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# Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults

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ABSTRACT

Animal models point towards a key role of brain-derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) in mediating exercise-induced structural and functional changes in the hippocampus. Recently, also platelet derived growth factor-C (PDGF-C) has been shown to promote blood vessel growth and neuronal survival. Moreover, reductions of these neurotrophic and angiogenic factors in old age have been related to hippocampal atrophy, decreased vascularization and cognitive decline. In a 3-month aerobic exercise study, forty healthy older humans (60 to 77 years) were pseudo-randomly assigned to either an aerobic exercise group (indoor treadmill, n = 21) or to a control group (indoor progressive-muscle relaxation/stretching, n = 19). As reported recently, we found evidence for fitness-related perfusion changes of the aged human hippocampus that were closely linked to changes in episodic memory function. Here, we test whether peripheral levels of BDNF, IGF-I, VEGF or PDGF-C are related to changes in hippocampal blood flow, volume and memory performance. Growth factor levels were not significantly affected by exercise, and their changes were not related to changes in fitness or perfusion. However, changes in IGF-I levels were positively correlated with hippocampal volume changes (derived by manual volumetry and voxel-based morphometry) