MNI) space and smoothing with an Gaussian kernel of 8 mm full width at half maximum (FWHM).

Outcome Variables and Data Analysis

The outcome variable for the neuroanatomical analysis was the structural difference in brain neuroanatomy as observed by VBM. In order to analyze the difference in GM volume changes between groups, an independent *t*-test with the factor group (ballet, control) was applied. Since the whole-brain between-group comparison was carried out, multiple comparison correction was also performed in the form of Family-wise-error (FWE) correction, where the results were considered significant at $p < 0.05$, unless otherwise specified (uncorrected $p < 0.001$). Data were analyzed with MatLab (Mathworks, United States) and SPM12 (UCL, Great Britain). The results of the VBM analyses are visualized using the xjView toolbox[1].

Analysis of the behavioral data was performed with SPSS v.21 (IBM, United States), with the group (control, ballet) as factor. Independent *t*-test was run after checking for assumptions of normality and homogeneity of variance. If the assumptions were not met, the non-parametric equivalent (Mann–Whitney *U*-test) was applied.

In tables and figures the respective means with 95% confidence intervals of the difference are presented. In addition the effect sizes are calculated and listed.

1 http://www.alivelearn.net/xjview

RESULTS

The final analysis included 19 participants in each group. All subjects were recruited from July 2015 to December 2016 and their characteristics are shown in **Table 2**. The participants of the two groups did significantly differ in weight ($p = 0.02$) and amount of training hours per week ($p < 0.001$), whereas in other characteristics there was no significant difference.

Behavioral Tests

As illustrated in the **Figure 1**, the ballet dancers performed significantly better on the CBT, which was true for all sub-conditions of the test except for the simplest task which involved standing on stable flat surface. In the **Table 3** are listed the mean values for the two groups as well as the effect sizes and confidence intervals of the difference between the two groups. The effect size was large to very large for all comparisons, including the condition where no significant difference was observed.

The **Figure 2** illustrates the difference between the two groups on the TCT. The results demonstrated that ballet dancers performed significantly better on this test, by having smaller error in both distance and angle, which was mainly attributable to their better performance on the wheelchair (vestibular) condition. The results in the **Table 3** depict medium effect sizes for this condition.

VBM Analysis

For the ballet group, the VBM analysis revealed significantly larger cluster-based FWE-corrected gray matter volumes within the inferior and posterior areas of the right cerebellar hemisphere, right parahippocampus, right cingulate motor cortex, and right insula (**Figure 3**). Additional tendencies at uncorrected level ($p < 0.001$) could be observed in the vermis, right posterior hippocampus, and right posterior thalamus. The respective MNI coordinates as well as the cluster sizes are listed in the **Table 4**.

The control group participants also had significantly larger volume (FWE-corrected at cluster and voxel levels) within the cerebellum when compared to the ballet group, which was located within the right anterior lobe (**Figure 4**). A small tendency could also be observed at a similar location within the left cerebellar

TABLE 2 | Characteristics of participants.

Characteristic	Training (n = 19)	Control (n = 19)
Age (years)	27.5 4.1	26.5 2.1
Age when training begun (min–max)	8.0 3.8 (3–16)	–
Sex (females)	10 (53%)	10 (53%)
Weight (kg)	59.4 11.6	67.9 10.4
Height (cm)	169.3 10.1	172.5 8.4
Hours of activity–per week	33.4 13.5	3.3 1.6
Handedness–right	18 (95%)	19 (100%)
Ethnic origin		
European	16 (84%)	17 (90%)
Asian (Indian)	0 (0%)	2 (10%)
Asian (Japanese)	3 (16%)	0 (0%)

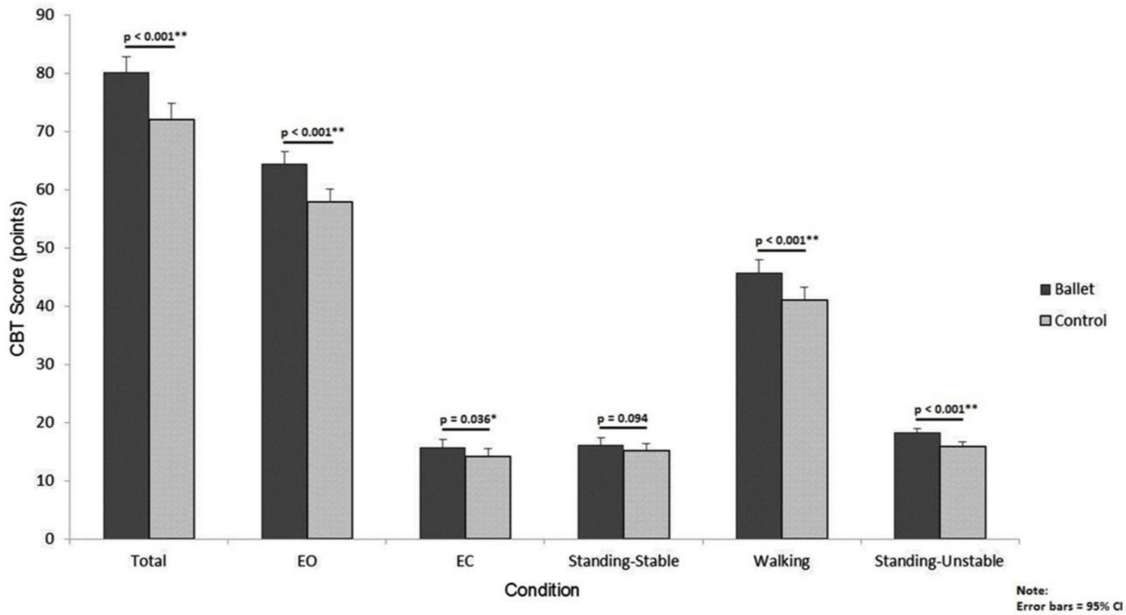


FIGURE 1 | Comparison of ballet and control groups on all conditions of the clinical balance test (CBT).

TABLE 3 | Comparison of ballet and control groups on all conditions of the clinical balance test (CBT).

Test (assessment scale)	Condition	Mean		95% CI of the difference	Effect size (d)
		Ballet	Control		
CBT (points)	Total	80.1	72.0	5.3 to 10.9	1.89
	Eyes open	64.4	57.9	4.3 to 8.6	1.98
	Eyes closed	15.7	14.1	0.1 to 3.1	0.70
	Standing-stable	16.2	15.1	0.2 to 2.3	0.56
	Walking	45.7	41.1	2.5 to 6.8	1.43
	Standing-unstable	18.2	15.8	1.6 to 3.2	1.94
TCT (degrees)	Total	15.7	20.9	8.2 to 2.1	0.22
	Walk	14.9	18.4	7.7 to 0.5	0.08
	Wheelchair	16.6	23.3	11.2 to 2.2	0.37
TCT (centimeters)	Total	106.9	122.5	28.8 to 2.5	0.31
	Walk	103.2	108.9	24.3 to 13.1	0.23
	Wheelchair	110.5	136.1	44.0 to 7.4	0.39

hemisphere. The respective MNI coordinates and sizes of these clusters are also presented in the **Table 4**.

DISCUSSION

The results of our study confirm both of our *a priori* hypotheses. That is, ballet dancers do have larger GM volumes in regions that contribute to balance and spatial orientation abilities, such as the posterior cerebellum and the vermis, insula, and hippocampal and parahippocampal regions, when compared to non-dancers; effects in the opposite direction, i.e., smaller GM volumes, were found in the cerebellar anterior lobes. On a behavioral level, in comparison with non-dancers, they demonstrated an increased ability to maintain balance in all conditions, as well as to orientate in space with closed eyes, especially in the mere vestibular-dependent condition, in which the blindfolded subjects did not walk themselves but were pushed in a wheelchair.

To date, to the best of our knowledge, there has been only one similar study on structural brain alterations in professional ballet dancers, however, this study only investigated females, and did not assess their spatial orientation and general balancing abilities, which prevents us from effectively comparing these with our findings. In the named study, Hänggi and colleagues (Hänggi et al., 2010) did not report any GM increments in ballet dancers compared to non-dancers. Instead they observed smaller volumes in several areas, including the supplementary motor and premotor areas and the putamen, all of which were located in the

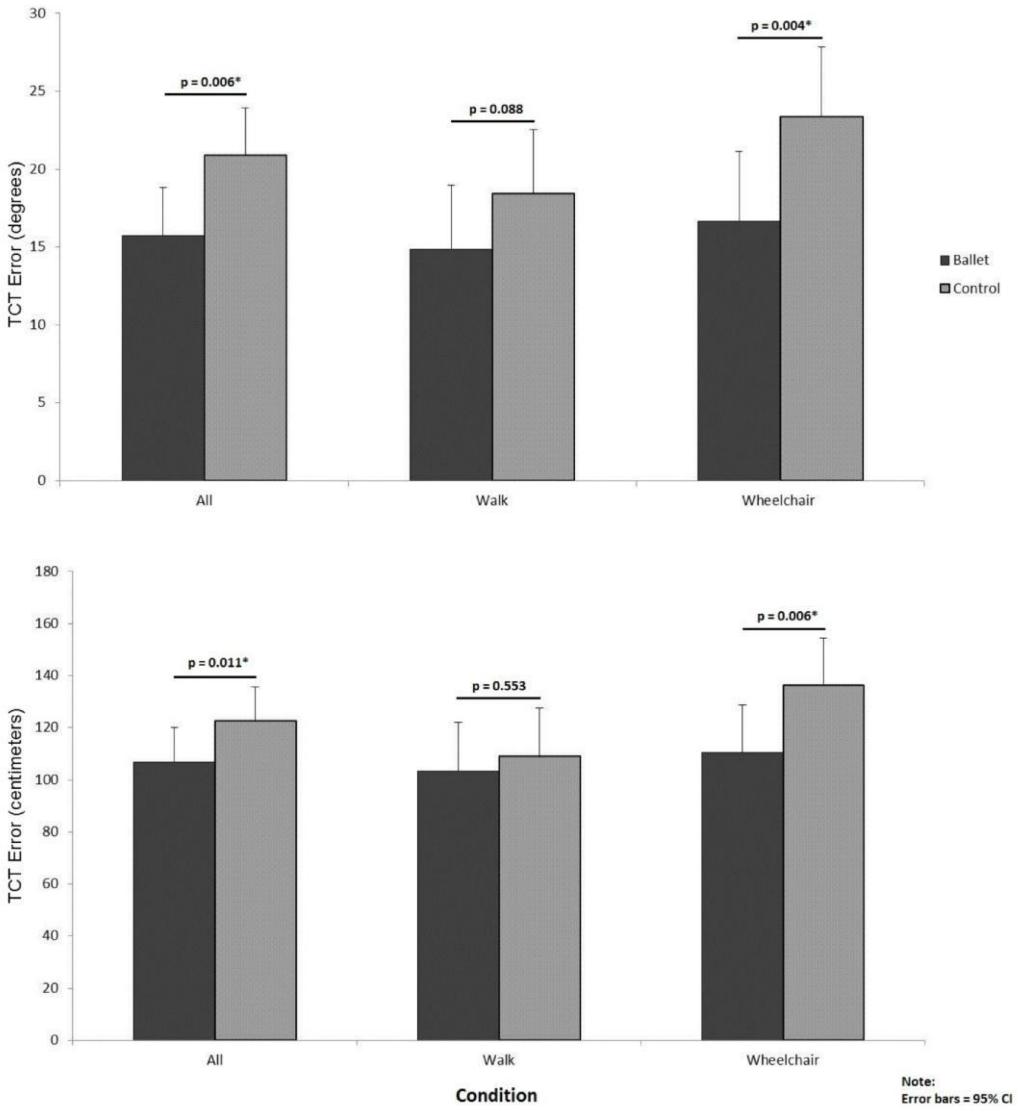


FIGURE 2 | Comparison of ballet and control groups on both conditions of the triangle completion task (TCT).

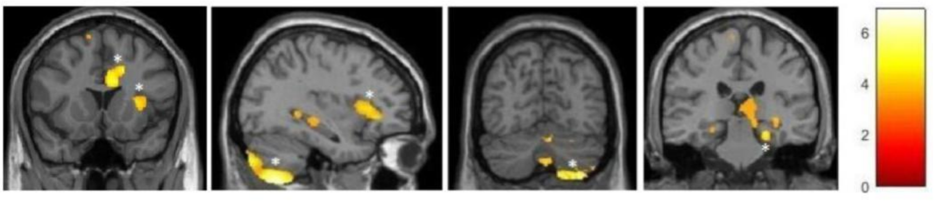


FIGURE 3 | VBM observed GM increments in the ballet group compared to the control group. FWE-corrected at the cluster level.

left hemisphere. This is in contrast to our findings, both regarding the localization and the direction of the effects. That is, apart from smaller GM volumes in the right and left cerebellar anterior lobes, we observed increments in the right posterior cerebellar hemisphere, vermis, right hippocampal and parahippocampal areas, and right posterior thalamus. This dissimilarity could be perhaps attributed to some of the cohort or methodological differences, including the sample, which in our case consisted of both genders, together with a higher mean age and the duration of professional activity of participants, as well as the VBM analysis procedure. For instance, the cerebellum and the pons are larger in men than in women and the difference is

TABLE 4 | MNI coordinates of VBM-detected gray matter changes in the ballet group as compared to the control group.

Brain region	Location	Direction of difference	MNI coordinates (*x*, *y*, and *z*)	Cluster size (in voxels)
Temporal	Right hippocampus	Larger	30, 26, 6	128
	Right parahippocampal gyrus	Larger	21, 32, 20	2,013
Temporo-parietal	Right insula	Larger	38, 24, 3	1,020
			27, 21, 2	
Cingulate cortex	Right hemisphere	Larger	6, 17, 24	800
			15, 17, 36	
Cerebellum	Right hemisphere	Larger	32, 68, 59	3,897
	Vermis	Larger	2, 68, 47	283
	Right hemisphere	Smaller	12, 65, 29	460
	Left hemisphere	Smaller	12, 63, 29	30

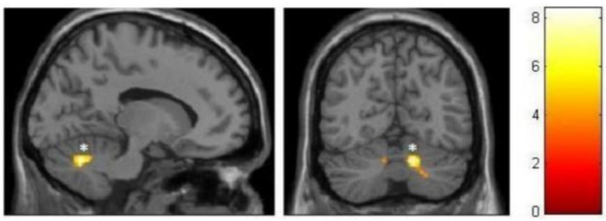


FIGURE 4 | VBM observed GM decrements in the ballet group compared to the control group. FWE corrected.

especially pronounced in the cerebellar hemispheres and the anterior vermis (Raz et al., 2001). Additionally, it has been shown not only that GM demonstrates a non-linear change during pre- and post-adolescence, but also that these developmental curves are not the same for all brain regions, with frontal lobe and parietal lobes peaking at the age of 12, the temporal at the age of 16, and the occipital only at the age of 20 (Giedd et al., 1999). Thus, the large discrepancies between the two studies may call for an additional study with a larger cohort so the structural brain differences between professional ballet dancers and non-dancers could be better delineated.

In an earlier study, we were able to detect some tendencies toward posterior hippocampal changes in a group of young healthy adults that had learned to slackline (Dordevic et al., submitted). The temporal dynamics of these brain changes partly resembled the balancing and spatial orientation skill levels of the participants which also showed a transient improvement. Increments in the posterior hippocampus have also been reported previously in ballet dancers by Hüffner and colleagues (Hüfner et al., 2011). Posterior hippocampal structural changes were also observed after year-long experience in taxi-driving and several sports with very high balancing demands, such as ballet dance, ice-skating, and slacklining (Maguire et al., 2000; Hüfner et al., 2011). Our sample of ballet dancers revealed similar results, indicating that long-term training might be necessary for structural changes in posterior hippocampus to persist. We could also demonstrate significant differences in the parahippocampal region between the ballet dancers and non-dancers. These were mainly located in the entorhinal cortex, particularly the medial entorhinal cortex. The entorhinal cortex is an interface between the three-layered hippocampal cortex and the six-layered neocortex and it provides the main cortical input to the hippocampus, with many reciprocal connections. More recently, an investigation of the medial entorhinal cortex (MEC) in animals, led to the discovery of grid cells that fire when the animal is in any of multiple locations that form a triangular grid (Moser et al., 2008, 2014; Sanders et al., 2015). Here the change in position can be computed based on vestibular information, sensorimotor information about self-motion, and optic flow. In our study on healthy older adults an increase in GM within parahippocampal regions was observed following an 18-month dance intervention (Müller et al., 2017). It is thus plausible to speculate that the better performance of ballet dancers in the TCT relies on these particular biological mechanisms, as well as that their long-term utilization causes neuroanatomical alterations which we were able to detect with VBM.

We observed highly significant differences in the caudal part of the cingulate cortex, area 24 of Broadman's classification. It is known from functional MRI studies that the cingulate motor cortex and the cerebellum are active during interlimb coordinative movements, together with primary and associative sensory and motor regions of the cortex (Debaere et al., 2001). Somatotopy similar to that of the larger sensory-motor cortices can be found in those cingulate cortex regions where the changes were detected (Vogt and Pandya, 1987; Vogt et al., 1992; Paus et al., 1993; Petit et al., 1993; Wu et al., 2000). Since such coordinative movements are a core element of ballet dancing, the finding of a larger volume of this brain region in dancers makes perfectly sense.

The changes we detected in the cerebellum were mainly in the expected direction, considering its close relationship with movement control and learning. Once learned, the skilled movements remain coded in the cerebellar memory cells for a long time. The cerebellum then provides speed, complexity, variety, stereotypy, and automaticity of the motor response so that one does not have to think consciously about the movement. It has been suggested that the learned programs are stored within the cerebellar cortex and that the memory capacity for storage is proportionate to the number of granule cells (Thach, 1998; Boyden et al., 2004). Our study revealed a major GM expansion in the superficial layers of the cerebellum, which could be perhaps due to the increment in the capacity to store complex movement-related memories in ballet dancers. On the other hand, we observed significantly smaller GM volumes in slightly deeper cerebellar structures of the ballet dancers, parts that are known to be involved in the control of limb movements, which can be interpreted in terms of automaticity/stereotypy.

One earlier study on ballet dancers has shown that the right hemispheric visual dominance is particularly useful for postural control in complex equilibrium conditions (Golomer et al., 2010). In our study, we could also observe differences that were mainly localized on the right side of the brain.

Main between-group differences in the thalamus were observed for its posterior part, and only on the right side. Earlier studies have proposed a functional importance of the posterolateral thalamus as a unique relay station for vestibular input to the cortex, but also the dominance of the right hemisphere in right-handedness, and of ipsilateral ascending pathways (Dieterich et al., 2005). Multiple thalamic nuclei are involved in vestibular processing, as well as somatosensory and visual, and this may explain the enlarged thalamus in the dancers (Morel et al., 1997; Lopez and Blanke, 2011).

Possible neurobiological mechanisms of the observed GM differences could be neurogenesis, synaptogenesis, hypertrophy of glia cells, and angiogenesis (Zatorre et al., 2012). The generation of new cells within the confines of our findings can only be expected for the hippocampus, but not for other areas of the ballet dancers' brain (Bhardwaj et al., 2006). Hippocampal neurogenesis in the adult has also been undermined somewhat by a recent publication (Sorrells et al., 2018). Instead, the observed GM increments are presumably based on the sensory experience which drives the formation and elimination of synapses and these changes might underlie adaptive remodeling of neural circuits (Trachtenberg et al., 2002). Importantly, animal studies have reported that motor learning of complex and acrobatic skills, and not repetitive use of synapses during simple physical exercise, generates new synapses in the cerebellar cortex (Black et al., 1990). In contrast, simple exercise leads to a greater density of blood vessels in the cerebellum.

The main limitation of our study is its cross-sectional nature, whereby no causal relationship for the effects observed can be established. Previous studies have, however, shown that training-induced neuroplastic adaptations are actually sport-specific rather than just sport-general. For instance, GM volumes in the hand representations are increased in handball players compared with ballet dancers, whereas GM volumes in the foot representations are increased in ballet dancers compared with handball players (Meier et al., 2016). Similarly, differences were observed between martial artists and endurance athletes (Schlaffke et al., 2014), but also musicians and non-musicians (Gaser and Schlaug, 2003), world class gymnasts (Huang et al., 2015), golfers with various skill levels and non-golfers (Jäncke et al., 2009), and sprinters and endurance runners (Wenzel et al., 2014), who differ in GM volumes of the anterior cerebellar lobe, and in vermian lobules compared to basketball players (Park et al., 2009). Considering the cross-sectional nature of these studies, some of these multiregional differences may be attributable to innate predisposition. Additionally, GM alterations in the cortex can occur as early as after 7 days of training (Driemeyer et al., 2008), and the temporal dynamics of the structural changes may lead to a partial or complete loss of these effects (May and Gaser, 2006; Taubert et al., 2010). Nevertheless, many researchers believe the neuroanatomical effects found in professional athletes may represent structural adaptations in response to long-term skill acquisition and the repetitive rehearsal of those skills. Some studies have shown that visual input is of a great importance for ballet dancers (Hugel et al., 1999; Perrin et al., 2002; Golomer et al., 2009), in order to successfully maintain balance and perform complex movements; however, we could not find any structural differences in visual cortex.

CONCLUSION

Our study demonstrated significant differences between professional ballet dancers and non-dancers in their neuroanatomy as well as in their abilities pertained to balancing and non-visual wayfinding. Some anticipated brain regions revealed structural alterations in professional ballet dancers, such as the cerebellum, the cingulate motor cortex, the posterior thalamus, as well as the posterior hippocampus and parahippocampus. In accordance with these structural observations, the dancers were able to better maintain balance, both in static and dynamic conditions, and to more accurately complete the triangular path both in the walking (somatosensory and vestibular) and particularly in the wheelchair (vestibular only) condition. We conclude that intensive life-long ballet training is presumably the main cause for the differences detected in our study.

AUTHOR CONTRIBUTIONS

MD organized the study, analyzed the data, and wrote and reviewed the paper. RS collected the data and wrote the paper. MT analyzed the data and reviewed the paper. PM reviewed the paper. AH organized the study and reviewed the paper. NM organized the study, and wrote and reviewed the paper.

REFERENCES

Allen, G. L., Kirasic, K. C., Rashotte, M. A., and Haun, D. B. M. (2004). Aging and path integration skill: kinesthetic and vestibular contributions to wayfinding. *Percept. Psychophys.* 66, 170–179. doi: 10.3758/BF03194870

Ashburner, J., and Friston, K. J. (2000). Voxel-based morphometry–the methods. *Neuroimage* 11, 805–821. doi: 10.1006/nimg.2000.0582

Bhardwaj, R. D., Curtis, M. A., Spalding, K. L., Buchholz, B. A., Fink, D., Bjork-Eriksson, T., et al. (2006). Neocortical neurogenesis in humans is restricted to development. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12564–12568. doi: 10.1073/pnas. 0605177103

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., and Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5568–5572. doi: 10.1073/pnas.87.14.5568

Boyden, E. S., Katoh, A., and Raymond, J. L. (2004). Cerebellum-dependent learning: the role of multiple plasticity mechanisms. *Annu. Rev. Neurosci.* 27, 581–609. doi: 10.1146/annurev.neuro.27.070203.144238

Brandt, T., Schautzer, F., Hamilton, D. A., Bruning, R., Markowitsch, H. J., Kalla, R., et al. (2005). Vestibular loss causes hippocampal atrophy and impaired spatial memory in humans. *Brain* 128, 2732–2741. doi: 10.1093/brain/awh617

Carter, N. D., Kannus, P., and Khan, K. M. (2001). Exercise in the prevention of falls in older people and the evidence. *Sport Med.* 31, 427–438. doi: 10.2165/ 00007256-200131060-00003

Debaere, F., Swinnen, S. P., Beatse, E., Sunaert, S., Van Hecke, P., and Duysens, J. (2001). Brain areas involved in interlimb coordination: a distributed network. *Neuroimage* 14, 947–958. doi: 10.1006/nimg.2001.0892

Dieterich, M., Bartenstein, P., Spiegel, S., Bense, S., Schwaiger, M., and Brandt, T. (2005). Thalamic infarctions cause side-specific suppression of vestibular cortex activations. *Brain* 128, 2052–2067. doi: 10.1093/brain/awh551

Dordevic, M., Hokelmann, A., Muller, P., Rehfeld, K., and Muller, N. G. (2017a). Improvements in orientation and balancing abilities in response to one month of intensive slackline-training. a randomized controlled feasibility study. *Front. Hum. Neurosci.* 11:55. doi: 10.3389/fnhum.2017.00055

Dordevic, M., Hökelmann, A., Müller, P., Rehfeld, K., and Müller, N. G. (2017b). Improvements in orientation and balancing abilities in response to one month of intensive slackline-training. a randomized controlled feasibility study. *Front. Hum. Neurosci.* 11:55. doi: 10.3389/fnhum.2017.00055/full

Driemeyer, J., Boyke, J., Gaser, C., Büchel, C., and May, A. (2008). Changes in gray matter induced by learning–revisited. *PLoS One* 3:e2669. doi: 10.1371/journal. pone.0002669

Gaser, C., and Schlaug, G. (2003). Brain structures differ between musicians and non-musicians. *J. Neurosci.* 23, 9240–9245. doi: 10.1523/JNEUROSCI.23-2 7-09240.2003

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nat. Neurosci.* 2, 861–863. doi: 10.1038/13158

Golomer, E., Mbongo, F., Toussaint, Y., Cadiou, M., and Israël, I. (2010). Right hemisphere in visual regulation of complex equilibrium: the female ballet dancers' experience. *Neurol. Res.* 32, 409–415. doi: 10.1179/174313209X 382476

Golomer, E. M. E., Gravenhorst, R. M., and Toussaint, Y. (2009). Influence of vision and motor imagery styles on equilibrium control during whole-body rotations. *Somatosens. Mot. Res.* 26, 105–110. doi: 10.3109/08990220903384968

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N., Friston, K. J., and Frackowiak, R. S. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 14, 21–36. doi: 10.1006/nimg.2001. 0786

Göttlich, M., Jandl, N. M., Sprenger, A., Wojak, J. F., Münte, T. F., Krämer, U. M., et al. (2016). Hippocampal gray matter volume in bilateral vestibular failure. *Hum. Brain Mapp.* 37, 1998–2006. doi: 10.1002/hbm.23152

Hänggi, J., Koeneke, S., Bezzola, L., and Jäncke, L. (2010). Structural neuroplasticity in the sensorimotor network of professional female ballet dancers. *Hum. Brain Mapp.* 31, 1196–1206. doi: 10.1002/hbm.20928

Hitier, M., Besnard, S., and Smith, P. F. (2014). Vestibular pathways involved in cognition. *Front. Integr. Neurosci.* 8:59. doi: 10.3389/fnint.2014.00059

Huang, R., Lu, M., Song, Z., and Wang, J. (2015). Long-term intensive training induced brain structural changes in world class gymnasts. *Brain Struct. Funct.* 220, 625–644. doi: 10.1007/s00429-013-0677-5

Hüfner, K., Binetti, C., Hamilton, D. A., Stephan, T., Flanagin, V. L., Linn, J., et al. (2011). Structural and functional plasticity of the hippocampal formation in professional dancers and slackliners. *Hippocampus* 21, 855–865. doi: 10.1002/ hipo.20801

Hugel, F., Cadopi, M., Kohler, F., and Perrin, P. (1999). Postural control of ballet dancers: a specific use of visual input for artistic purposes. *Int. J. Sports Med.* 20, 86–92. doi: 10.1055/s-2007-971098

Jäncke, L., Koeneke, S., Hoppe, A., Rominger, C., and Hänggi, J. (2009). The architecture of the golfer's brain. *PLoS One* 4:e4785. doi: 10.1371/journal.pone. 0004785

Kannus, P., Sievänen, H., Palvanen, M., Järvinen, T., and Parkkari, J. (2005). Prevention of falls and consequent injuries in elderly people. *Lancet* 366, 1885–1893. doi: 10.1016/S0140-6736(05)67604-0

Kremmyda, O., Hüfner, K., Flanagin, V. L., Hamilton, D. A., Linn, J., Strupp, M., et al. (2016). Beyond dizziness: virtual navigation, spatial anxiety and hippocampal volume in bilateral vestibulopathy. *Front. Hum. Neurosci.* 10:139. doi: 10.3389/fnhum.2016.00139/abstract

Lopez, C., and Blanke, O. (2011). The thalamocortical vestibular system in animals and humans. *Brain Res. Rev.* 67, 119–146. doi: 10.1016/j.brainresrev.2010. 12.002

Maguire, E. A., Gadian, D. G., Johnsrude, I. S., Good, C. D., Ashburner, J., Frackowiak, R. S. J., et al. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4398–4403. doi: 10.1073/pnas.070039597

May, A., and Gaser, C. (2006). Magnetic resonance-based morphometry: a window into structural plasticity of the brain. *Curr. Opin. Neurol.* 19, 407–411. doi: 10.1097/01.wco.0000236622.91495.21

Meier, J., Topka, M. S., and Hänggi, J. (2016). Differences in cortical representation and structural connectivity of hands and feet between professional handball players and ballet dancers. *Neural Plast.* 2016:6817397. doi: 10.1155/2016/ 6817397

Morel, A., Magnin, M., and Jeanmonod, D. (1997). Multiarchitectonic and stereotactic atlas f the human thalamus. *J. Comp. Neurol.* 387, 588–630. doi: 10.1002/(SICI)1096-9861(19971103)387:4<588::AID-CNE8>3.0.CO;2-Z

Moser, E. I., Kropff, E., and Moser, M.-B. (2008). Place cells, grid cells, and the brain's spatial representation system. *Annu. Rev. Neurosci.* 31, 69–89. doi: 10.1146/annurev.neuro.31.061307.090723

Moser, E. I., Roudi, Y., Witter, M. P., Kentros, C., Bonhoeffer, T., and Moser, M.-B. (2014). Grid cells and cortical representation. *Nat. Rev. Neurosci.* 15, 466–481. doi: 10.1038/nrn3766

Müller, P., Rehfeld, K., Schmicker, M., Hökelmann, A., Dordevic, M., Lessmann, V., et al. (2017). Evolution of neuroplasticity in response to physical activity in old age: the case for dancing. *Front. Aging Neurosci.* 9:56. doi: 10.3389/fnagi.2017. 00056

Park, I., Lee, K., Han, J., Lee, N., Lee, W., Park, K., et al. (2009). Experience-dependent plasticity of cerebellar vermis in basketball players. *Cerebellum* 8, 334–339. doi: 10.1007/s12311-009-0100-1

Paus, T., Petrides, M., Evans, A. C., and Meyer, E. (1993). Role of the human anterior cingulate cortex in the control of oculomotor, manual, and speech responses: a positron emission tomography study. *J. Neurophysiol.* 70, 453–469. doi: 10.1152/jn.1993.70.2.453

Perrin, P., Deviterne, D., Hugel, F., and Perrot, C. (2002). Judo, better than dance, develops sensorimotor adaptabilities involved in balance control. *Gait Posture* 15, 187–194. doi: 10.1016/S0966-6362(01)00149-7

Petit, L., Orssaud, C., Tzourio, N., Salamon, G., Mazoyer, B., and Berthoz, A. (1993). PET study of voluntary saccadic eye movements in humans: basal ganglia-thalamocortical system and cingulate cortex involvement. *J. Neurophysiol.* 69, 1009–1017. doi: 10.1152/jn.1993.69.4.1009

Raz, N., Gunning-Dixon, F., Head, D., Williamson, A., and Acker, J. D. (2001). Age and sex differences in the cerebellum and the ventral pons: a prospective MR study of healthy adults. *Am. J. Neuroradiol.* 22, 1161–1167.

Russell, N. A., Horii, A., Smith, P. F., Darlington, C. L., and Bilkey, D. K. (2003). Long-term effects of permanent vestibular lesions on hippocampal spatial firing. *J. Neurosci.* 23, 6490–6498. doi: 10.1523/JNEUROSCI.23-16-06490.2003

Sanders, H., Rennó-Costa, C., Idiart, M., and Lisman, J. (2015). Grid cells and place cells: an integrated view of their navigational and memory function. *Trends Neurosci.* 38, 763–775. doi: 10.1016/j.tins.2015.10.004

Schlaffke, L., Lissek, S., Lenz, M., Brüne, M., Juckel, G., Hinrichs, T., et al. (2014). Sports and brain morphology - A voxel-based morphometry study with endurance athletes and martial artists. *Neuroscience* 259, 35–42. doi: 10.1016/j. neuroscience.2013.11.046

Sherrington, C., Tiedemann, A., Fairhall, N., Close, J. C. T., and Lord, S. R. (2011). Exercise to prevent falls in older adults: an updated meta-analysis and best practice recommendations. *N. S. W. Public Health Bull.* 22, 78–83. doi: 10.1071/ NB10056

Sorrells, S. F., Paredes, M. F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K. W., et al. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature* 555, 377–381. doi: 10.1038/nature25975

Stackman, R. W., Clark, A. S., and Taube, J. S. (2002). Hippocampal spatial representations require vestibular input. *Hippocampus* 12, 291–303. doi: 10.1002/hipo.1112

Taubert, M., Draganski, B., Anwander, A., Muller, K., Horstmann, A., Villringer, A., et al. (2010). Dynamic properties of human brain structure: learning-related changes in cortical areas and associated fiber connections. *J. Neurosci.* 30, 11670–11677. doi: 10.1523/JNEUROSCI.2567-10.2010

Thach, W. T. (1998). A role for the cerebellum in learning movement coordination. *Neurobiol. Learn. Mem.* 70, 177–188. doi: 10.1006/nlme.1998.3846

Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E., et al. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 420, 788–794. doi: 10.1038/nature01273

Vogt, B. A., Finch, D. M., and Olson, C. R. (1992). Functional heterogeneity in cingulate cortex: the anterior executive and posterior evaluative regions. *Cereb. Cortex* 2, 435–443.

Vogt, B. A., and Pandya, D. N. (1987). Cingulate Cortex of the Rhesus Monkey?: 11. Cortical Merents. *J. Comp. Neurol.* 262, 271–289. doi: 10.1002/cne.902620208

Wenzel, U., Taubert, M., Ragert, P., Krug, J., and Villringer, A. (2014). Functional and structural correlates of motor speed in the cerebellar anterior lobe. *PLoS One* 9:e96871. doi: 10.1371/journal.pone.0096871

Wu, C. W. H., Bichot, N. P., and Kaas, J. H. (2000). Converging evidence from microstimulation, architecture, and connections for multiple motor areas in the frontal and cingulate cortex of prosimian primates. *J. Comp. Neurol.* 423, 140–177. doi: 10.1002/1096-9861(20000717)423:1<140::AID-CNE12>3.0. CO;2-3

Zatorre, R. J., Fields, R. D., and Johansen-Berg, H. (2012). Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat. Neurosci.* 15, 528–536.doi: 10.1038/nn.3045

Zu Eulenburg, P., Stoeter, P., and Dieterich, M. (2010). Voxel-based morphometry depicts central compensation after vestibular neuritis. *Ann. Neurol.* 68, 241–249. doi: 10.1002/ana.22063

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Human Neuroscience

ORIGINAL RESEARCH
publishedFebruary 2017
doi: 10.3389/fnhum.2017.00055

Improvements in Orientation and Balancing Abilities in Response to One Month of Intensive Slackline-Training. A Randomized Controlled Feasibility Study

Milos Dordevic[1,2]*, Anita Hökelmann[2], Patrick Müller[1], Kathrin Rehfeld[2] and Notger G. Müller[1,3]

[1]Department of Neuroprotection, German Center for Neurodegenerative Diseases, Magdeburg, Germany, [2] Institute of Sports Science, Otto von Guericke University, Magdeburg, Germany, [3] Center for Behavioral Brain Sciences, Magdeburg, Germany

Background: Slackline-training has been shown to improve mainly task-specific balancing skills. Non-task specific effects were assessed for tandem stance and preferred one-leg stance on stable and perturbed force platforms with open eyes. It is unclear whether transfer effects exist for other balancing conditions and which component of the balancing ability is affected. Also, it is not known whether slackline-training can improve non-visual-dependent spatial orientation abilities, a function mainly supported by the hippocampus.

Objective: To assess the effect of one-month of slackline-training on different components of balancing ability and its transfer effects on non-visual-dependent spatial orientation abilities.

Materials and Methods: Fifty subjects aged 18–30 were randomly assigned to the training group (T) (n = 25, 23.2 2.5 years; 12 females) and the control group (C) (n = 25, 24.4 2.8 years; 11 females). Professional instructors taught the intervention group to slackline over four consecutive weeks with three 60-min-trainings in each week. Data acquisition was performed (within 2 days) by blinded investigators at the baseline and after the training. Main outcomes Improvement in the score of a 30-item clinical balance test (CBT) developed at our institute (max. score = 90 points) and in the average error distance (in centimeters) in an orientation test (OT), a triangle completion task with walking and wheelchair conditions for 60 , 90 , and 120 .

Results: Training group performed significantly better on the closed-eyes conditions of the CBT (1.6 points, 95% CI: 0.6 to 2.6 points vs. 0.1 points, 95% CI: –1 to 1.1 points; p = 0.011, $!^2_p$ = 0.128) and in the wheelchair (vestibular) condition of the OT (21 cm, 95% CI: 8–34 cm vs. 1 cm, 95% CI: –14–16 cm; p = 0.049, $!^2_p$= 0.013).

Conclusion: Our results indicate that one month of intensive slackline training is a novel approach for enhancing clinically relevant balancing abilities in conditions with closed eyes as well as for improving the vestibular-dependent spatial orientation capability; both of the benefits are likely caused by positive influence of slackline-training on the vestibular system function.

Keywords: balance, slackline-training, orientation, vestibular system, hippocampus

OPEN ACCESS

Edited by:
Claudia Voelcker-Rehage, Chemnitz University of Technology, Germany

Reviewed by:
Oliver Faude,
University of Basel, Switzerland
Astrid Zech,
Jena University, Germany

***Correspondence:**
Milos Dordevic
milos.dordevic@dzne.de

Received: 15 September 2016
Accepted: 26 January 2017
Published: 10 February 2017

Citation:
Dordevic M, Hökelmann A, Müller P, Rehfeld K and Müller NG (2017) Improvements in Orientation and Balancing Abilities in Response to One Month of Intensive Slackline-Training. A Randomized Controlled Feasibility Study.
Front. Hum. Neurosci. 11:55. doi: 10.3389/fnhum.2017.00055

INTRODUCTION

Intact balance control is required not only to maintain postural stability but also to assure safe mobility-related activities during daily life (Mancini and Horak, 2010). Approximately 1.5% of healthcare expenditures in European countries are caused by falls, which mainly occur because of impaired balance, aging and cognitive decline (Ambrose et al., 2013); this large number does not take into account any additional indirect costs. Prevention in the earliest stages, already at young age, is hence justified. Balance and strength training is considered to be by far the most efficient intervention for fall prevention (Karlsson et al., 2013) and it can be effective for postural and neuromuscular control improvements; in addition, balance training is considered to be an effective intervention for improvement in static postural sway and dynamic balance in both athletes and non-athletes (Zech et al., 2010). Moreover, both gray and white matter alterations have been reported in young people in response to only six weeks of balance training (Taubert et al., 2010). The optimal interaction between visual, vestibular and somatosensory systems is the key to stability of the body. While the visual factor can be corrected in many different ways, the other two can be best enhanced through optimal training interventions.

Several recent studies have demonstrated particularly beneficial effect of slacklining on balancing abilities in both younger and older populations (Pfusterschmied et al., 2013; Thomas and Kalicinski, 2016), through enhancement in postural control and functional knee joint stability. Although in other studies mainly task-specific effects were found in response to six weeks of slackline training, larger non-task specific effects on postural control could not be found in these studies for only several relatively simple testing assignments, such as one-leg and tandem stance on stable force platform surface (Donath et al., 2013, 2016b); moreover, the amount of training in these studies was limited to approximately only one hour per week. Slackline length in previous studies was set to between 5 and over 15 m, which proportionally decreases the rate of turns per training, limiting thereby the stimulation of the vestibular system and its output pathways mainly to the otolith organs; in other words, important function of semicircular canals and related brain regions might have been underemployed and an additional potential effect overseen (Highstein, 1991; Cullen and Minor, 2002). Earlier research using several other types of balance-training interventions found transfer effects of training on performance in clinical tests of balance, and these tests are considered very important for both diagnostic and therapeutic purposes (Mancini and Horak, 2010). Some questions remain, however, still unanswered: (1) can slackline-training cause such non-specific transfer effects on performance in a comprehensive clinical balance test clinical balance test (CBT) and (2) what component of balancing ability, as assessed by this test, is mainly affected by slackline-training, with the vestibular component being of particular interest here.

The hippocampus and neighboring cortical regions are the main loci where the onset of Alzheimer's disease pathology occurs (Raskin et al., 2015), followed by their progressive degeneration, and early prevention treatments (in younger age) concerning this problem are strongly encouraged (Brookmeyer et al., 2007). Several previous animal and human studies have pointed towards a strong link between the vestibular system and orientation centers of the brain, considered to be located in the hippocampus and neighboring regions (Stackman et al., 2002; Russell et al., 2003; Brandt et al., 2005; Jahn et al., 2009). These studies found serious deficits in the orientation function of the temporal lobe as a result of disturbed or lost vestibular input. Many other studies also suggested that the vestibular system provides self-motion information which is important for the hippocampus and related brain regions to develop spatial memories; when this input is lost, spatial memory becomes impaired (Smith et al., 2010). Moreover, professionals who intensively make use of their vestibular system during their daily artistic performances, such as ballet/ice(Pfusterschmied et al., 2011) dancers and slackliners, have differently structured temporal brain regions, including the hippocampus, compared to non-professionals (Hüfner et al., 2011). A study by Allen et al. (2004) clearly demonstrated a reduction in vestibular-kinesthetic dependent orientation abilities with aging, by comparing performance of younger and older adults on the triangle completion task; the older adults performed particularly worse on this task when their input was restricted to the vestibular system only (passively pushed in a wheelchair), implying deterioration of this system with aging. A question that remains unanswered here is if an intensive slackline-training can lead to significant improvement in the vestibular system's function, which can then be beneficial for spatial orientation abilities in a trained person. Therefore, here we wanted to find out whether an especially challenging balance training program (learning to slackline) can also induce transfer effects on cognitive function, namely spatial orientation. The idea behind this assumption was that a) a strong connection between the vestibular system (which is important for balancing) and the hippocampus has been suggested and b) that spatial orientation is a function that is to a great extent supported by the hippocampus (Hitier et al., 2014). We chose intensive slacklining in young adults as an intervention measure under the assumption that if this training is not capable of inducing transfer effects then other, less demanding regimen (such as those typically used to enhance balancing skills in elderly, sick patients) will surely not be able to do so either. In other words, this was a feasibility pilot study, using a young population.

Thus, having in mind the close connection between the vestibular and orientation systems, we asked whether intensive slackline training can improve not only one's ability to maintain balance but also has transfer effects on the capability to successfully orientate in space. Up to this point we are not aware of any longitudinal studies that investigated whether the vestibular-dependent temporal lobe orientation function can be enhanced through an intervention aimed towards improvements in balancing skills. The goal of this study was to find out whether learning how to slackline over a period of one month can be of benefit for both stability and orientation skills.

MATERIALS AND METHODS

Ethics Statement

This study was carried out in accordance with the recommendations of and was approved by the Medical Faculty Ethics Committee at the Otto von Guericke University (approval number: 156/14). Each participant signed a document of informed consent before the beginning of the study.

Subjects

Fifty healthy young (18 to 30 years old) subjects were recruited for this study and randomly assigned (without stratification) into two groups, control (12 females and 13 males; mean age D 23.2 years; *SD* D 2.6 years) and training (11 females and 14 males; mean age D 24.4 years; SD D 2.7 years) (**Table 1**). The two groups did not significantly differ in any of the recorded demographic and other characteristics, including age, height, weight, years of education, handedness etc. Physical activity was assessed by asking subjects how many hours they spend on sports weekly on average; all sports were taken into consideration, including jogging, various team sports, cycling etc., but not walking. Participants of both groups were paid the same amount of money for their participation in the study. Sample size and characteristics, as well as the balance-training duration have been justified by several previous slackline- and other balance-training studies (Zech et al., 2010; Pfusterschmied et al., 2013).

Eligible subjects for this study were all those aged from 18 to 30 years who had no previous experience in slacklining or similar activity (i.e., highly demanding balancing activities, such as ballet dancing, rhythmic gymnastics etc.) and normal or corrected to normal vision. Exclusion criteria were injuries to the musculoskeletal system and systemic diseases (e.g., cardiovascular, metabolic, nervous system diseases etc.). Participants were recruited through advertisement in the buildings of Otto von Guericke University in Magdeburg, both at the main and medical campus.

TABLE 1 | Characteristics of participants.

Characteristic	Training (*n* D 25)	Control (*n* D 25)
Age (years)	24.5 2.7	23.2 2.6
Sex (females)	11(44%)	12(48%)
Weight (kg)	69.112.5	65.010.0
Height (cm)	173.4 9.2	170.3 8.4
Hours of activity (per week)	3.01.8	3.22.5
Handedness (right)	24(96%)	23(92%)
Profession (student)	22(88%)	23(92%)
Suffered a small injury (e.g., ankle sprain)	5(20%)	5(20%)
Ethnic origin		
European	20 (80%)	19 (76%)
Asian (Indian)	5(20%)	5(20%)
Arabic	0 (0%)	1 (4%)

Study Design

Flow diagram of the study is shown in the **Figure 1**. This study was planned and organized as a randomized controlled single-blinded trial with factorial design (factors: time and group). Participants were randomly assigned to the training and control groups using computer-based randomization procedure[1]. The computer-based randomization and assignment of participants to groups were performed by MD (not involved in data collection), with all other investigators blinded to the outcome of the randomization.

The study consisted of measurements at two time points: baseline and one month (2 days) after baseline. All trainings took place in the movement lab of our institute (German Center for Neurodegenerative Diseases) from February to April 2015.

Intervention

During this one month period the training group underwent intensive balance training consisting of 12 trainings (three trainings/week with each training lasting 1 h; max. 2 consecutive non-training days) on a 3-m long slackline ("Power-wave 2.0" slackline rack), whilst the control group was instructed to abstain from any type of similar activity; the abstinence from this type of activity was confirmed by control group participants at the post-test.

Trainings were led and supervised by an experienced instructor, whose assignment was to achieve the best possible skill level in the training group participants; content of teaching is shown in the **Table 2**. Minimum requirement to be achieved was set to walking forward two slackline lengths with turn at the end of the first length; each participant must have achieved this minimum requirement to be considered for the analysis, and all participants were successful in achieving this. Each training unit consisted of a 10-min warm up session and 50-min training session. Maximum group size allowed was four participants, so the instructor could dedicate enough time to each trainee. Moreover, the trainings were highly individualized, according to the skill and progression levels of each of the participant. At the end of each training session the instructor collected the information about skill progression, by writing down the achieved skill level of each participant. To do so, the amount of time every participant needed to walk up to four slackline lengths forward, backward, sideways, and turn in between was recorded.

The slackline tension was also individualized, so that when standing in the middle and applying a light vertical force (as during walking) the slackline would not get more than several centimeters away from the metal bar located 15 cm underneath. Our goal here was to increase difficulty of training by keeping the slackline slack and thus more unstable, rather than tight and stable, which would otherwise resemble walking on a firm surface. The length of the slackline was also intentionally set to 3 meters; in this way we wanted to achieve a higher rate of turns on the slackline, and thus a higher rate of semicircular canal stimulation. This is in contrast to earlier studies which used moderately to much longer slacklines (5 to over 15 m in length) (Granacher et al., 2010; Pfusterschmied et al., 2013; Donath et al., 2016b),

www.randomizer.org

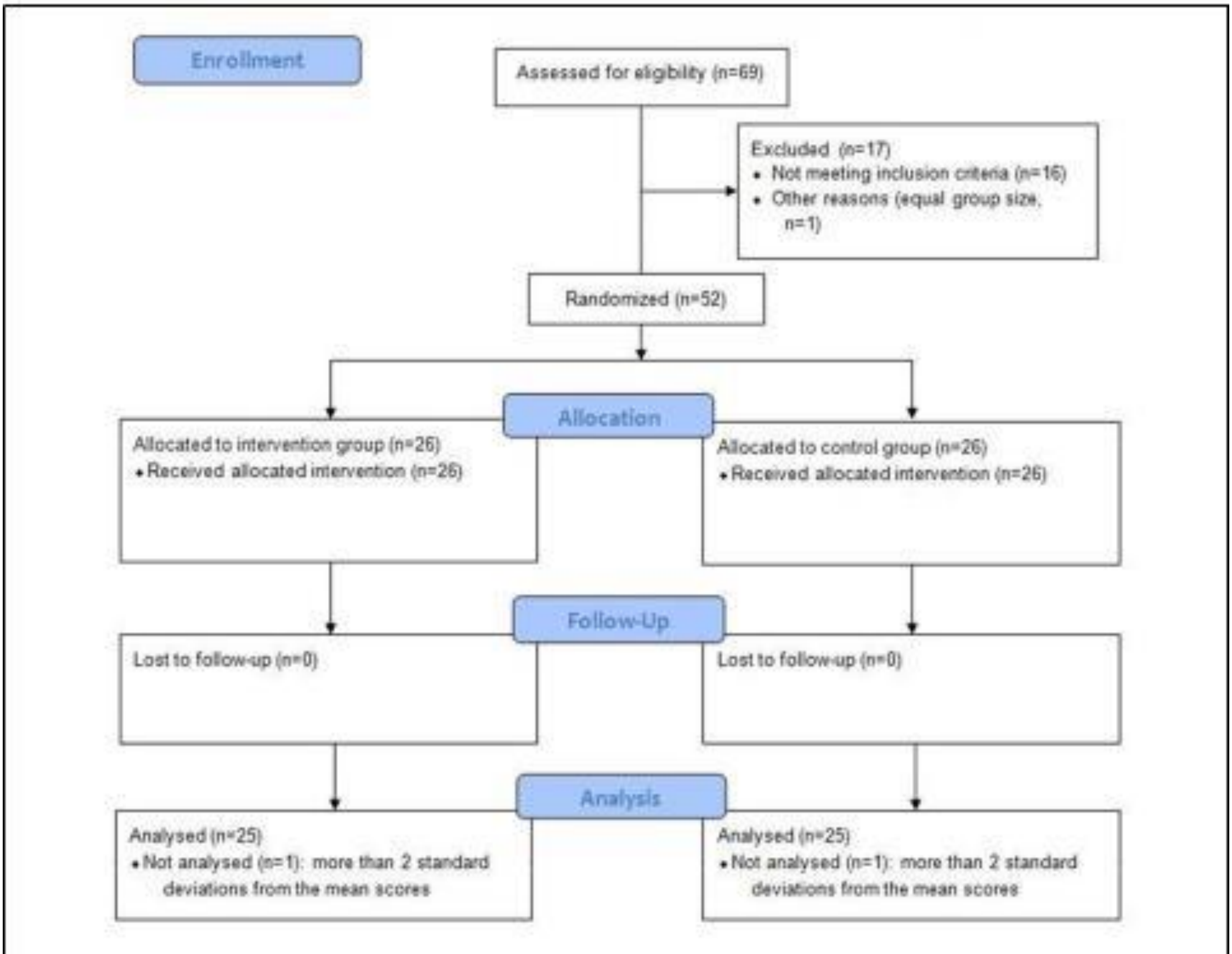


FIGURE 1 | Study flow diagram.

stimulating thereby mainly otolith organs and related central vestibular pathways.

Tests

All tests were performed by two trained members of our institute and the results of the following sets of measurements were recorded before and after the training:

TABLE 2 | Contents participants were taught during slackline-trainings; the minimum difficulty level they had to achieve in order to be considered for the final analysis is also presented.

Task	Difficulty levels/Minimum to be achieved
Stable tandem stance	5–10 s/5 s
Stable one-leg stance	5–10 s/5 s
Turn	1–4 times/2 times
Walk forward (with turn for 2)	1–4 lengths/2 lengths
Walk backward (with turn for 2)	1–4 lengths/1 length
Walk sideways (with turn for 2)	1–4 lengths/1 length

Clinical Balance Test

Considering that every CBT has its advantages and disadvantages (Mancini and Horak, 2010), this comprehensive test was developed by experts in our institute (DZNE) with the goal to assess different components of patients' ability to maintain equilibrium, in both standing and gait conditions. Many of the test conditions are consistent with similar comprehensive CBTs (Mancini and Horak, 2010) used in other clinics. The inter-rater reliability of the test (determined with ICC coefficient) is 0.98 0.04 (SEM D 0.003), and its validity is still to be evaluated. The conditions can be briefly divided into standing and walking (**Figure 2**), both of which further contain sub-conditions with open and closed eyes (for detailed list of conditions see the **Table 3**).

Standing conditions include:

two- and one-leg stance on both stable (floor) and unstable (soft pad) surfaces, with both open and closed eyes

Walking conditions included:

FIGURE 2 | Examples of clinical balance test (CBT) conditions: unstable surface one-leg stand (left) and balance beam walking (right) conditions.

Walking forwards, backwards, and turning inside a 30 cm wide and 4 m long polygon with open eyes, followed by the same test on a 5 cm wide line as well as on 10 cm wide balance beam.
Walking forward on 5 cm line with closed eyes.

In total, there are 30 assessment items within this test, 14 of which assess standing and 16 walking; 8 of all measurements are performed with closed eyes. The maximum amount of points that could be collected on the test was 90, with each condition carrying the minimum of 0 and the maximum of 3 points, similar to other comprehensive CBT batteries (Horak et al., 2009). Assessment was based on the subjective opinion of trained assessor who graded postural sway during each of the conditions; to avoid potential differences in subjective opinion between the assessors, each participant was tested by only one assessor at both pre-and post-test. In each of the standing conditions participants were instructed to maintain the required position for 15 s, whereas in walking conditions there was no time requirement and participants were asked to walk at their own pace.

Orientation Test (OT)

Orientation test was a modified version of the test described by (Allen et al., 2004), whereby the only modification was the inclusion of only three conditions (turning angles) from this study, due to time and space limitations. In brief, six triangular paths were marked on the floor of a room, three in the left and three in the right direction, giving thus three pairs of triangular paths. Lengths of the segments of each triangular path as well as the turn angles between the segments of the triangles are presented in the **Table 4**, with examples of the polygon and test conditions shown in the **Figures 3** and **4**. The test consisted of two conditions: active-walking and passive-wheelchair.

In the active-walking condition, while being guided on foot, the participant's movement was controlled by leading him or her along two sides of the triangular path as he or she held onto a wooden bar. The passive-wheelchair condition included transport along the same routes with the use of a standard wheelchair with attached footpads.

Each participant was walked (active) and pushed (passive) only once along each of the paths, giving thus 12 trials per participant in total (3 to the left and 3 to the right, times 2 conditions).

Once the participant was walked/pushed in the wheelchair along two sides of each triangle, his or her task was to walk along the third one, back to the starting point, using thus the shortest possible way back; that is, the participants were instructed not to walk back along the two sides that were used to bring them to the drop-off point, but to use the shortest possible way back to the starting point instead, which is actually always the third side of the respective triangle.

The main outcome variable was the distance error on each trial, which was assessed by marking the participant's stopping point with adhesive dots on the floor and later measuring the distance from that stopping point to the starting point, from which the respective movement was initiated. The dots were placed on the floor exactly between the feet, aiming thus for the center of pressure, by second assessor, so that the first assessor could focus on giving instructions and guiding participants. After each trial participants were led or pushed back from the stopping point to the starting point, which was for the whole test at the same location, so the next trial could begin.

For the whole duration of the test participants were blindfolded in a quiet room and thereby could not use any visual nor auditory cues that might help them in finding their way back to the starting point. It can thus be assumed that the only cues

TABLE 3 | Test conditions of the clinical balance test (CBT).

No.	Condition	Task		Points (min D 0, max D 3)			
				0	1	2	3
K.	Static – stable surface (floor)	Stand with feet together – open eyes					
L.		Stand with feet together – closed eyes					
M.		One leg stance – left – open eyes					
N.		One leg stance – right – open eyes					
O.		One leg stance – left – closed eyes					
P.		One leg stance – right – closed eyes					
7.	Static – unstable surface (pad)	Stand normally (hip width stance) – open eyes					
8.		Stand with feet together – open eyes					
9.		Stand normally (hip width stance) – closed eyes					
10.		Stand with feet together – closed eyes					
11.		One leg stance – left – open eyes					
12.		One leg stance – right – open eyes					
13.		One leg stance – left – closed eyes					
14.		One leg stance – right – closed eyes					
15.	Dynamic	Walk inside the zone (4 m 30 cm)	Forward				
16.		Turn (90)					
17.		Backward					
18.		Walk on the line (4 m 5 cm)	Forward				
19.		Turn (90)					
20.		Backward					
21.		Walk on the line with feet one after the other (4 m 5 cm)	Forward				
22.		Turn (90)					
23.		Backward					
24.		Walk on the beam (4 m 10 cm)	Forward				
25.		Turn (90)					
26.		Backward					
27.		Walk on the beam sideways (4 m 10 cm)	Rightward				
28.		Turn (90)					
29.		Leftward					
30.		Walk on the line with closed eyes(4 m 5 cm)	Forward				

they could use were somatosensory and vestibular in the active-walking condition and vestibular only in the passive-wheelchair condition.

Outcome Variables and Data Analysis

Pre-specified primary outcomes were improvement in score (in points) on the CBT and decrement in average error distance (in cm) on the orientation test (OT).

TABLE 4 | Length of segments and turning angles of triangular paths in the orientation test (OT).

Direction	Turning angle ( )	Segment 1 (cm)	Segment 2 (cm)	Segment 3 (cm)
Right	60		203	201
	90	203	196	286
	120		250	377
Left	60		203	201
	90	203	196	286
	120		250	377

Data were analyzed with MatLab (Mathworks, USA) and SPSS (IBM, USA) software. Statistical analysis included paired *t*-tests for within group analyses and repeated-measures-ANOVAs with time and group as factors for between group and interaction effects analyses. The significance level was set to a D 0.05. The descriptive results are shown as mean standard deviation; in addition, effect sizes ($!^{2}_{p}$) and 95% confidence intervals of change are reported; the effect size magnitude of 0.01 indicated small, 0.059 medium and 0.138 large effects(Cohen, 1988; Donath et al., 2013). All of the datasets were checked for normal distribution and homogeneity of variance before running parametric tests.

RESULTS

Final analysis included 25 participants in each group. Two participants (one from each group) were not considered for the analysis because of major outliers, reaching more than 2 standard deviations away from the mean score of all participants. All subjects were recruited from December 2014 until March 2015 and their characteristics are shown in the **Table 1**.

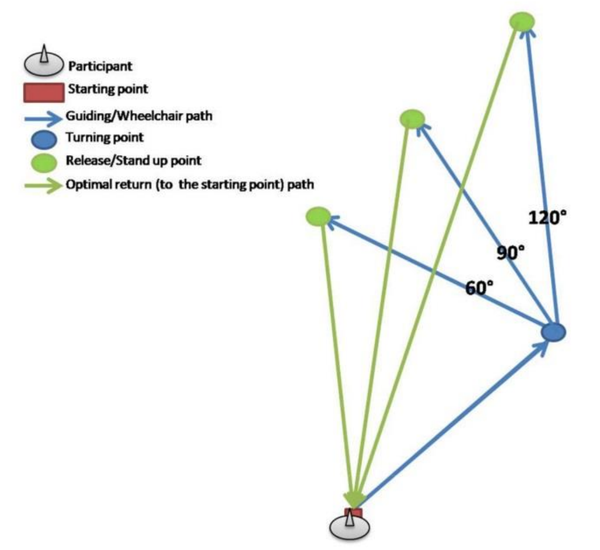


FIGURE 3 | Example of three triangular paths in right direction used for the orientation test (OT); blue arrows mark two sides of respective triangular paths along which participants where guided or pushed in a wheelchair, whereas green arrows show the optimal route for walking back to the starting point from the respective release/stand up point.

Clinical Balance Test

Figure 5 shows both results of the overall test as well as the results for closed eyes condition of the CBT; the respective significance levels are summarized in the **Table 5**.

When overall results are considered, both of the groups demonstrated pre- to post-training improvements. In the training group this improvement was on average 5.1 points (71.8 5.2 to 77.0 4.5) whereas in the control group it amounted to 2.4 points on average (71.1 6.4 to 73.50 4.4). The interaction effect here was not large enough to reach our preset significance level and the effect size was small (*p* D 0.166, !^2_p D 0.039) (**Figure 5**; **Table 5**).

In contrast to the overall test results, when only those conditions were analyzed in which the participants had their eyes closed, a significant interaction effect with medium to large effect size was observed (*p* D 0.011, !^2_p D 0.128), as can be seen from the **Figure 5** and **Table 5**. In these conditions the training group improved (13.7 1.8 to 15.4 2.2) while the control group performed slightly worse on the post-test (13.7 2.6 to 13.6 2.4).

The results from test conditions where participants had their eyes open did not reach significant interaction effect (*p* D 0.594). A learning effect could be observed here, with very similar improvements of about 3 points in both the training and control group (**Table 5**).

Orientation Test

Overall OT results gave a non-significant interaction effect with very small effect size (*p* D 0.063, !^2_p D 0.006) (**Figure 6**; **Table 5**). Errors in the training group decreased by 11 cm (114 68 to 103 62) whereas the error in the control group increased slightly by 2 cm (111 74 to 113 75) (**Figure 6**; **Table 5**).

Further analysis of the wheelchair condition results revealed a much larger improvement in the training group compared to the control group; the training group improved by about 21 cm (131 75 to 110 63) in comparison to a very small 1 cm (121 68 to 120 79) improvement in the control group. This difference in improvements between the two groups that occurred over time led also to a significant interaction effect with small effect size (*p* D 0.049, !^2_p D 0.013) (**Figure 6**; **Table 5**).

Lastly, the condition where participants were walking while actively guided over the polygon did not reveal a significant time x group interaction effect (*p* D 0.591). Within this condition of the OT the training group remained at about the same level of

FIGURE 4 | Examples of OT conditions: guided walking (left) and wheelchair sitting (right) conditions.

TABLE 5 | Summary of mean improvements over time, interaction effects and effect sizes on CBT and OT; cm – centimeters, pts – points, –*p* < a.

Test	Condition	Mean improvements over time (95% CI)		*F*	*p*	!p 2
		Training	**Control**			
CBT	All conditions	5.1pts (2.5, 7.7pts)	2.4pts (0.4, 5.3pts)	1.93	0.171	0.039
	Closed eyes	1.7pts (0.6, 2.7pts)	0.1pts (–1, 1.1pts)	7.06	0.011	0.128
	Open eyes	3.5pts (0.9, 6.0pts)	2.5pts (0, 5.1pts)	0.20	0.594	0.004
OT	All conditions	11 cm (2, 19 cm)	–2 cm (–11, 8 cm)	3.46	0.063	0.006
	Wheelchair	21 cm (8, 34 cm)	1 cm (–14, 16 cm)	3.91	0.049	0.013
	Walking	0 cm (–10, 10 cm)	–4cm (–16, 8 cm)	0.29	0.591	0.001

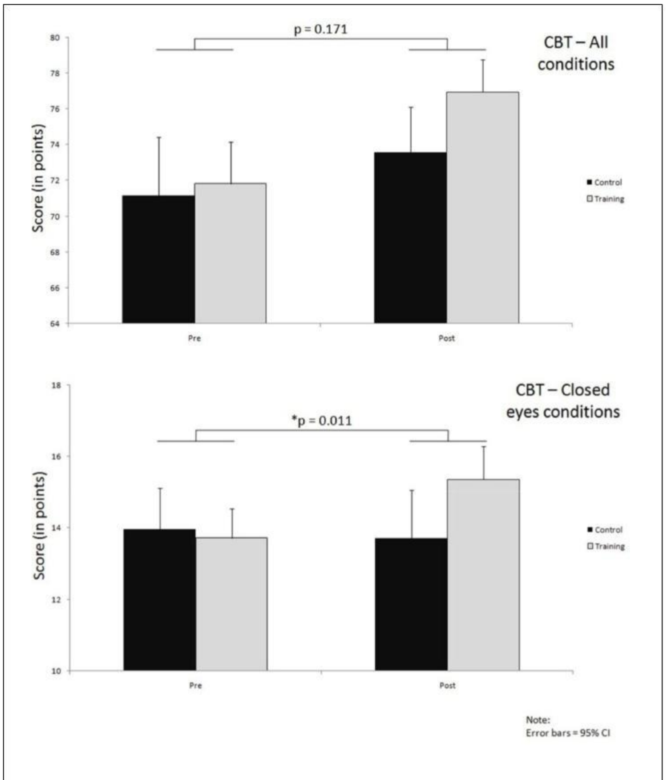


FIGURE 5 | Improvements over time in both groups on CBT, for all conditions together and closed eyes conditions only; significance levels (*p*) indicate time group interaction effects.

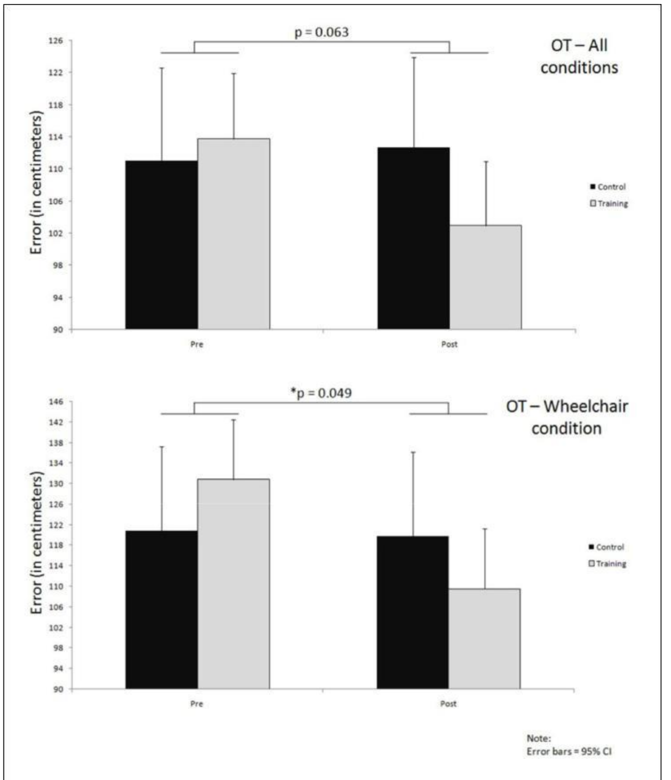


FIGURE 6 | Improvements over time in both groups on OT, for all conditions together and wheelchair condition only; significance levels (*p*) indicate time group interaction effects.

error while the control group performed worse at the post test by about 4 cm (**Table 5**).

DISCUSSION

The main findings of this study are twofold, both of which are supportive of the a priori hypothesized improvements of vestibular system function in response to intensive balance training.

Firstly, 1 month of intensive balance training during which participants learned how to slackline, led to significantly better performance of our training group participants on the CBT compared to their control counterparts, but only on those measurements where their visual input was blocked, i.e., where they had to balance with eyes closed. The magnitude of the effect of slackline-training here was medium to large. In contrast, on tasks where visual input was not blocked, both groups improved about the same, thus revealing a potential practice effect which might have taken place between pre- and post-test. Considering that the input from three systems involved in balance maintenance is present normally in a moving person (visual, vestibular and somatosensory) (Horak, 2006), it appears from our test results that the vestibular and somatosensory systems were particularly affected by the slackline-training.

Secondly and similarly to the previous finding, the training group performed significantly better on the OT compared to the control group, but again only in one condition, namely the passive-wheelchair condition (passively pushed along the designated

routes). In this condition the input was intentionally limited to the vestibular system, and the performance thus depended solely on the function of the vestibular system and related brain regions which process this input. Many connections have been proposed to exist between the vestibular system and temporal lobe, in particular the hippocampus, for the purpose of processing these spatial and orientation inputs (Hitier et al., 2014). Once more, the results of the OT used in our study allow us to speculate that vestibulo-hippocampal spatial orientation function has been positively affected by the slackline-training, with a small effect size.

Many earlier studies used numerous diverse approaches to enhance balancing skills in various target groups (Zech et al., 2010; Sherrington et al., 2011). The majority of balance trainings were reported to be successful in improving outcome variables in healthy young (Zech et al., 2010) and elderly (Sherrington et al., 2011; Cadore et al., 2013; El-Khoury et al., 2015) participants, athletes (Hubscher et al., 2010; Boccolini et al., 2013), as well as patients suffering from Alzheimer's (Ries et al., 2015) and Parkinson's disease (Sehm et al., 2014), post-stroke patients (Lubetzky-Vilnai and Kartin, 2010) and patients with vestibular disorders (Porciuncula et al., 2012). A literature review pertained to our first finding (stability improvement in closed-eyes conditions of CBT) revealed that similar studies (involving slackline-training) published before suggested large task-specific improvements (standing on slackline) in response to training but only small to moderate non-task specific improvements (for meta-analytical review see (Donath et al., 2016a)). However, these studies used different training and evaluation methodologies; that is, the only non-task specific transfer effects evaluated were postural sway displacement and velocity changes, while participants stood with open eyes on a firm or suddenly perturbed flat surface of a force platform, mostly in one-leg and tandem stance modes. In contrast to these studies, for our analysis outcome from comprehensive clinical balance assessment was used, in which the standing conditions included standing on both and each leg separately (not only one by own choice) in open and closed eyes conditions, on a firm flat but also on a soft, unstable surface. In fact, our main finding here was related to the larger improvement in the closed eyes conditions, which was not even assessed by these studies; for the open-eyes conditions we could also not find any significant effects. Furthermore, our training methodology differed from that applied in previous studies in at least two points: (a) it involved more hours spent on the slackline (around 600 min vs. an average of 380 min in other studies) and was implemented on slacklines of shorter length (3 m vs. 5 to over 15 m in other studies). As we already mentioned earlier, this slackline length was intentionally chosen for the purpose of stimulating semicircular canal function, in addition to that of otolith organs; this important input (Highstein, 1991; Cullen and Minor, 2002) might have been neglected in other training interventions and its effects could hence have been overlooked. Regarding the training intensity, variation in intensity of motor training has already been shown to differentially affect the skill learning and brain structure (Sampaio-Baptista et al., 2014), an effect which could have also contributed to our results. One of previous studies investigated improvements in balancing skills with both open and closed eyes in response to 6 weeks of balance training (Strang et al., 2011). Their results from postural movement measurement were, interestingly, very similar to our results; in the eyes closed condition they noticed a significant improvement while in the eyes open condition no significant change could be observed. The authors argued that this finding was to be expected, because only imposing a constraint during test, such as blockading visual input, would allow the effects of training to emerge. Another study on basketball players also reported improvements in tests with closed eyes in response to a 6-week balance training (Zemkova and Hamar, 2010). Whereas in that study improvements were seen mainly in dynamic balance tests we found them in the static balance tests only, consisting of various conditions on stable and unstable surfaces, which might be due to methodological differences between the studies; that is, the training methods differed and only one dynamic test condition was performed with closed eyes in our CBT, whereas all the other closed eyes conditions of the CBT belonged to the static group. Since the participants improved significantly on the closed eyes conditions, this had to be to the greatest extent within the static conditions. Had, however, our test involved more dynamic conditions with closed eyes, it appears from our results that it would have been reasonable to expect a significant difference in the amount of improvement between groups there as well.

The importance of stimulating both the rotational (semicircular canal function) and the translational (otolith organs) component of the vestibular system becomes obvious and is also crucial for our second finding. Namely, this was to show that the link between the vestibular system and its central vestibular-dependent spatial-orientation brain regions, primarily hippocampal regions (Hitier et al., 2014), can be affected by an adequately designed slackline-training. No previous studies investigated this possibility, making consequently our results novel in that sense. After learning how to slackline, our participants were able to return to the starting position more precisely after being taken away from it in a wheelchair along three different triangular paths. The triangle completion task was already used by many previous studies, mainly to examine the difference between younger and older persons in their ability to navigate in space (Allen et al., 2004; Adamo et al., 2012) or to investigate functions of the medial temporal lobe (Wolbers et al., 2007; Wiener et al., 2011). Consequently, the design of these studies was cross-sectional and no particular treatment was used to improve this ability over time. Our study is the first one to our knowledge to show transfer effects of slackline-training on orientation abilities in young people assessed with this task. Several authors studied rats to demonstrate the importance of the vestibular system for successful orientating in space (Stackman and Herbert, 2002; Russell et al., 2003; Smith et al., 2005). It has been shown that peripheral vestibular deficiency leads to impairments in functioning of the medial temporal lobe in spatial orientation tasks as well as in spatial learning. These impairments are due to alterations in electrophysiological and neurochemical signaling between the two systems. Other previous studies went on further to investigate the importance of the vestibular system for orientation in humans (Brandt et al., 2005; Hüfner et al., 2011; Previc et al., 2014), thereby confirming

the findings of animal studies. The structure of the hippocampal formation has been found to be altered in persons who suffer from vestibular deficiency, but also in persons who need to rely heavily on their vestibular system because of their profession, for example ballet dancers. It has even been proposed that vestibular system degeneration might be a significant contributor to development of the Alzheimer's disease (Previc, 2013). Although our study sample consisted of young and healthy subjects, considering neuroplasticity principles in response to motor task learning over the entire lifespan (Dayan and Cohen, 2011), it is legitimate to hypothesize that similar results could be expected in older populations, particularly as a prevention strategy in those at early stages of dementia. Some studies could not find significant relevant transfer effects of slackline-training in this population (Donath et al., 2016b), but, as discussed earlier, the methodological issues might have contributed to such findings; in our opinion additional research on this topic is required to answer this question.

Therefore, as far as the external validity or generalizability of our findings is concerned, our sample consisted of young and healthy (18–30 years old) subjects, and the results are thus mostly applicable to the same population. Considering, however, that many balance-training interventions can benefit both healthy and diseased populations of various ages in their original form (Taubert et al., 2010; Sehm et al., 2014), it is reasonable to assume that similar interventions to that used in our study could be beneficial for healthy older or even non-healthy older populations, in the direction obtained with our younger sample. It would be of a particular interest for us to see if similar interventions would demonstrate a significant gain in patients suffering from various stages of neurodegeneration, from those with mild cognitive impairment to those with Alzheimer's disease, in whom the spatial orientation capabilities are considerably reduced (Allen et al., 2004).

There are several limitations of our study which we would like to list here. First, our CBT has not yet been validated; however, many of its items resemble those applied in other validated CBTs and its inter-rater reliability is very high. Another argument against could be that the subjective nature of our postural sway assessment is less accurate than the quantitative assessments at force platforms; most CBTs, however, are subjective opinion-based tests, but are comprehensive and specifically designed to assess various components of balancing abilities, and remain important and valid assessment tools for this purpose (Mancini and Horak, 2010). Our OT comes from the extensively used triangle completion test that assesses orientation abilities; still, only a subset of conditions was applied in our study, in accordance with availability of facilities at our institute; although the error distance measurement has been performed thoroughly, reliability data is still to be provided. Secondly, we did not report any follow up results which would signify a potential of this training to cause eventual retention of the achieved effects over a longer period of subsequent inactivity. Third limitation can be considered the fact that we yet have to show neural correlates of our behavioral improvements, by analyzing pre/post MR data. Finally, our participants performed the training with open eyes; it would be interesting to know whether the same training performed with closed eyes would bring any different results, compared to both the control group and the actual training group, since in this third group visual input would be blocked. We will attempt to successfully deal with these limitations in our future work.

CONCLUSION

Our results indicate that 1 month of intensive balance training, through learning how to slackline, is a successful novel approach for enhancing clinically relevant balancing abilities in conditions with closed eyes and simultaneous improvements in vestibular-dependent spatial orientation capability; both of the benefits are possibly caused by positive influence of slackline-training on vestibular system function, and possibly its connectivity with temporal lobe regions responsible for orienting in space, such as the hippocampus. We can highly recommend this method, both its intensity and type, to all young persons who need to improve functioning of their vestibular system, either for the purpose of increasing stability, upgrading spatial orientation abilities or both. Modifying the training protocol could be also potentially of advantage for healthy elderly and those at risk of neurodegeneration of the medial temporal lobe orientation-system, such as in AD, but this is yet to be proven by future studies.

AUTHOR CONTRIBUTIONS

MD: Study planning and organization, data collection, data analysis, paper writing, paper revision, paper submission. AH: Study planning and organization, paper revision. PM: Data collection, paper revision. KR: Study planning and organization, paper revision. NM: Study planning and organization, data analysis, paper writing, paper revision.

REFERENCES

Adamo, D. E., Briceño, E. M., Sindone, J. A., Alexander, N. B., and Moffat, S. D. (2012). Age differences in virtual environment and real world path integration. *Front. Aging Neurosci* 4:26. doi: 10.3389/fnagi.2012.00026

Allen, G. L., Kirasic, K. C., Rashotte, M. A., and Haun, D. B. M. (2004). Aging and path integration skill: kinesthetic and vestibular contributions to wayfinding. *Percept. Psychophys.* 66, 170–179. doi: 10.3758/BF0319 4870

Ambrose, A. F., Paul, G., and Hausdorff, J. M. (2013). Risk factors for falls among older adults: a review of the literature. *Maturitas* 75, 51–61. doi: 10.1016/j. maturitas.2013.02.009

Boccolini, G., Brazzit, A., Bonfanti, L., and Alberti, G. (2013). Using balance training to improve the performance of youth basketball players. *Sport Sci. Health* 9, 37–42. doi: 10.1007/s11332-013-0143-z

Brandt, T., Schautzer, F., Hamilton, D. A., Brüning, R., Markowitsch, H. J., Kalla, R., et al. (2005). Vestibular loss causes hippocampal atrophy and impaired spatial memory in humans. *Brain* 128, 2732–2741. doi: 10.1093/brain/awh617

Brookmeyer, R., Johnson, E., Ziegler-Graham, K., and Arrighi, H. M. (2007). Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement.* 3, 186–191. doi: 10.1016/j.jalz.2007.04.381

Cadore, E. L., Rodríguez-Mañas, L., Sinclair, A., and Izquierdo, M. (2013). Effects of different exercise interventions on risk of falls, gait ability, and balance in physically frail older adults: a systematic review. *Rejuvenation Res.* 16, 105–114. doi: 10.1089/rej.2012.1397

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*, 2nd Edn. Cambridge, MA: Academic Press, doi: 10.1234/12345678

Cullen, K. E., and Minor, L. B. (2002). Semicircular canal afferents similarly encode active and passive head-on-body rotations: implications for the role of vestibular efference. *J. Neurosci.* 22:RC226.

Dayan, E., and Cohen, L. G. (2011). Neuroplasticity subserving motor skill learning. *Neuron* 72, 443–454. doi: 10.1016/j.neuron.2011.10.008

Donath, L., Roth, R., Rueegge, A., Groppa, M., Zahner, L., and Faude, O. (2013). Effects of slackline training on balance, jump performance & muscle activity in young children. *Int. J. Sports Med.* 34, 1093–1098. doi: 10.1055/s-0033-1337949

Donath, L., Roth, R., Zahner, L., and Faude, O. (2016a). Slackline training (Balancing Over Narrow Nylon Ribbons) and balance performance: a meta-analytical review. *Sports Med.* doi: 10.1007/s40279-016-0631-9 [Epub ahead of print].

Donath, L., Roth, R., Zahner, L., and Faude, O. (2016b). Slackline training and neuromuscular performance in seniors: a randomized controlled trial. *Scand. J. Med. Sci. Sports* 26, 275–283. doi: 10.1111/sms.12423

El-Khoury, F., Cassou, B., Latouche, A., Aegerter, P., Charles, M.-A., and Dargent-Molina, P. (2015). Effectiveness of two year balance training programme on prevention of fall induced injuries in at risk women aged 75-85 living in community: ossébo randomised controlled trial. *BMJ* 351:h3830. doi: 10.1136/ bmj.h3830

Granacher, U., Iten, N., Roth, R., and Gollhofer, A. (2010). Slackline training for balance and strength promotion. *Int. J. Sports Med*. 31, 717–723. doi: 10.1055/ s-0030-1261936

Highstein, S. M. (1991). The central nervous system efferent control of the organs of balance and equilibrium. *Neurosci. Res.* 12, 13–30. doi: 10.1016/0168-0102(91)90096-H

Hitier, M., Besnard, S., and Smith, P. F. (2014). Vestibular pathways involved in cognition. *Front. Integr. Neurosci.* 8:59. doi: 10.3389/fnint.2014.00059

Horak, F. B. (2006). Postural orientation and equilibriumL What do we need to know about neural control of balance to prevent falls? *Age Ageing* 35, (Suppl. 2), ii7–ii11.

Horak, F. B., Wrisley, D. M., Frank, J., Horak, F. B., and Wrisley, D. M. (2009). The balance evaluation systems test (BESTest) to differentiate balance deficits. *Phys. Ther.* 89, 484–498. doi: 10.2522/ptj.20080071

Hubscher, M., Zech, A., Pfeifer, K., Hansel, F., Vogt, L., and Banzer, W. (2010). Neuromuscular training for sports injury prevention: a systematic review. *Med. Sci. Sports Exerc.* 42, 413–421. doi: 10.1249/MSS.0b013e3181b88d37

Hüfner, K., Binetti, C., Hamilton, D. A., Stephan, T., Flanagin, V. L., Linn, J., et al. (2011). Structural and functional plasticity of the hippocampal formation in professional dancers and slackliners. *Hippocampus* 21, 855–865. doi: 10.1002/ hipo.20801

Jahn, K., Wagner, J., Deutschländer, A., Kalla, R., Hüfner, K., Stephan, T., et al. (2009). Human hippocampal activation during stance and locomotion: FMRI study on healthy, blind, and vestibular-loss subjects. *Ann. N. Y. Acad. Sci.* 1164, 229–235. doi: 10.1111/j.1749-6632.2009.03770.x

Karlsson, M. K., Vonschewelov, T., Karlsson, C., Cöster, M., and Rosengen, B. E. (2013). Prevention of falls in the elderly: a review. *Scand. J. Public Health* 41, 442–454. doi: 10.1177/1403494813483215

Lubetzky-Vilnai, A., and Kartin, D. (2010). The effect of balance training on balance performance in individuals poststroke: a systematic review. *J. Neurol. Phys. Ther.* 34, 127–137. doi: 10.1097/NPT.0b013e3181ef764d

Mancini, M., and Horak, F. B. (2010). The relevance of clinical balance assessment tools to differentiate balance deficits. *Eur. J. Phys. Rehabil. Med.* 46, 239–248.

Pfusterschmied, J., Buchecker, M., Keller, M., Wagner, H., Taube, W., and Müller, E. (2011). Supervised slackline training improves postural stability. *Eur. J. Sport Sci.* 13, 1–9. doi: 10.1080/17461391.2011.583991

Pfusterschmied, J., Stöggl, T., Buchecker, M., Lindinger, S., Wagner, H., and Müller, E. (2013). Effects of 4-week slackline training on lower limb joint motion and muscle activation. *J. Sci. Med. Sports* 16, 562–566. doi: 10.1016/j.jsams.2012. 12.006

Porciuncula, F., Johnson, C. C., and Glickman, L. B. (2012). The effect of vestibular rehabilitation on adults with bilateral vestibular hypofunction: a systematic review. *J. Vestib. Res.* 22, 283–298. doi: 10.3233/VES-120464

Previc, F. H. (2013). Vestibular loss as a contributor to Alzheimer's disease. *Med. Hypotheses* 80, 360–367. doi: 10.1016/j.mehy.2012.12.023

Previc, F. H., Krueger, W. W., Ross, R. A., Roman, M. A., and Siegel, G. (2014). The relationship between vestibular function and topographical memory in older adults. *Front. Integr. Neurosci.* 8:46. doi: 10.3389/fnint.2014. 00046

Raskin, J., Cummings, J., Hardy, J., Schuh, K., and Dean, R. A. (2015). Neurobiology of Alzheimer's Disease: integrated molecular, physiological, anatomical, biomarker, and cognitive dimensions. *Curr. Alzheimer Res.* 12, 712–722. doi: 10.2174/1567205012666150701 103107

Ries, J. D., Hutson, J., Maralit, L. A., and Brown, M. B. (2015). Group balance training specifically designed for individuals with Alzheimer Disease
: impact on berg balance scale, timed up and go, gait speed, and mini-mental status examination. *J. Geriatr. Phys. Ther.* 37, 1–11. doi: 10.1519/JPT. 0000000000000030

Russell, N. A., Horii, A., Smith, P. F., Darlington, C. L., and Bilkey, D. K. (2003). Long-term effects of permanent vestibular lesions on hippocampal spatial firing. *J. Neurosci.* 23, 6490–6498.

Sampaio-Baptista, C., Scholz, J., Jenkinson, M., Thomas, A. G., Filippini, N., Smit, G., et al. (2014). Gray matter volume is associated with rate of subsequent skill learning after a long term training intervention. *Neuroimage* 96, 158–166. doi: 10.1016/j.neuroimage.2014.03.056

Sehm, B., Taubert, M., Conde, V., Weise, D., Classen, J., Dukart, J., et al. (2014). Structural brain plasticity in parkinson's disease induced by balance training. *Neurobiol. Aging* 35, 232–239. doi: 10.1016/j.neurobiolaging.2013. 06.021

Sherrington, C., Tiedemann, A., Fairhall, N., Close, J. C. T., and Lord, S. R. (2011). Exercise to prevent falls in older adults: an updated meta-analysis and best practice recommendations. *N. S. W. Public Health Bull.* 22, 78–83. doi: 10.1071/ NB10056

Smith, P. F., Darlington, C. L., and Zheng, Y. (2010). Move it or lose it–is stimulation of the vestibular system necessary for normal spatial memory? *Hippocampus* 20, 36–43. doi: 10.1002/hipo.20588

Smith, P. F., Horii, A., Russell, N., Bilkey, D. K., Zheng, Y., Liu, P., et al. (2005). The effects of vestibular lesions on hippocampal function in rats. *Prog. Neurobiol.* 75, 391–405. doi: 10.1016/j.pneurobio.2005.04.004

Stackman, R. W., Clark, A. S., and Taube, J. S. (2002). Hippocampal spatial representations require vestibular input. *Hippocampus* 12, 291–303. doi: 10. 1002/hipo.1112

Stackman, R. W., and Herbert, A. M. (2002). Rats with lesions of the vestibular system require a visual landmark for spatial navigation. *Behav. Brain Res.* 128, 27–40. doi: 10.1016/S0166-4328(01)00270-4

Strang, A. J., Haworth, J., Hieronymus, M., Walsh, M., and Smart, L. J. (2011). Structural changes in postural sway lend insight into effects of balance training, vision, and support surface on postural control in a healthy population. *Eur. J. Appl. Physiol.* 111, 1485–1495. doi: 10.1007/s00421-010-1770-6

Taubert, M., Draganski, B., Anwander, A., Müller, K., Horstmann, A., Villringer, A., et al. (2010). Dynamic properties of human brain structure: learning-related changes in cortical areas and associated fiber connections. *J. Neurosci.* 30, 11670–11677. doi: 10.1523/JNEUROSCI.2567-10.2010

Thomas, M., and Kalicinski, M. (2016). The effects of slackline balance training on postural control in older adults. *J. Aging Phys. Act*. 24, 393–398. doi: 10.1123/ japa.2015-0099

Wiener, J. M., Berthoz, A., and Wolbers, T. (2011). Dissociable cognitive mechanisms underlying human path integration. *Exp. Brain Res.* 208, 61–71. doi: 10.1007/s00221-010-2460-7

Wolbers, T., Wiener, J. M., Mallot, H. A., and Büchel, C. (2007). Differential recruitment of the hippocampus, medial prefrontal cortex, and the human motion complex during path integration in humans. *J. Neurosci.* 27, 9408–9416. doi: 10.1523/JNEUROSCI.2146-07.2007

Zech, A., Hübscher, M., Vogt, L., Banzer, W., Hänsel, F., and Pfeifer, K. (2010). Balance training for neuromuscular control and performance enhancement: a systematic review. *J. Athl. Train.* 45, 392–403. doi: 10.4085/1062-6050-45. 4.392

Zemkova, E., and Hamar, D. (2010). The effect of 6-week combined agility-balance training on neuromuscular performance in basketball players. *J. Sports Med. Phys. Fitness* 50, 262–267.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 14 March 2017
doi: 10.3389/fnagi.2017.00056

Evolution of Neuroplasticity in Response to Physical Activity in Old Age: The Case for Dancing

Patrick Müller[1]*, Kathrin Rehfeld[2], Marlen Schmicker[1], Anita Hökelmann[2], Milos Dordevic[1], Volkmar Lessmann[3,4], Tanja Brigadski[3,4,5], Jörn Kaufmann[6] and Notger G. Müller[1,4,6]

[1]Neuroprotection Laboratory, German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany, [2]Institute of Sport Science, Otto-von-Guericke-University, Magdeburg, Germany, [3]Medical Faculty, Institute of Physiology, Otto-von-Guericke University, Magdeburg, Germany, [4]Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany, [5]Informatics and Microsystem Technology, University of Applied Science Kaiserslautern, Kaiserslautern, Germany, [6]Medical Faculty, Clinic for Neurology, Otto-von-Guericke University, Magdeburg, Germany

From animal research, it is known that combining physical activity with sensory enrichment has stronger and longer-lasting effects on the brain than either treatment alone. For humans dancing has been suggested to be analogous to such combined training. Here we assessed whether a newly designed dance training program that stresses the constant learning of new movement patterns is superior in terms of neuroplasticity to conventional fitness activities with repetitive exercises and whether extending the training duration has additional benefits. Twenty-two healthy seniors (63–80 years) who had been randomly assigned to either a dance or a sport group completed the entire 18-month study. MRI, BDNF and neuropsychological tests were performed at baseline and after 6 and 18 months of intervention. After 6 months, we found a significant increase in gray matter volume in the left precentral gyrus in the dancers compared to controls. This neuroplasticity effect may have been mediated by the increased BDNF plasma levels observed in the dancers. Regarding cognitive measures, both groups showed significant improvements in attention after 6 months and in verbal memory after 18 months. In addition, volume increases in the parahippocampal region were observed in the dancers after 18 months. The results of our study suggest that participating in a long-term dance program that requires constant cognitive and motor learning is superior to engaging in repetitive physical exercises in inducing neuroplasticity in the brains of seniors. Therefore, dance is highly promising in its potential to counteract age-related gray matter decline.

Keywords: neuroplasticity, VBM, exercise, dancing, neurodegeneration, BDNF

Edited by:
Aurel Popa-Wagner, University of Rostock, Germany

Reviewed by:
Daniel Ortuño-Sahagún, Centro Universitario de Ciencias de la Salud, Mexico
Raluca Sandu Vintilescu, University of Medicine and Pharmacy of Craiova, Romania

****Correspondence:***
Patrick Müller
patrick.mueller@dzne.de

Received: 23 December 2016
Accepted: 27 February 2017
Published: 14 March 2017

Citation:
Müller P, Rehfeld K, Schmicker M, Hökelmann A, Dordevic M, Lessmann V, Brigadski T, Kaufmann J and Müller NG (2017) Evolution of Neuroplasticity in Response to Physical Activity in Old Age: The Case for Dancing. Front. Aging Neurosci. 9:56. doi: 10.3389/fnagi.2017.00056

INTRODUCTION

The current demographic change in Western societies, involving both a relative and an absolute increase in the number of older people, has sparked increasing scientific interest in geriatric issues. In this context, concepts of successful aging are becoming increasingly important. Several studies have indicated that physical exercise may play a key role in healthy aging and in the prevention of cognitive decline and neurodegenerative diseases (Colcombe et al., 2006; Erickson et al., 2011). Recent reviews summarizing epidemiological, cross-sectional and interventional studies support physical activity as a propitious method to induce neuroplasticity in late adulthood (Gregory et al., 2012; Voelcker-Rehage and Niemann, 2013; Bamadis et al., 2014). Erickson et al. (2012) have concluded that higher cardiorespiratory fitness and aerobic physical activity levels are associated with larger gray matter volumes in the prefrontal regions and the hippocampus. However, not only cardiovascular fitness but also coordinative exercise (Niemann et al., 2014) and cognitive training (Bamadis et al., 2014) have been shown to induce gray matter plasticity and enhance cognitive functions in older adults.

Animal research has suggested that a combination of physical exercise with sensory enrichment has the strongest effect on the genesis of new neurons—predominantly in the hippocampus—and that only this combination ensures the enduring survival of the newborn cells (Kempermann et al., 1997; van Praag et al., 2005). Kattenstroth et al. (2013) have suggested that ''dancing activities should be regarded as an equivalent of enriched environmental conditions for humans since they provide an individual with increased sensory, motor and cognitive demands.'' Despite this encouraging statement, studies examining the effects of dance training on brain structure or function are scarce. After a 6-month dancing intervention, Kattenstroth et al. (2010) have reported significant improvements in cognitive, tactile and motor performance in participating seniors. The results of a prospective study of 469 subjects older than 75 years over a median follow-up period of 5.1 years have indicated that dancing is associated with a markedly reduced risk of dementia (Verghese et al., 2003). However, Hüfner et al. (2011) have reported reduced volumes in several brain regions including the anterior hippocampal formation and parts of the parieto-insular vestibular cortex in professional dancers and slackliners compared with non-professionals. This finding may indicate that intensive and repetitive training of the same motor skills leads in context of specialization to reduced volumes of some brain regions. In this study, we therefore design a special dance training program that constantly required the participants to learn new movement patterns (Müller et al., 2016). To assess the specific benefits of this intervention, we compared our newly designed dance program to an active rather than a passive control group, which took part in a conventional health sport fitness program in which participants typically performed repetitive physical exercises such as bicycling on an ergometer. Furthermore, because we were interested in the temporal dynamics of the interventions, we assessed the effects on brain structure and function after 6 and 18 months of training. In doing so, we sought to assess whether it is beneficial to extend interventions, because some brain regions may require more training than others, or whether there is a limit after which more training becomes detrimental. Finally, in search of a potential mechanism underlying neuroplasticity, we measured the BDNF levels in the peripheral blood. Several studies have suggested that BDNF promotes the differentiation of new neurons and synapses (Huang and Reichardt, 2001; Lessmann and Brigadski, 2009; Park and Poo, 2013; Edelmann et al., 2014). BDNF, therefore, has been proposed to be a mediator of adult neuroplasticity (Flöel et al., 2010).

MATERIALS AND METHODS

Participants and Experimental Design

The study was designed as an 18-month controlled intervention. The study was approved by the ethics committee of Otto-von-Guericke University, Magdeburg, and all subjects signed a written informed and they did not receive payment for their participation. Sixty-two healthy elderly individuals (63–80 years) recruited via announcements in local newspapers were screened for the study. The exclusion criteria were claustrophobia, tinnitus, metal implants, tattoos, diabetes mellitus, depression (Beck-Depressions Inventory, BDI-II > 13), cognitive deficits (Mini-Mental State Examination, MMSE < 27), neurological diseases and regular exercising (1 h/week). On the basis of these criteria, 10 subjects were excluded. The remaining 52 participants were then randomly assigned to either the dance or the sport group by using the website www.randomization.com and controlling for age, MMSE status and physical fitness. Assessments were performed at baseline, after 6 and after 18 months of training (**Figure 1**). Twenty-two participants completed the entire intervention and all measurements. **Table 1** provides detailed demographic data for these participants. No group differences regarding the demographic data were found.

Interventions

The interventions were separated into two periods. In the first period, the subjects trained twice per week in 90-min sessions for

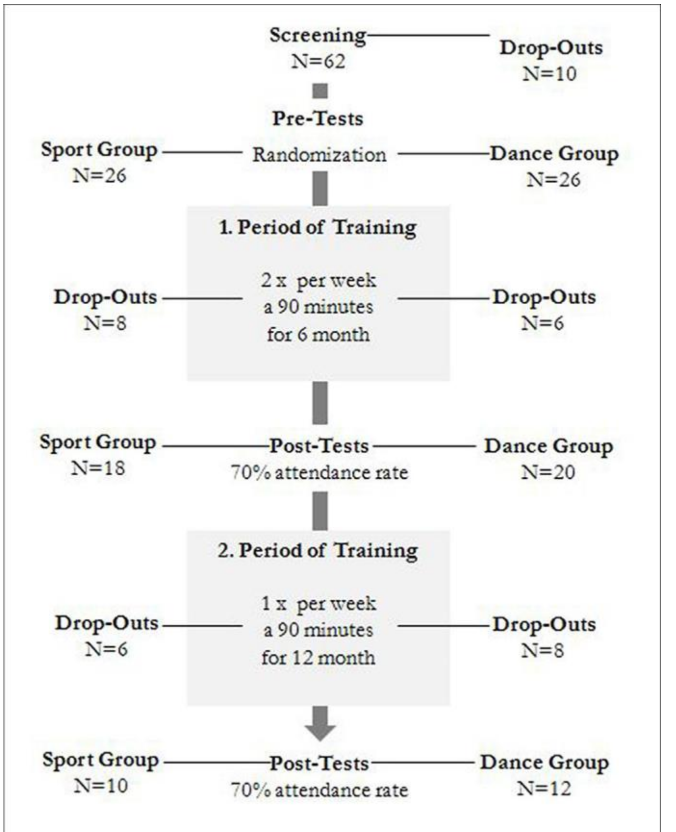


FIGURE 1 | Flow chart of participants' recruitment.

TABLE 1 | Demographic information on the participants at baseline.

Measure	Dancing group	Sport group
N	12	10
Age (years)	68.25 .3.91/	68.60 .2.79/
Gender (% female)	50%	40%
BMI	27.51 .3.87/	27.24 .2.94/
BDI-II	5.50 .2.94/	3.00 .3.77/
Education	15.50 .2.11/	16.40 .1.35/
MMSE	28.33 .1.07/	29.10 .0.57/

BMI, body mass index; MMSE, Mini-Mental State Examination; BDI-II, Beck-Depressions Inventory.

6 months. For practical reasons (availability of participants and trainers), the second 12-month intervention period comprised a reduced training frequency of once per week. Both intervention programs were performed in a group context with music to control for psychosocial interactions. Conditional load was examined by recording the pulse values during the training sessions and by calculating the individual training heart rate according to Karvonen et al. (1957) with a factor 0.6 for extensive endurance training. Hence, the two training programs were comparable in terms of intensity, duration and frequency. Both were supervised by experienced instructors.

Dance Group

Participants in the dance group attended a newly designed training program in which they were constantly asked to learn new movement sequences. These choreographies required the coordination of different body parts (i.e., legs, arms, trunk) in space under different strain conditions (physical strain, precision, situation and time pressure). The subjects had to learn the choreographies by heart, thus imposing high demands on memory as well. The program comprised five different genres (line dance, jazz dance, rock ‘‘n’’ roll and square dance), which were switched after every fourth session. Over the course of the intervention, coordinative demands and time pressure were increased by introducing more complex dance moves and choreographies and by increasing the beats per minute in the music.

Sport Group

Participants in the sport group completed a conventional strength-endurance training program with mainly repetitive exercises and low demands in terms of whole-body coordination and memory. Each session comprised 20-min units of endurance, strength-endurance and flexibility training. The endurance training was performed on cycle ergometers. In the strength-endurance unit, alternating movements (e.g., biceps curls, squats, sit-ups) were performed, but complex whole body movements were avoided to keep coordinative demands low. The flexibility unit mainly consisted of stretching exercises.

Outcome Measures

Cardiovascular Fitness

Cardiovascular fitness was assessed by the Physical Working Capacity 130 Test (PWC 130). PWC 130 is the power output (measured in watts) that a subject is able to achieve on a cycle ergometer under a heart rate of 130 bpm. For calculations, we used resting heart rate [min^{1}] and relative physical capacity [watt/kg].

Neuropsychological Testing

An extensive battery of neuropsychological tests were performed on the subjects. For the purpose of the current study, only the results of a verbal short- and long-term memory test, the ‘‘VLMT’’ (an adapted German version of the ‘‘Rey Auditory Verbal Learning Test’’; Helmstaedter and Durwen, 1990) and an attention test battery (Test of Attentional Performance (TAP); Zimmermann and Fimm, 2002) are reported.

BDNF

Fasting blood samples were taken in the mornings of the neuropsychological assessments. From the blood samples, plasma concentrations of BDNF were determined by sandwich ELISAs (BDNF DuoSets; R&D Systems, Wiesbaden, Germany) as previously described (Schega et al., 2016).

MRI

MR images were acquired on a 3 Tesla Siemens MAGNETOM Verio (Syngo MR B17) using a 32-channel head coil. High-resolution T1-weighted MPRAGE sequences were acquired using a 3D magnetization-prepared rapid gradient echo imaging protocol (224 sagittal slices, voxel size: 0.8 0.8 0.8 mm^{3}, TR: 2500 ms, TE: 3.47 ms, TI: 1100 ms, flip angle: 7). The MR images were analyzed using voxel-based morphometry (VBM) implemented in SPM 12 (Welcome Department of Cognitive Neurology, London, UK). VBM is a whole-brain unbiased technique for analysis of regional gray matter volume and tissue changes (Ashburner and Friston, 2000).

Preprocessing involved gray-matter segmentation, template creation via DARTEL, spatial normalization to standardized Montreal Neurological Institute (MNI) space and smoothing with an Gaussian kernel of 8 mm full width at half maximum (FWHM).

To analyze the difference in gray matter volume changes between groups, a full-factorial design with the factors group (dance, sport) and time (0, 6 and 18 months) was applied. In the case of significant group time interactions, *post hoc t*-tests between consecutive pairs of time points (0 vs. 6; 0 vs. 18; 6 vs. 18 months) were calculated separately for each group. A threshold of $p < 0.001$ (uncorrected) was applied for all analyses.

RESULTS

The presentation of the results is structured as follows. We first looked for a general intervention effect (factor time), then looked for differential effects of the two interventions (interaction group time); finally, a more detailed analysis of the temporal dynamics was performed via *post hoc* pairwise comparisons.

Cardiovascular Fitness

Cardiovascular fitness as measured by the PWC 130 (**Table 2**, relative physical capacity) did not differ between groups at baseline. Furthermore, the PWC 130 score did not increase significantly throughout the time course of either intervention.

Neuropsychological Tests

A significant main effect of time was observed for verbal short-term memory (VLMT early recall; $F_{.2,19/}$ = 6.438, p = 0.004, ^{2} = 0.253), verbal long-term free recall (VLMT late recall; $_{.2,19/}$ = 3.387, p = 0.049, ^{2} = 0.244), verbal long-term recognition (VLMT recognition; $F_{.2,19/}$ = 5.352, p = 0.009, ^{2} = 0.220) and attention reaction time (subtest flexibility; $_{.2,19/}$ = 19.156, p < 0.001, ^{2} = 0.489). No significant time group interactions emerged.

Post hoc pairwise comparisons showed significant improvements from baseline to 18 months and from 6 to 18 months in all three VLMT subcategories in both groups. Regarding TAP reaction times, a significant improvement was seen in the comparison of the baseline to the 18-month data in both groups.

BDNF

Plasma levels of BDNF were analyzed in blood samples before the onset of training as well as 6 months and 18 months after the onset of training. Absolute BDNF plasma levels are summarized in **Table 2**. The intraindividual changes in BDNF level revealed a significant increase in the dancing group, whereas the intraindividual BDNF level remained constant after 6 months of training in the sport group. No further changes in BDNF occurred after 18 months in any group (**Figure 2**).

MRI

A significant group time interaction was observed in the left precentral gyrus and the right parahippocampal. *Post hoc t*-tests between the baseline and 6-month data showed a significant increase in gray matter volume in the left precentral gyrus of only the dancers. In the comparison of the baseline and the 18-month data in addition to the precentral gyrus, the dancers exhibited a significant gray matter volume increase in the right parahippocampal gyrus, which was also the only significant change in the interval from 6 to 18 months. Thus, the volume increase in the precentral gyrus emerged after 6 months and remained stable over the remaining dance training interval, whereas the change in the parahippocampal gyrus occurred during the later training interval only (**Figure 3**).

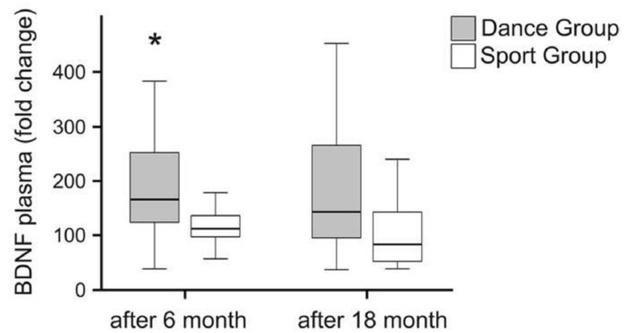


FIGURE 2 | Intraindividual changes in BDNF plasma levels after intervention. BDNF plasma levels were analyzed in blood samples of participants performing a dancing training program or a sport training program before the onset of training, after 6 months of training and after 18 months of training. The relative increase in BDNF levels was quantified. The BDNF levels significantly increased in the dance group after 6 months of training (Mann-Whitney U-test, p < 0.004) and declined nearly to baseline of the pretreatment value after 18 months of dancing training. There was no change over the entire time course in the sport group (Friedman test, p = 0.319), whereas a significant change over the entire time course was observed in the dance group (Friedman test, p = 0.028). Box plots: minimum, 25th percentile, median, 75th percentile. p 0.05.

DISCUSSION

In this study, we compared the effects of participation in either a dance program or a conventional physical fitness sport program on brain function and volume in healthy seniors. The dance program was a newly designed intervention that required constantly learning new dance choreographies. The conventional sport program focused mainly on repetitive motor exercises. As a main finding, we observed that after 6 months of training, the volumes in the left precentral gyrus of the dancers had increased more than those in the sport group. After another 12 months of training, an additional volume increase was observed in the right parahippocampal gyrus of the dancers. BDNF levels increased during the first 6 months of dance training and returned to the pre-treatment values after 18 months. In the conventional sport group, a similar

TABLE 2 | Means and (SD) for fitness, cognitive functioning, BDNF plasma levels and total gray matter volume within training groups over the intervention.

	Dance group			Sport group		
Variable	Baseline	6 months	18 months	Baseline	6 months	18 months
Relative physical capacity (Watt/kg)	1.28.0.33/	1.19.0.29/	1.36.0.38/	1.19.0.33/	1.39.0.30/	1.21.0.31/
Resting heart frequency (min ^{1})	77.50.12.64/	76.08.10.21/	73.83.7.66/	72.00.14.86/	69.75.15.96/	75.00.12.58/
VLMT early recall (points)	47.83.10.24/	43.92.8.29/	52.42.6.86/	53.10.8.00/	53.30.8.68/	56.22.5.19/
VLMT late recall (points)	10.25.3.34/	9.08.2.94/	9.90.4.15/	12.00.3.23/	11.70.3.09/	14.00.1.73/
VLMT recognition (points)	10.25.3.08/	8.92.3.42/	11.17.2.21/	11.40.3.20/	11.80.2.86/	12.89.1.965/
TAP flexibility reaction time (ms)	978.58.241.15/	901.75.288.62/	772.92.168.97/	873.70.234.04/	863.50.277.63/	792.70.162.17/
BDNF plasma	1469.57 .1038.87/	2189.59 .1116.28/	1725.83.778.27/	1861.17 .1284.69/	2170.76 .1285.04/	1610.80.848.59/
Gray matter volume (mm^3)	601.95.32.26/	597.03.33.66/	611.27.33.23/	593.75.40.44/	585.89.34.54/	602.16.40.75/

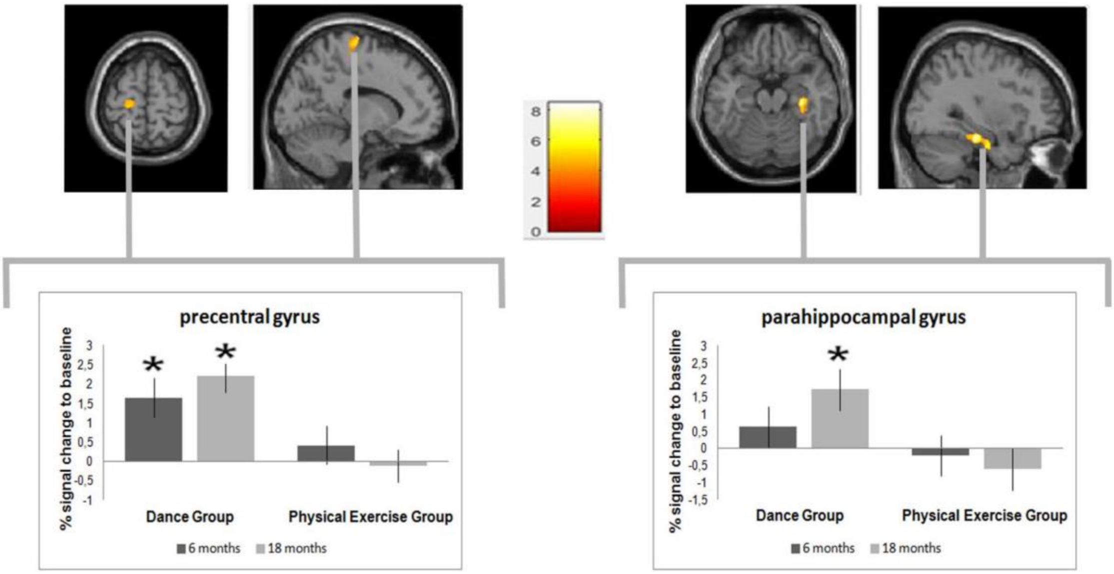


FIGURE 3 | Time by group interaction analysis, testing for greater volume changes in the dance compared with the sport group. A significant increase in gray matter was found in the precentral gyrus (Montreal Neurological Institute (MNI)-coordinates: x = 16; y = 18; z = 77) and in the parahippocampal gyrus gyrus (MNI-coordinates: x = 34; y = 26; z = 20). The box plots show the relative gray matter changes in the peak voxel. p 0.05.

increase in BDNF was not evident. Because the cardiovascular fitness levels over the course of the interventions remained constant in both groups, the observed effects could not be attributed to improvements in physical fitness but instead seemed to be related to the specific features of the dance program. These features included the requirement to constantly learn new choreographies (i.e., memory), to integrate multisensory information, to coordinate the whole body and to navigate in space.

Brain Changes

The precentral gyrus is essential for the control of voluntary motor functions. The increase in gray matter volume in the precentral gyrus in the dance group may, therefore, have been based on the complex and ever-changing movement patterns that the dancers had to perform. These movements required the simultaneous coordination of several parts of the body in different directions and adjustment to the varying rhythms of the music (polycentric and polyrhythmic). Reflecting on these complex coordination requirements, Brown et al. (2006) have reported dance-induced activations in the putamen, the primary motor cortex and the supplementary motor area (SMA), as shown by PET. Other studies have indicated an association between coordination demands (e.g., balancing, juggling) and neuroplasticity in the precentral region (Boyke et al., 2008; Taubert et al., 2010). Hence, the dance-related volume increase in this area was consistent with expectations based on the literature.

The parahippocampal gyrus is part of the outer arc of the limbic system and plays an important role in working memory and episodic memory retrieval (Pantel et al., 2003). According to Bliss and Lomo (1973), the parahippocampal gyrus constitutes the interface between memory and the experiential consciousness of the present, because it is interconnected by the perforant tract both to regions of the frontal lobe, which are associated with working memory, and to the hippocampus, the central structure in episodic memory encoding and spatial navigation. Many VBM studies have reported an age-related volume loss in parahippocampal regions (Tisserand et al., 2002). Furthermore, Echávarri et al. (2011) have suggested that parahippocampal atrophy is an early biomarker of AD. The observation that engaging in a dance program for a longer period can induce neuroplastic processes in this crucial memory region, therefore, particularly encouraging in terms of developing prevention strategies.

Temporal Dynamics of Gray Matter Brain Plasticity

The observed volume increases in the two brain regions developed at different times. Dancing led to a volume increase in the motor areas after 6 months (see also Rehfeld et al., 2016), which remained stable during the subsequent 12 months. The dance-associated volume increase in the parahippocampal gyrus emerged later and was observed only in the 18-month data. The different temporal dynamics in the evolution of the two brain regions may be related to differences in the underlying cellular mechanisms. Animal research has suggested that angiogenesis and the formation of new dendrites (Thomas et al., 2012) occur rapidly, whereas changes in the neuropil occur much slower (Black et al., 1990). In humans, rapid increases in gray matter volume in the prefrontal regions have been observed after only 2 weeks of motor learning in younger adults (Taubert et al., 2010). To induce neuroplasticity in the hippocampus, longer training periods have been recommended (Erickson et al., 2012; Niemann et al., 2014).

Interestingly, the aforementioned volume increases from our study are in contrast to the results of Hänggi et al. (2010), which revealed that professional ballet dancers have decreased gray matter volumes in the left premotor cortex, SMA, Putamen and superior frontal gyrus and the results of Hüfner et al. (2011), which report reduced volumes in several brain regions including the anterior hippocampal formation in professional dancers and slackliners compared with non-professionals. However, these studies were cross-sectional observational studies which compared the brains of professional dancers to those of non-professionals. In addition, our dance group consisted of old aged novices.

It has been shown that learning of a new skill first leads to recruitment of additional neural resources. Later, when the skill becomes more automatic, less neuronal resources are needed which may lead to volume decreases in those with long-term experience. Based on these initial conditions (novices, older adults) and our special designed dancing training program, which required constantly new learning of movement patterns, it is possible that effects of specialization induced volume decreases as reported by Hänggi et al. (2010) are not observed.

It is generally thought that motor training initially induces brain volume increases. However, prolonged training leads to automatization, which may have the opposite effects on cortical volume, because less cortical control is needed after the motor skills have been fully established (Taube, 2008). Our dance training program, therefore, was specially designed to avoid such automatization, which may explain why, at least within 18 months, no cortical volume decreases were observed in our study.

Cognitive functions also showed nonlinear development, whereby verbal memory increased only during the second training period. Regarding attention performance, significant improvements were observable after only 6 months in both groups (Rehfeld et al., 2016). These findings support prior reports regarding the beneficial effects of physical interventions on neuropsychological tests (Bamadis et al., 2014). However, in cognitive ability data, in contrast to the brain data, no group differences emerged. Others have reported superior effects of combined cognitive and physical training as opposed to single interventions (Oswald et al., 2006). We will extend our interventions further to test whether group differences in cognition might emerge at even later time points.

Underlying Cellular and Molecular Mechanisms of Gray Matter Plasticity

Although VBM is an imaging modality that reveals volume changes in the brain, this technique does not allow causal conclusions regarding the underlying neurophysiological processes. Neurogenesis, synaptogenesis and angiogenesis are just a few of the mechanisms that have been suggested to be the basis of brain volume changes (Zatorre et al., 2012).

As mediators of the effects of cardiovascular fitness on the brain, growth factors such as BDNF, insulin-like growth factor (IGF) and nerve growth factor (NGF) are being studied (Kirk-Sanchez and McGough, 2014). However, in our study, in contrast to previous ones (Erickson et al., 2011; Maass et al., 2015), no differences in cardiovascular fitness were present between groups, and the fitness levels did not change during the interventions. The latter observation was probably related to our control of the individual heart frequency, which we aimed to maintain in the aerobic zone. However, BDNF changes have also been associated with physical activity, social interaction and positive stress (Mattson, 2008), and not all studies have observed a BDNF increase after cardiovascular training (Vital et al., 2014). Finally, animal research has suggested that coordination but not endurance training induces synaptogenesis and glial changes (Black et al., 1990). Together, the named additional factors that drive BDNF secretion may have been more crucial during dancing than during fitness activities, thus explaining why only dancers showed a BDNF increase in the first 6 months. The observation that BDNF levels returned to baseline in the following 12 months while volume increases were simultaneously observed in the parahippocampal gyrus, however, indicates that there must be other factors involved in adult brain plasticity than the ones represented by BDNF levels in the peripheral blood.

Regarding neurobiological mechanisms of exercised-induced plasticity also concept of brain reserve (Satz et al., 2011) should be considered. The concept of brain reserve describe individual differences in an increased baseline adaptive neuroplasticity, which provide greater dynamic capacity for remodeling cortical circuits to different stressors (Barulli and Stern, 2013; Freret et al., 2015).

Perspectives

The results of our study suggest that a long-term dancing intervention could be superior to repetitive physical exercise in inducing neuroplasticity in the aging human brain. We presume that this advantage is related to the multimodal nature of dancing, which combines physical, cognitive and coordinative challenges. To our knowledge, this is the first longitudinal, randomized study to recommend dancing programs as a means of preventing gray matter and cognitive decline in the elderly. Further research is needed to clarify in greater detail the temporal dynamics and the underlying neurobiological mechanisms of dance-induced neuroplasticity and whether this intervention truly has the potential to reduce the risk of neurodegenerative diseases such as Alzheimer's.

AUTHOR CONTRIBUTIONS

PM designed and performed the research, analyzed the data, wrote the article. KR designed and performed the research. MS analyzed the data. AH and VL designed the research. MD: data collection, article revision. TB and JK designed the research, analyzed the data. NGM designed the research, wrote the article.

REFERENCES

Ashburner, J., and Friston, K. J. (2000). Voxel-based morphometry—the methods. *Neuroimage* 11, 805–821. doi: 10.1006/nimg.2000.0582

Bamadis, P. D., Vivas, A. B., Styliadis, C., Frantzidis, M., Klados, M., Schlee, W., et al. (2014). A review of physical and cognitive interventions in aging. *Neurosci. Biobehav. Rev.* 44, 206–220. doi: 10.1016/j.neubiorev.2014.03.019

Barulli, D., and Stern, Y. (2013). Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts in cognitice reserve. *Trends Cogn. Sci.* 17, 502–509. doi: 10.1016/j.tics.2013.08.012

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., and Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc. Natl. Acad. Sci. U S A* 87, 5568–5572. doi: 10.1073/pnas.87.14.5568

Bliss, T. V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate of the aneasthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232, 331–356. doi: 10.1113/jphysiol.1973. sp010273

Boyke, J., Driemeyer, J., Gaser, C., Büchel, C., and May, A. (2008). Training-induced brain structure changes in the elderly. *J. Neurosci.* 28, 7031–7035. doi: 10.1523/JNEUROSCI.0742-08.2008

Brown, S., Martinez, M., and Parsons, M. (2006). The neuronal basis of human dance. *Cereb. Cortex* 16, 1157–1167. doi: 10.1093/cercor/bhj057

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., et al. (2006). Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. A. Biol. Sci. Med. Sci.* 61, 1166–1170. doi: 10.1093/gerona/ 61.11.1166

Echávarri, C., Aalten, P., Uyling, H. B. M., Jacobs, H. I. L., Visser, P. J., Gronenschild, M., et al. (2011). Atrophy in the parahippocampal gyrus as an early biomarker of Alzheimer's disease. *Brain Struct. Funct.* 215, 265–271. doi: 10.1007/s00429-010-0283-8

Edelmann, E., Lessmann, V., and Brigadski, T. (2014). Pre- and postsynaptic twists in BDNF secretion and action in synaptic plasticity. *Neuropharmacology* 76, 610–627. doi: 10.1016/j.neuropharm.2013.05.043

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. U S A* 108, 3017–3022. doi: 10.1073/pnas.1015950108

Erickson, K. I., Weinstein, A. M., and Lopez, O. L. (2012). Physical activity, brain plasticity, and Alzheimer's disease. *Arch. Med. Res.* 43, 615–621. doi: 10.1016/j. arcmed.2012.09.008

Flöel, A., Ruscheweyh, R., Krüger, K., Willemer, C., Winter, B., Völker, K., et al. (2010). Physical activity and memory functions: are neurotrophins and cerebral gray matter volume the missing link? *Neuroimage* 49, 2756–2763. doi: 10.1016/j.neuroimage.2009.10.043

Freret, T., Gaudreau, P., Schumann-Bard, P., Billard, J. M., and Popa-Wagner, A. (2015). Mechanisms underlying the neuroprotective effect of brain reserve against late life depression. *J. Neural. Transm. (Vienna)* 122, S55–S61. doi: 10.1007/s00702-013-1154-2

Gregory, S. M., Parker, B., and Thompson, P. D. (2012). Physical activity, cognitive function, and brain health: what is the role of exercise training in the prevention of dementia? *Brain Sci.* 2, 684–708. doi: 10.3390/brainsci2040684

Hänggi, J., Koeneke, S., Bezzola, L., and Jäncke, L. (2010). Structural neuroplasticity in the sensorimotor network of professional female ballet dancers. *Hum. Brain Mapp.* 31, 1196–1206. doi: 10.1002/hbm.20928

Helmstaedter, C., and Durwen, H. F. (1990). The verbal learning and retention test. A useful and differentiated tool in evaluating verbal memory performance. *Schweiz. Arch. Neurol. Psychiatr. (1985)* 141, 21–30.

Huang, E. J., and Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* 24, 677–736. doi: 10.1146/annurev.neuro.24.1.677

Hüfner, K., Binetti, C., Hamilton, D. A., Stephan, T., Flanagin, V. L., Linn, J., et al. (2011). Structural and functional plasticity of the hippocampal formation in professional dancers and slackliners. *Hippocampus* 21, 855–865. doi: 10.1002/hipo.20801

Karvonen, M. J., Kentala, E., and Mustala, O. (1957). The effects of training on heart rate: a longitudinal study. *Ann. Med. Exp. Biol. Fenn.* 35, 307–315.

Kattenstroth, J. C., Kalisch, T., Holt, S., Tegethoff, M., and Dinse, H. R. (2013). Six months of dance intervention enhances postural, sensorimotor, and cognitive performance in elderly without affecting cardio-respiratory functions. *Front. Aging Neurosci.* 5:5. doi: 10.3389/fnagi.2013.00005

Kattenstroth, J. C., Kolankowska, I., Kalisch, T., and Dinse, H. R. (2010). Superior sensory, motor, and cognitive performance in elderly individuals with multi-year dancing activities. *Front. Aging Neurosci.* 2:31. doi: 10.3389/fnagi.2010.00031

Kempermann, G., Kuhn, H. G., and Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature.* 386, 493–495. doi: 10.1038/386493a0

Kirk-Sanchez, N. J., and McGough, E. L. (2014). Physical exercise and cognitive performance in the elderly: current perspectives. *Clin. Interv. Aging* 9, 51–62. doi: 10.2147/CIA.S39506

Lessmann, V., and Brigadski, T. (2009). Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neurosci. Res.* 65, 11–22. doi: 10.1016/j. neures.2009.06.004

Maass, A., Düzel, S., Goerke, M., Becke, A., Sobieray, U., Neumann, K., et al. (2015). Relationship between peripheral IGF-1, VEGF and BDNF levels and exercise-related changes in memory, hippocampal perfusion and volumes in older adults. *Neuroimage* 131, 142–154. doi: 10.1016/j.neuroimage.2015. 10.084

Mattson, M. P. (2008). Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann. N Y Acad. Sci.* 1144, 97–112. doi: 10.1196/annals.1418.005 Müller, P., Rehfeld, K., Lüders, A., Schmicker, M., Hökelmann, A., Kaufmann, J., et al. (2016). Effekte eine Tanz- und eines Gesundheitssporttrainings auf die graue Hirnsubstanz gesunder Senioren. *Sportwiss.* 46, 213–222. doi: 10.1007/s12662-016-0411-6

Niemann, C., Godde, B., and Voelcker-Rehage, C. (2014). Not only cardiovascular, but also coordinative exercise increases hippocampal volume in older adults. *Front. Aging Neurosci.* 6:170. doi: 10.3389/fnagi.2014.00170

Oswald, W. D., Gunzelmann, T., Rupprecht, R., and Hagen, B. (2006). Differential effects of single versus combined cognitive and physical training with older adults: the SimA study in a 5-year perspective. *Eur. J. Ageing* 3, 179–192. doi: 10.1007/s10433-006-0035-z

Pantel, J., Kratz, B., Essig, M., and Schröde, J. (2003). Parahippocampal volume deficits in subjects with aging-associated cognitive decline. *Am. J. Psychiatry* 160, 379–382. doi: 10.1176/appi.ajp.160.2.379

Park, H., and Poo, M. M. (2013). Neurotrophin regulation of neural circuit development and function. *Nat. Rev. Neurosci.* 14, 7–23. doi: 10.1038/ nrn3379

Rehfeld, K., Hökelmann, A., Kaufmann, J., and Müller, N. G. (2016). ''Effects of a dance vs. a fitness training on brain plasticity, balance performance and attention in healthy seniores: a new approach with SPM 12 for pairwise longitudinal group comparison,'' in *Proceeding-International Conference of Sports and Neuroscience* (Magdeburg), 127–138.

Satz, P., Cole, M. A., Hardy, D. J., and Rassovsky, Y. (2011). Brain and cognitive reserve: mediator(s) and construct validity, a critique. *J. Clin. Exp. Neuropsychol.* 33, 121–130. doi: 10.1080/13803395.2010.493151

Schega, L., Peter, B., Brigadski, T., Leßmann, V., Isermann, B., Hamacher, D., et al. (2016). Effect of intermittent normobaric hypoxia on aerobic capacity and cognitive function in older people. *J. Sci. Med. Sport* 19, 941–945. doi: 10.1016/j. jsams.2016.02.012

Taube, W. (2008). Neurophysiological adaptations in response to balance training. *Dtsch. Z. Sportmed.* 63, 273–277. doi: 10.5960/dzsm. 2012.030

Taubert, M., Draganski, B., Anwander, A., Müller, K., Horstmann, A., Villringer, A., et al. (2010). Dynamic properties of human brain structure: learning related changes in cortical areas and associated fiber connections. *J. Neurosci.* 30, 11670–11677. doi: 10.1523/JNEUROSCI.2567-10.2010

Thomas, A. G., Dennis, A., Bandettini, P. A., and Johansen-Berg, H. (2012). The effects of aerobic activity on brain structure. *Front. Psychol.* 3:86. doi: 10.3389/fpsyg.2012.00086

Tisserand, D. J., Pruessner, J. C., Sanz Arigita, E. J., van Boxtel, M. P., Evans, A. C., Jolles, J., et al. (2002). Regional frontal cortical volumes decrease differentially in aging: an MRI study to compare volumetric approaches and voxel-based morphometry. *Neuroimage* 17, 657–669. doi: 10.1016/s1053-8119(02)91173-0 van Praag, H., Shubert, T., Zhao, C., and Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *J. Neurosci.* 25, 8680–8685. doi: 10.1523/JNEUROSCI.1731-05.2005

Verghese, J., Lipton, R. B., Katz, M. J., Hall, C. B., Dervy, C. A., Kuslansky, G., et al. (2003). Leisure activities and the risk of dementia in the elderly. *N. Engl. J. Med.* 348, 2508–2516. doi: 10.1056/NEJMoa022252

Vital, T. M., Stein, A. M., de Melo Coelho, F. G., Arantes, F. J., Teodorov, E., Santos-Galduróz, R. F., et al. (2014). Physical exercise and vascular endothelial growth factor (VEGF) in elderly: a systematic review. *Arch. Gerontol. Geriatr.* 59, 234–239. doi: 10.1016/j.archger.2014.04.011

Voelcker-Rehage, C., and Niemann, C. (2013). Structural and functional brain changes related to different types of physical activity across the life span. *Neurosci. Biobehav. Rev.* 37, 2268–2295. doi: 10.1016/j.neubiorev.2013. 01.028

Zatorre, R. J., Fields, R. D., and Johansen-Berg, H. (2012). Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat. Neurosci.* 15, 528–536. doi: 10.1038/nn.3045

Zimmermann, P., and Fimm, B. (2002). *Testbatterie zur Aufmerksamkeitsprüfung (TAP), Version 1.7, Handbuch—Teil1.* Würselen: Psytest.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Human Neuroscience

ORIGINAL RESEARCH
published: 15 June 2017
doi: 10.3389/fnhum.2017.00305

Dancing or Fitness Sport? The Effects of Two Training Programs on Hippocampal Plasticity and Balance Abilities in Healthy Seniors

Kathrin Rehfeld[1,2]*†, Patrick Müller[1,3]†, Norman Aye[1,2], Marlen Schmicker[1], Milos Dordevic[1,2], Jörn Kaufmann[4], Anita Hökelmann[2] and Notger G. Müller[1,3,5]

[1] German Center for Neurodegenerative Diseases, Magdeburg, Germany, [2] Institute for Sport Science, Otto von Guericke University Magdeburg, Magdeburg, Germany, [3] Medical Faculty, Otto von Guericke University Magdeburg, Magdeburg, Germany, [4] Department of Neurology, Otto von Guericke University Magdeburg, Magdeburg, Germany, [5] Center for Behavioral Brain Sciences, Magdeburg, Germany

Age-related degenerations in brain structure are associated with balance disturbances and cognitive impairment. However, neuroplasticity is known to be preserved throughout lifespan and physical training studies with seniors could reveal volume increases in the hippocampus (HC), a region crucial for memory consolidation, learning and navigation in space, which were related to improvements in aerobic fitness. Moreover, a positive correlation between left HC volume and balance performance was observed. Dancing seems a promising intervention for both improving balance and brain structure in the elderly. It combines aerobic fitness, sensorimotor skills and cognitive demands while at the same time the risk of injuries is low. Hence, the present investigation compared the effects of an 18-month dancing intervention and traditional health fitness training on volumes of hippocampal subfields and balance abilities. Before and after intervention, balance was evaluated using the Sensory Organization Test and HC volumes were derived from magnetic resonance images (3T, MP-RAGE). Fourteen members of the dance (67.21 3.78 years, seven females), and 12 members of the fitness group (68.67 2.57 years, five females) completed the whole study. Both groups revealed hippocampal volume increases mainly in the left HC (CA1, CA2, subiculum). The dancers showed additional increases in the left dentate gyrus and the right subiculum. Moreover, only the dancers achieved a significant increase in the balance composite score. Hence, dancing constitutes a promising candidate in counteracting the age-related decline in physical and mental abilities.

Keywords: dancing, fitness training, balance, hippocampus, aging

OPEN ACCESS

Edited by:
Claudia Voelcker-Rehage, Technische Universität Chemnitz, Germany

Reviewed by:
Dieter J. Meyerhoff, University of California, San Francisco, United States Marco Taubert, Otto von Guericke University Magdeburg, Germany

***Correspondence:**
Kathrin Rehfeld
kathrin.rehfeld@ovgu.de

† These authors have contributed equally to this work.

Received: 08 November 2016
Accepted: 26 May 2017
Published: 15 June 2017

Citation:
Rehfeld K, Müller P, Aye N, Schmicker M, Dordevic M, Kaufmann J, Hökelmann A and Müller NG (2017) Dancing or Fitness Sport? The Effects of Two Training Programs on Hippocampal Plasticity and Balance Abilities in Healthy Seniors. Front. Hum. Neurosci. 11:305. doi: 10.3389/fnhum.2017.00305

INTRODUCTION

The human hippocampus (HC) is affected not only by pathological aging such as in Alzheimer's disease but also by the normal aging process resulting in deficits in memory, learning, and spatial navigation at old age (Driscoll et al., 2003; Barnes et al., 2009). Magnetic resonance-studies indicate an atrophy rate of the hippocampus and the nearby parahippocampal gyrus of 2–3% per decade (Raz et al., 2004, 2005), which is further accelerated in the very old age where there is an annual loss of 1% over the age of 70 (Jack et al., 1998). On the other hand more recent research has shown that the HC counts among the few brain regions with the ability to generate new neurons throughout the lifespan (Kempermann et al., 2010; Spalding et al., 2013). In animal models physical activity has been identified as a key mechanism that can drive this adult neuroplasticity (van Praag et al., 1999; Kronenberg, 2003). In humans, research has focused on the effects of aerobic fitness and training on volumes and perfusion of the HC. Results reveal that higher cardiorespiratory fitness levels (VO_2 max) are associated with larger hippocampal volumes in late adulthood, and that larger hippocampal volumes may, in turn, contribute to better memory function (Erickson et al., 2011; Szabo et al., 2011; Bugg et al., 2012; Maass et al., 2015). Furthermore, some investigations also assessed possible physiological mediators of the observed neuroplasticity, such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) (Flöel et al., 2010; Erickson et al., 2011; Ruscheweyh et al., 2011; Maass et al., 2016). Whereas Erickson et al. (2011) reported a positive correlation between levels of serum BDNF, hippocampal volume and cardiorespiratory fitness during 1 year of aerobic training, neither Ruscheweyh et al. (2011) nor Maass et al. (2016) found fitness-related BDNF changes after 6 or 3 months of training, respectively. Moreover, other studies failed to find correlations between volumes of the medial temporal lobe area or the hippocampus and cardiovascular fitness in healthy elderly (Honea et al., 2009; Smith et al., 2011). Therefore, the role of cardio-respiratory fitness in modulating hippocampal gray matter volume is still under debate.

The hippocampus is also involved in spatial navigation (O'Keefe, 1990) and in motor sequence consolidation (Albouy et al., 2008) suggesting that motor skill learning and motor fitness can have impact on hippocampal volume without any cardio-respiratory change. In this respect, Niemann et al. (2014) tested whether 12 months of cardiovascular or coordination training induces larger increases in hippocampal volume in healthy older participants. After training, the cardio-vascular group revealed a significant volume increase in the left HC of 4.22% and a non-significant increase of 2.98% for the right HC. Effects of the coordinative training were more pronounced in the right HC with an increase of 3.91%, whereas the changes in the left HC (1.78%) were non-significant. Further correlation analyses between motor fitness and hippocampal volume failed to reach significant results. Still there is compelling evidence that the human brain undergoes morphological alterations in response to motor-skill learning (Draganski et al., 2004; Boyke et al., 2008; Taubert et al., 2010; Sehm et al., 2014). Along these lines, a recent study demonstrated structural brain changes already after two sessions of dynamic balance training that correlated with the individual motor skill learning success of the participants (Taubert et al., 2010). Sehm et al. (2014) could demonstrate that 6 weeks of balance training induced increases in the gray matter of the left HC in healthy seniors. These findings highlight the behavioral relevance of structural brain plasticity in the HC for the learning process. Hüfner et al. (2011) stated that long-term balance training with its extensive vestibular, visual and sensorimotor stimulation is associated with altered hippocampal formation volumes in professional ballet dancers and slackliners. Hence, the HC seems not only crucial for long-term memory consolidation, learning and spatial navigation, but also for balancing. Intact balance is essential for social mobility and quality of life in aging (Dordevic et al., 2017). Hence, physical intervention programs should take this function into account, too.

In this respect dancing seems to be a promising intervention since it requires the integration of sensory information from multiple channels (auditory, vestibular, visual, somatosensory) and the fine-grained motor control of the whole body. Behavioral studies have already provided evidence of better performance in balance and memory tasks in elderly dancers (Kattenstroth et al., 2010, 2013; Rehfeld et al., 2014), but the underlying neural mechanisms have not been addressed comprehensively so far. Knowing that aerobic, sensorimotor and cognitive training contribute to hippocampal volume, which also seems to be associated with balancing capabilities, we initialized a prospective, randomized longitudinal trial over a period of 18 months in healthy seniors. Two interventions were compared: a specially designed dance program, during which subjects constantly had to learn new choreographies, and a traditional fitness program with mainly repetitive exercises, such as cycling on an ergometer or Nordic walking. Whole-brain analyzes of the acquired data using voxel based morphometry had shown dance-associated volume increases mainly in the precentral and the parahippocampal gyrus (Müller et al., 2016). Knowing that dancing/slacklining (Hüfner et al., 2011) and endurance sport (e.g., Erickson et al., 2011) have different impact on anterior and posterior parts of the hippocampus in the present analysis we ran a region of interest analysis of this specific brain region. To do so we first computed a restricted VBM analysis with a hippocampal mask. In the next step we divided the hippocampus in five subfields in order to allow a detailed analysis of the interventions' effects on different parts of the HC. The hippocampus is not a homogeneous structure but consists of histologically specialized subfields, such as the subiculum, cornu ammonis (CA) 1–4 and dentate gyrus (DG). The subiculum has been implicated in working memory and spatial relations (Riegert et al., 2004; O'Mara, 2005). CA3 and DG have been suggested to be involved in memory and early retrieval, whereas CA1 in late retrieval, consolidation and recognition. Especially the DG is one of the few regions of the adult brain where neurogenesis takes places, which is important in the formation of new memories and spatial memory (Saab et al., 2009). Nevertheless all these subfields are tightly interconnected (Duvernoy, 2005). Since dancing seems to promote spatial orientation, working memory and might promote neurogenesis, we expected volume changes in more subfields of the HC after this intervention. Moreover, given the importance of intact balance for successful aging on the one hand and its dependence on the hippocampus on the other hand, we also assessed effects of the interventions on balancing capabilities and their relation to hippocampal subfield volumes.

MATERIALS AND METHODS

Study Design and Subjects

This investigation, comprising hippocampal volume alterations and changes in balance abilities, is part of a large prospective longitudinal study which compares the effects of dancing versus aerobic training on brain structure and function, mediating neuroplasticity factors, such as BDNF, as well as cognitive and motor performances in healthy elderly seniors. The cognitive development and BDNF changes are highlighted in our recent report (see Müller et al., 2017). The intervention was provided for 18 months and contained three time-points of measurement: baseline pre-test, first post-test after 6 months of training and second post-test after 18 months of training (see **Figure 1**). Again, the temporal dynamics of gray matter brain plasticity are already stated by Müller et al. (2017), showing a significant increase of gray matter volume in parahippocampal gyrus only for the dancers. Based on that finding, we assume only changes from baseline to the second post-test (18 months).

The approval for the study was obtained from the ethics committee of the Otto von Guericke University, Magdeburg. All subjects signed a written informed consent and received a reimbursement for their participation.

The timeline of the study can be depicted from **Figure 1**. Primarily, we invited 62 healthy elderly volunteers aged 63–80 years for cognitive and physical screening as well as for verification of magnetic resonance imaging suitability. Exclusion criteria were defined as follows: any history of severe neurological conditions, metal implants, claustrophobia, tinnitus, intensive physical engagement (more than 1 h/week), cognitive impairments as evidenced in the MMSE (Folstein et al., 1975) and depressive symptoms (BDI-II > 13) (Beck et al., 2006). Fifty two seniors met the inclusion criteria and were then randomly assigned to the experimental dance group and the control sport group. After 18 months of training we were left with 26 complete data sets, including 14 dancers and 12 sportsmen. Both groups (mean age D 67.9 3.3 years) did not differ concerning age, sex, education, and BMI. For detailed information about demographic data see **Table 1**.

Interventions

The precise description of the interventions is published elsewhere (Müller et al., 2016). In brief, the first period of training was provided for 6 months, twice a week for both groups. Each dancing or fitness class lasted 90 min. Because of organizational reasons we had to change the training frequency from twice a week to once a week after 6 months of training. The second training period was run for 12 months and the training sessions were reduced to once a week for 90 min in both groups. The content of the dance classes induced a permanent learning situation with constantly changing choreographies, which participants had to memorize accurately. The training focused on elementary longitudinal turns, head-spins, shifts of center of gravity (COG), single-leg stances, skips and

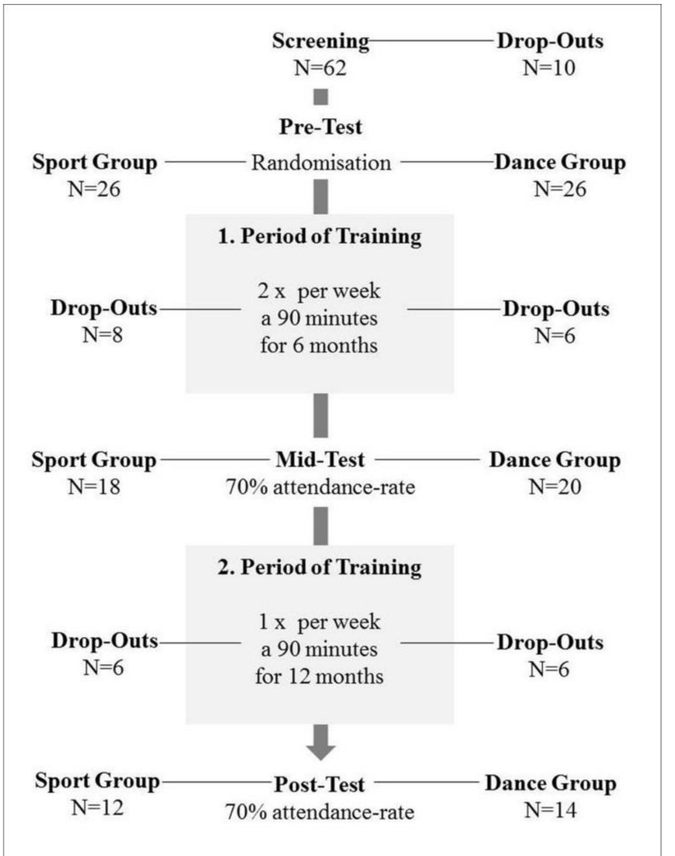


FIGURE 1 | Study design presenting: voluntary recruitment, drop-outs, time-points of measurement, duration of training periods.

hops, different steps like chassée, mambo, cha cha, grapevine, jazz square to challenge the balance system. Additional arm-patterns enforced imbalances (moving arms away from center of pressure).

The program for the sport group was adjusted according to the recommended guidelines for health sport (Brehm et al., 2006) and included endurance training, strength-endurance training, and flexibility training (stretching and mobility). Each part of the mentioned topics (endurance; strength-endurance; and flexibility) was exercised for 20 min, whereby a 10 min warm-up, a 10 min cool-down and short breaks between the different exercises adding to another 10 min completed each 90 min lasting session. So both groups exercised for 90 min in each training session. In the first 6 months, endurance training was performed on bicycle ergometers with the intensity adjusted to the individual training heart rate (HR) using the Karvonen Formula:

Target training HR D

Resting HR C .0:6Tmaximum HR resting HRU/:

The factor 0.6 is a representative for an extensive aerobic training (Davis and Convertino, 1975). In the second training period (12 months) the participants completed a Nordic Walking program. The strength-endurance training aimed to strengthen major muscles of the muscular skeleton. In this program we

TABLE 1 | Demographic information at baseline of analyzed participants (N D 26).

	Dance group [*N* D 14]		Sport group [*N* D 12]			
	M	*SD*	*M*	*SD*	*t*-value	*p*-value
Age [years]	67.21	3.78	68.67	2.57	1:12	0.272
Sex [%]	50% male		58% male		0:154	0.695
MMSE [points]	28.07	0.92	29.17	0.58	3:011	0.003
BDI-II [points]	4.5/5.54	3.37/2.97	3.75/3.39	3.79/3.77	0:53	0.598
Education [years]	15.40	2.05	16.10	1.45	1:395	0.175
BMI [kg/m^2]	27.51	3.87	27.24	2.94	0:275	0.786

BMI, Body-Mass-Index; BDI-II, Becks-Depressions-Inventar II; MMSE, Mini Mental State Examination; M, Mean; SD, Standard deviation; p 0.05 D statistical significance.

avoided combined arm and leg movements in order to keep coordinative demands low.

Structural MRI Acquisition, Preprocessing, and Analysis

Magnetic resonance (MR) images were acquired on a 3 Tesla Siemens MAGNETOM Verio (Syngo MR B17) using a 32-channel head coil. T-1 weighted MPRAGE sequence (224 sagital slices, voxel size: 0.8 mm 0.8 mm 0.8 mm, TR: 2500 ms, TE: 3.47 ms, TI: 1100 ms, flip angle: 7) were analyzed using region of interest (ROI) defined voxel-based morphometry with SPM 12 (Welcome Department of Cognitive Neurology, London, United Kingdom) running under Matlab (The Math Works). The data preprocessing involved gray matter segmentation, DARTEL based template creation, spatial normalization to MNI-Space and an 5 mm smoothing with a Gaussian kernel as previously described.

Voxel-Based Morphometry with Hippocampal Mask

In order to incorporate our *a priori* hypotheses concerning hippocampal gray matter volume changes we first conducted a ROI-VBM with hippocampal masks. The longitudinal analysis for hippocampal gray matter volume changes was performed using repeated measurement ANOVAs in a full factorial design. We applied a threshold of $p < 0.05$ (FDR corrected).

Hippocampal Subfield Volume Measurements

In a second step we analyzed volume changes in five subfields of the HC. Up to now there is no real gold standard in analyzing HC subfield volumes and each of the current manifold analytic techniques has its strengths and weaknesses (Bandettini, 2009; Kuhnt et al., 2013). Here for the hippocampal subfield segmentation in order to obtain ROI volumes we chose the SPM ANATOMY Toolbox v.2.2.c (Eickhoff et al., 2007) with normalized images. This segmentation included the cornu ammonis (CA1–CA3), the dentate gyrus (DG, including CA4) and the subiculum (**Figure 2**). In SPM Anatomy toolbox, definition of anatomical regions is based on maximum probability cytoarchitectonic maps.

Postural Control

Postural control was assessed with the Sensory Organization Test (SOT) implemented in the Balance Master System (Neurocom International, Inc., United States). This test provides information about the contribution of the visual, somatosensory, and vestibular system to the maintenance of balance. The system consists of a dual force platform including force transducers measuring the angular displacement of the COG under certain conditions and visual surround. Both, visual surround and platform enable anterior/posterior sway and this sway can be assessed under different conditions. The six conditions are: normal vision and fixed support (condition 1), absent vision and fixed support (condition 2), sway-referenced vision and fixed support (condition 3), normal vision and sway-referenced support (condition 4), absent vision and sway referenced support (condition 5), and sway-referenced vision and sway-referenced support (condition 6). These conditions were performed in three trials for 20 s, resulting in equilibrium scores. Those equilibrium scores range from 0% (balance loss) to 100% (perfect stability). From the equilibrium scores a sensory analysis was performed by calculating average scores of specific pairs of SOT conditions: the participant's ability to use input from the somatosensory system to maintain balance is reflected by the average of condition 2 divided by the average of condition 1, the contribution of the visual system by the average of condition 4 divided by the average of condition 1 and that of the vestibular system by the average of condition 5 divided by the average of condition 1.

The composite score was calculated by averaging the score for conditions 1 and 2; adding these two scores to the equilibrium scores from each trial of sensory conditions 3, 4, 5, and 6; and dividing that sum by the total number of trials (NeuroCom Natus Medical Incorporated, 2008).

Statistical Analysis

Statistical analysis of hippocampal volumes and balance data were performed with SPSS (SPSS 22, inc./IBM). Intervention effects were tested using repeated-measurement ANOVAs with group (dance, sport) as between-subject factor and time (pre, post) as within-subject factor. Hereby, age, gender, and total hippocampal volume were included as covariates. Additionally, hypothesis driven *t*-tests (with Bonferroni

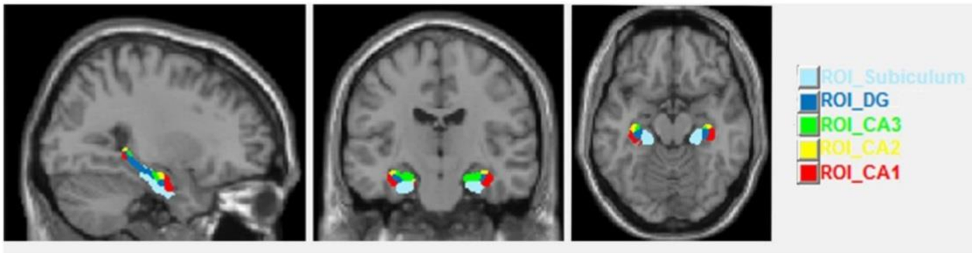


FIGURE 2 | Sagittal, coronal and axial views of the hippocampal subfields.

adjustment) were performed to determine longitudinal changes in the dance and the sports group separately. In case of missing normal distribution we used the Mann–Whitney-*U*-test or Wilcoxon instead of *t*-tests. Pearson-Correlation analysis was performed between percentage change of hippocampal subfield volumes and the balance composite score.

RESULTS

The presentation of the results is structured as follows. We first tested for hippocampal volume differences after intervention using both masked VBM and subfield volume measurements. In the next step we investigated balance data and finally we looked for correlations between improvements in balance and hippocampal volume.

Voxel-Based Morphometry with Hippocampal Mask

A two-sample *t*-test revealed no group differences at baseline. To explore hippocampal gray matter volume changes during intervention we used repeated measurement ANOVA for comparison between baseline and post-test. There was a significant interaction effect in the right hippocampus [MNI-coordinates: *x* D 28, *y* D 16, *z* D 23; *p*(FDR) D 0.049, *F* D 17.03]. *Post hoc* paired *t*-tests showed only in the dance group significant volume increases in the right hippocampus [MNI-coordinates: *x* D 29, *y* D 16, *z* D 27; *p*(FDR) D 0.001, *t* D 6.10] (**Figure 3**).

Hippocampal Subfield Volume Measurements

A two-sample *t*-test revealed no group differences of total hippocampal volumes at baseline [*t*(25) D 1.078, *p* D 0.658, *d* D 0,424]. The repeated measurement ANOVA of hippocampal subfield volumes showed a main effect of time regarding left CA1, left CA2, left and right subiculum and left CA4/dentate gyrus (**Table 2**). There were no significant interactions with group. Paired *t*-tests showed significant volume increases for the dancers in left CA1, left CA2, left CA4/dentate gyrus and left and right subiculum and for the sportsmen in the left CA1, left CA2, and left subiculum (**Figure 4**).

Postural Control

Repeated measurement ANOVAs of balance data showed an interaction effect with group for the composite equilibrium score (see **Table 3** and **Figure 5**).

There was a main effect of time regarding the somatosensory and vestibular contribution but no significant time group interaction effects after 18 months of training (see **Table 3**). *Post hoc* tests revealed that the dancers improved in the use of all three sensory systems somatosensory system [*t*(13) D 2.902, *p* D 0.004], visual system [*t*(12) D 2.525, *p* D 0.027] vestibular system [*t*(12) D 3.271, *p* D 0.007] to maintain balance. Members of the sports group improved in the use of the somatosensory system [*t*(9) D 3.579, *p* D 0.006] and the vestibular system [*t*(9) D 3.881, *p* D 0.004] but not in the visual system. **Table 3** presents an overview of significant alterations related to employment of sensory information to maintain balance from baseline to post-intervention for both groups.

Correlation Analysis

Correlation analysis between all hippocampal subfields and balance did not yield any significant results irrespective of whether the groups were analyzed separately or jointly.

DISCUSSION

Animal research has shown that combining aerobic training with sensory enrichment has a superior effect on inducing neuroplasticity in the HC compared to physical exercise or sensory stimulation alone (Kempermann et al., 2010). This sparked our idea to investigate the impact on neuroplasticity in elderly humans of a specially designed, sensorimotor and cognitive challenging dance program in comparison to a classical cardiovascular fitness program. In addition to our previous work (Müller et al., 2017), in the present study we ran a dedicated ROI analysis, which was focused on subfield volumes of the HC. The HC is of special interest as this brain structure is (a) especially affected by normal and pathological aging and (b) plays a key role in major cognitive processes, e.g., memory and learning and (c) is also involved in keeping one's balance, a function which is crucial for well-being and quality of life.

We observed that both, dancing and fitness training led to increases in hippocampal subfield volumes. Although there was no significant group time interaction in the ANOVA omnibus

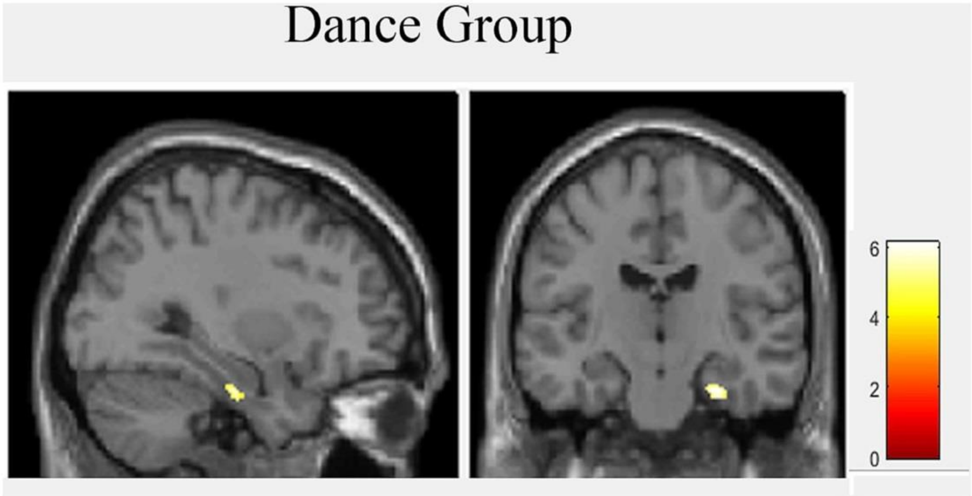


FIGURE 3 | Hippocampal volume increases after 18 month of intervention in dance group.

TABLE 2 | Statistical values of repeated-measures ANOVAs for hippocampal subfields.

Hippocampal subfield	Main effect of time				Main effect of group				Interaction (time group)			
	F	**df**	***p***	$!^{2}$	***F***	**df**	***p***	$!^{2}$	***F***	**df**	***p***	$!^{2}$
Left CA1	16.920	1.25	**0.001**	0.413	0.000	1.25	0.985	0.000	0.469	1.25	0.500	0.019
Right CA1	2.952	1.25	0.099	0.110	0.116	1.25	0.736	0.005	2.189	1.25	0.152	0.084
Left CA2	21.126	1.25	**0.001**	0.468	0.427	1.25	0.520	0.017	0.941	1.25	0.342	0.038
Right CA2	2.020	1.25	0.162	0.078	0.212	1.25	0.650	0.009	1.431	1.25	0.243	0.056
Left CA3	4.237	1.25	0.51	0.150	0.347	1.25	0.561	0.014	0.986	1.25	0.331	0.039
Right CA3	2.680	1.25	0.115	0.100	0.011	1.25	0.916	0.000	1.376	1.25	0.252	0.054
Left subiculum	49.926	1.25	**0.001**	0.675	0.959	1.25	0.337	0.038	0.094	1.25	0.762	0.004
Right subiculum	12.976	1.25	**0.001**	0.351	2.025	1.25	0.168	0.079	0.001	1.25	0.976	0.000
Left CA4/DG	9.480	1.25	**0.005**	0.283	0.541	1.25	0.469	0.022	0.001	1.25	0.961	0.000
Right CA4/DG	0.002	1.25	0.963	0.000	0.595	1.25	0.448	0.024	2.32	1.25	0.141	0.088

CA, cornu ammonis; DG, dentate gyrus; p 0.05 D statistical significance. Level of significance: 0.01 a < 0.05: "significant"; 0.001 a < 0.01: "high significant"; a < 0.001: "highly significant".

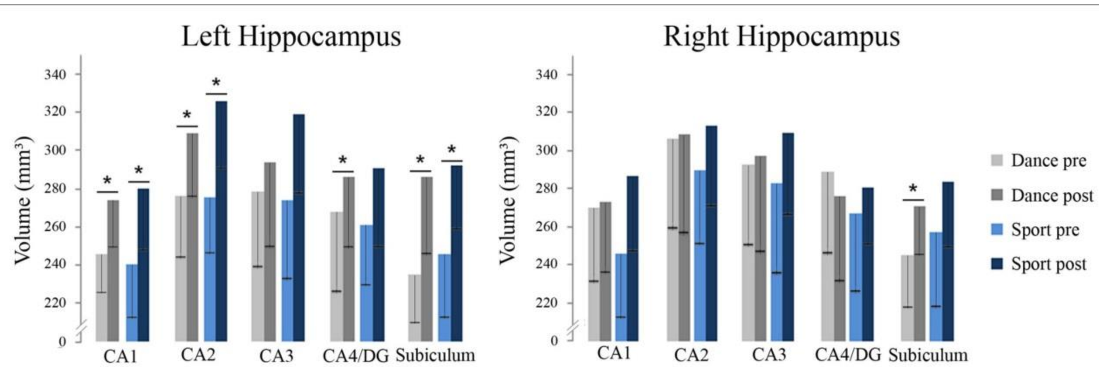


FIGURE 4 | Volumes of hippocampal subfields in dance and sport group at baseline and after 18 months of intervention including standard deviation (DG, dentate gyrus; CA, cornu ammonis; p < 0.05).

TABLE 3 | Statistical values of repeated-measures ANOVAs for sensory organization of balance.

Sensory organization of balance [%]	Time				Group				Interaction (time group)			
	F	**df**	***p***	!2	***F***	**df**	***p***	!2	***F***	**df**	***p***	!2
Somatosensory system	30.340	1,25	**0.001**	0.591	1.208	1,25	0.284	0.054	0.692	1,25	0.415	0.032
Visual system	6.094	1,25	0.022	0.225	0.363	1,25	0.553	0.017	0.296	1,25	0.592	0.014
Vestibular system	17.722	1,25	**0.001**	0.458	0.229	1,25	0.637	0.011	1.326	1,25	0.262	0.059
Composite equilibrium score	0.514	1,25	0.481	0.024	0.092	1,25	0.764	0.004	4.851	1,25	0.039	0.188

Level of significance: 0.01 a < 0.05: "significant"; 0.001 a < 0.01: "high significant"; a < 0.001: "highly significant".

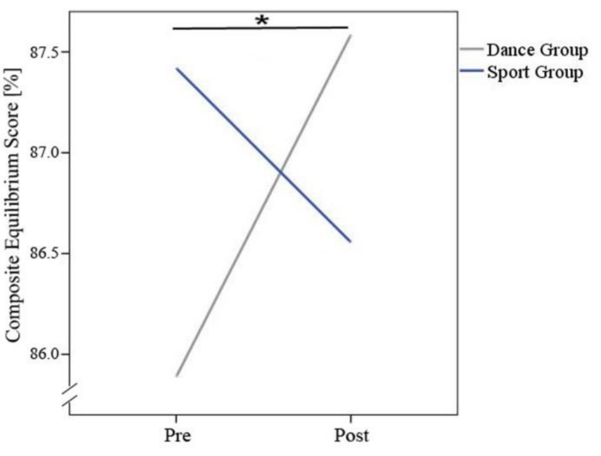


FIGURE 5 | Interaction-effect (time group) for composite equilibrium score. Means significant interaction-effect.

analysis, exploratory *post hoc t*-tests indicated that participants of the dance group showed volume increases in more subfields (four out of five, including the DG) of the left HC and that only dancing led to an increase in one subfield of the right HC, namely the subiculum. Regarding balance abilities dancing was superior to standard fitness as expressed by a larger increase in the composite score of our balance test and improved use of all three sensory systems. We, however, did not observe a correlation between changes in HC subfield volumes changes and those in balance; in other words whether the observed skill improvement can be attributed to the HC cannot be fully answered yet.

Regarding the HC volume increases observed in both groups, our results support the assumption that HC volume can be enhanced by physical fitness alone, as this was the overlapping feature of both trainings. Animal studies have shown that adult neurogenesis takes place mainly in the DG part of the HC (van Praag et al., 1999). Interestingly, only the dancers showed an increase in this brain region. Whether adult neurogenesis was indeed the basis of the here observed volume change, however, must remain an open question as there is no direct way in addressing this process in humans.

The dancers showed increases in some HC subfields where there was no change to be observed in the sports group. This indicates that apart from physical fitness, other factors inherent in dancing, contribute to HC volume changes, too. Animal research has suggested that sensory enrichment may be such a factor whereby physical fitness and enrichment have different effects on HC neurons: running in a wheel generates new neurons in the HC of mice but these only survive when sensory stimulation is also present (Kempermann et al., 2010). Again, with our own data we cannot differentiate between these different processes. We nevertheless can conclude that the additional challenges involved in our dance program, namely cognitive and sensorimotor stimulation, induced extra HC volume changes in addition to those attributable to physical fitness alone. It is noteworthy, that other studies in elderly humans, which did not boost physical fitness but which were sensorimotor demanding, such as learning to juggle (Boyke et al., 2008), have observed HC volume increases as well.

Only the dancers showed an increased balance composite score and they improved in all three involved sensory systems. This indicates that dancing drives all three senses and presumably also improves the integration of sensorimotor, visual and vestibular information. Balancing is an important everyday function, crucial for example for social mobility. Impaired balance often results in falls, which constitutes a major health risk factor with consequences both on morbidity (and even mortality) and health care costs (see also Dordevic et al., 2017). Although the ability to balance has been also linked to the HC and its connections, for example, to the vestibular system (Brandt et al., 2005), we did not observe a correlation between HC subfield volumes and improvements in balance. Given the small size of our sample, this needs to be interpreted with care but may suggest that other brain regions, probably those described in our earlier analysis (Müller et al., 2017) were involved in these improvements or that changes in the HC other than those expressed in measurable volumes, e.g., synaptic function, perfusion, etc. contributed to this effect.

There are other limitations in the present study which should not be left unmentioned. As already mentioned above (see Materials and Methods), we had to change the training intervention frequency from twice a week to once a week after 6 months of training. Hence, it must remain unclear whether more pronounced effects could have been observed if we had been able to stick to the initial training intensity. Next the ANOVAs failed to reach significant group interaction effects, only the exploratory *t*-tests became significant which may be a consequence of the large number of factors and levels in the ANOVAs on the one hand, and the small sample size on the other hand. A further limitation can be seen in the use of fully automated segmentation tools. Finally, the small sample size accompanied by a high drop-out rate as well as the highly selective inclusion, a missing inactive control group and exclusion criteria must be mentioned as they limit the generalizability of our results.

In sum, the present results indicate that both dance and fitness training can induce hippocampal plasticity in the elderly, but only dance training improved balance capabilities. However, larger studies with more representative samples are required in the future. They should include additional analysis of mediating factors and they should try to find ways to optimally adjust the training protocol to an individual's needs and preferences. Most of all, it needs to be investigated in longitudinal randomized clinical trials whether the proposed interventions indeed have the potential to reduce or postpone the risk of neurodegenerative diseases such as Alzheimer's as suggested in large non-interventional studies (Verghese et al., 2003).

AUTHOR CONTRIBUTIONS

KR was responsible for the study organization and execution, as well as writing the text of the manuscript (Introduction, Discussion, and some parts of the Materials and Methods: Study Design and Subjects). PM contributes equally to this work. He has written some parts of the manuscript (Materials and Methods, Results, Discussion). NA assessed balance abilities and analyzed the data. He has written some parts of the Materials and Methods and Results (postural control). MS contributes to the Statistical Analysis and did some corrections of this manuscript. MD supported hippocampal subfield analysis and corrected this manuscript. JK contributes to the MRI measurements and for structural brain analysis. He corrected this manuscript. AH is the chief coordinator of this study and selected the motor skill tasks and organized the framework. NM is the second chief coordinator of this study and provided MRI measurements. He also worked on the Introduction and Discussion of this manuscript.

REFERENCES

Albouy, G., Sterpenich, V., Balteau, E., Vandewalle, G., Desseilles, M., Dang-Vu, T., et al. (2008). Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron* 58, 261–272. doi: 10.1016/j.neuron.2008. 02.008

Bandettini, P. A. (2009). What's new in neuroimaging methods? *Ann. N. Y. Acad. Sci.* 1156, 260–293. doi: 10.1111/j.1749-6632.2009.04420.x

Barnes, J., Bartlett, J. W., van de Pol, L. A., Loy, C. T., Scahill, R. I., Frost, C., et al. (2009). A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol. Aging* 30, 1711–1723. doi: 10.1016/j.neurobiolaging.2008.01.010

Beck, A. T., Brown, G. K., and Steer, R. A. (2006). *Beck-Depressions-Inventar. BDI-II, Manual.* 2. Auflage. Frankfurt: Harcourt Test Services.

Boyke, J., Driemeyer, J., Gaser, C., Büchel, C., and May, A. (2008). Training-induced brain structure changes in the elderly. *J. Neurosci.* 28, 7031–7035. doi: 10.1523/JNEUROSCI.0742-08.2008

Brandt, T., Schautzer, F., Hamilton, D. A., Brüning, R., Markowitsch, H. J., Kalla, R., et al. (2005). Vestibular loss causes hippocampal atrophy and impaired spatial memory in humans. *Brain* 128, 2732–2741. doi: 10.1093/brain/ awh617

Brehm, W., Janke, A., Sygusch, R., and Wagner, P. (2006). *Gesund durch Gesundheitssport*. München: Juventa, 16–23.

Bugg, J. M., Shah, K., Villareal, D. T., and Head, D. (2012). Cognitive and neural correlates of aerobic fitness in obese older adults. *Exp. Aging Res.* 38, 131–145. doi: 10.1080/0361073X.2012.659995

Davis, J. A., and Convertino, V. A. (1975). A comparison of heart rate methods for predicting endurance training intensity. *Med. Sci. Sports* 7, 295–298. doi: 10.1249/00005768-197500740-00010

Dordevic, M., Hökelmann, A., Müller, P., Rehfeld, K., and Müller, N. G. (2017). Improvements in orientation and balancing abilities in response to one month of intensive slackline training. a randomized controlled feasibility study. *Front. Hum. Neurosci.* 11:55. doi: 10.3389/fnhum.2017.00055

Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U., and May, A. (2004). Neuroplasticity: changes in grey matter induced by training. *Nature* 427, 311–312. doi: 10.1038/427311a

Driscoll, I., Hamilton, D. A., Petropoulos, H., Yeo, R. A., Brooks, W. M., Baumgartner, R. N., et al. (2003). The aging hippocampus: cognitive, biochemical and structural findings. *Cereb. Cortex* 13, 1344–1351. doi: 10.1093/ cercor/bhg081

Duvernoy, H. M. (2005). *The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI*. Berlin: Springer Science and Business Media.

Eickhoff, S. B., Paus, T., Caspers, S., Grosbras, M. H., Evans, A. C., Zilles, K., et al. (2007). Assignment of functional activations to probabilistic cytoarchitectonic areas revisited. *Neuroimage* 36, 511–521. doi: 10.1016/j.neuroimage.2007. 03.060

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3017–3022. doi: 10.1073/pnas.1015950108

Flöel, A., Ruscheweyh, R., Krüger, K., Willemer, C., Winter, B., Völker, K., et al. (2010). Physical activity and memory functions: are neurotrophins and cerebral gray matter volume the missing link? *Neuroimage* 49, 2756–2763. doi: 10.1016/ j.neuroimage.2009.10.043

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental" state. A practical method for grading the state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Honea, R., Thomas, G. P., Harsha, A., Anderson, H. S., Donnelly, J. E., Brooks, W. M., et al. (2009). Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer's disease. *Alzheimer Dis. Assoc. Disord.* 23, 188–197. doi: 10.1097/WAD.0b013e31819cb8a2

Hüfner, K., Binetti, C., Hamilton, D. A., Stephan, T., Flanagin, V. L., Linn, J., et al. (2011). Structural and functional plasticity of the hippocampal formation in professional dancers and slackliners. *Hippocampus* 21, 855–865. doi: 10.1002/ hipo.20801

Jack, C. R., Petersen, R. C., Xu, Y., O'Brien, P. C., Smith, G. E., Ivnik, R. J., et al. (1998). Rate of medial temporal lobe atrophy in typical aging and Alzheimer's disease. *Neurology* 51, 993–999. doi: 10.1212/WNL.51.4.993

Kattenstroth, J. C., Kalisch, T., Holt, S., Tegenthoff, M., and Dinse, H. R. (2013). Six months of dance intervention enhances postural, sensorimotor, and cognitive performance in elderly without affecting cardio-respiratory functions. *Front. Aging Neurosci.* 5:5. doi: 10.3389/fnagi.2013.00005

Kattenstroth, J. C., Kolankowska, I., Kalisch, T., and Dinse, H. R. (2010). Superior sensory, motor, and cognitive performance in elderly individuals with multi-year dancing activities. *Front. Aging Neurosci.* 2:31. doi: 10.3389/fnagi.2010. 00031

Kempermann, G., Fabel, K., Ehninger, D., Babu, H., Leal-Galicia, P., Garthe, A., et al. (2010). Why and how physical activity promotes experience-induced brain plasticity. *Front. Neurosci.* 4:189. doi: 10.3389/fnins.2010.00189

Kronenberg, H. M. (2003). Developmental regulation of the growth plate. *Nature* 423, 332–336. doi: 10.1038/nature01657

Kuhnt, D., Bauer, M. H., Egger, J., Richter, M., Kapur, T., Sommer, J., et al. (2013). Fiber tractography based on diffusion tensor imaging compared with high-angular-resolution diffusion imaging with compressed sensing: initial experience. *Neurosurgery* 72 165–175. doi: 10.1227/NEU.0b013e318270d9fb

Maass, A., Düzel, S., Brigadski, T., Goerke, M., Becke, A., Sobieray, U., et al. (2016). Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. *Neuroimage* 131, 142–154. doi: 10.1016/j.neuroimage.2015.10.084

Maass, A., Düzel, S., Goerke, M., Becke, A., Sobieray, U., Neumann, K., et al. (2015). Vascular hippocampal plasticity after aerobic exercise in older adults. *Mol. Psychiatry* 20, 585–593. doi: 10.1038/mp.2014.114

Müller, P., Rehfeld, K., Lüders, A., Schmicker, M., Hökelmann, A., Kaufman, J., et al. (2016). Effekte eines Tanz-und eines Gesundheitssporttrainings auf die graue Hirn-substanz gesunder Senioren. *Sportwissenschaft* 46, 213–222. doi: 10.1007/s12662-016-0411-6

Müller, P., Rehfeld, K., Schmicker, M., Hökelmann, A., Dordevic, M., Lessmann, V., et al. (2017). Evolution of neuroplasticity in response to physical activity in old age: the case for dancing. *Front. Aging Neurosci.* 9:56. doi: 10.3389/fnagi.2017. 00056

NeuroCom Natus Medical Incorporated (2008). *Balance Manager Systems, Clinical Interpretation Guide*. Clackamas, OR: NeuroCom International, Inc.

Niemann, C., Godde, B., and Voelcker-Rehage, C. (2014). Not only cardiovascular, but also coordinative exercise increases hippocampal volume in older adults. *Front. Aging Neurosci.* 6:120. doi: 10.3389/fnagi.2014.00170

O'Keefe, J. (1990). A computational theory of the hippocampal cognitive map. *Prog. Brain Res.* 83, 301–312. doi: 10.1016/S0079-6123(08)61258-3

O'Mara, S. (2005). The subiculum: what it does, what it might do, and what neuroanatomy has yet to tell us. *J. Anat.* 207, 271–282. doi: 10.1111/j.1469-7580. 2005.00446.x

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., et al. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb. Cortex* 15, 1676–1689. doi: 10.1093/cercor/bhi044

Raz, N., Rodrigue, K. M., Head, D., Kennedy, K. M., and Acker, J. D. (2004). Differential aging of the medial temporal lobe a study of a five-year change. *Neurology* 62, 433–438. doi: 10.1212/01.WNL.0000106466.09835.46

Rehfeld, K., Hökelmann, A., Lehmann, W., and Blaser, P. (2014). Auswirkungen einer Tanz- und Kraft-Ausdauer-Intervention auf kognitive Fähigkeiten älterer Menschen. *Z. Neuropsychol.* 25, 99–108. doi: 10.1024/1016-264X/a000124

Riegert, C., Galani, R., Heilig, S., Lazarus, C., Cosquer, B., and Cassel, J. C. (2004). Electrolytic lesions of the ventral subiculum weakly alter spatial memory but potentiate amphetamine-induced locomotion. *Behav. Brain Res.* 152, 23–34.

Ruscheweyh, R., Willemer, C., Krüger, K., Duning, T., Warnecke, T., Sommer, J., et al. (2011). Physical activity and memory functions: an interventional study. *Neurobiol. Aging* 32, 1304–1319. doi: 10.1016/j.neurobiolaging.2009.08.001

Saab, B. J., Georgiou, J., Nath, A., Lee, F. J., Wang, M., Michalon, A., et al. (2009). NCS-1 in the dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory. *Neuron* 63, 643–656. doi: 10.1016/j.neuron.2009. 08.014

Sehm, B., Taubert, M., Conde, V., Weise, D., Classen, J., Dukart, J., et al. (2014). Structural brain plasticity in Parkinson's disease induced by balance training. *Neurobiol. Aging* 35, 232–239. doi: 10.1016/j.neurobiolaging.2013.06.021

Smith, J. C., Nielson, K. A., Woodard, J. L., Seidenberg, M., Durgerian, S., Antuono, P., et al. (2011). Interactive effects of physical activity and APOE-+4 on BOLD semantic memory activation in healthy elders. *Neuroimage* 54, 635–644. doi: 10.1016/j.neuroimage.2010.07.070

Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., et al. (2013). Dynamics of hippocampal neurogenesis in adult humans. *Cell* 153, 1219–1227. doi: 10.1016/j.cell.2013.05.002

Szabo, A. N., McAuley, E., Erickson, K. I., Voss, M., Prakash, R. S., Mailey, E. L., et al. (2011). Cardiorespiratory fitness, hippocampal volume, and frequency of forgetting in older adults. *Neuropsychology* 25, 545–553. doi: 10.1037/a00 22733

Taubert, M., Draganski, B., Anwander, A., Müller, K., Horstmann, A., Villringer, A., et al. (2010). Dynamic properties of human brain structure: learning-related changes in cortical areas and associated fiber connections. *J. Neurosci.* 30, 11670–11677. doi: 10.1523/JNEUROSCI.2567-10.2010

van Praag, H., Christie, B. R., Sejnowski, T. J., and Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13427–13431. doi: 10.1073/pnas.96.23. 13427

Verghese, J., Lipton, R. B., Katz, M. J., Hall, C. B., Dervy, C. A., Kuslansky, G., et al. (2003). Leisure activities and the risk of dementia in the elderly. *N. Engl. J. Med.* 348, 2508–2516. doi: 10.1056/NEJMoa022252

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MT declared a shared affiliation, though no other collaboration, with several of the authors KR, NA, MD, JK and AH to the handling editor, who ensured that the process met the standards of a fair and objective review.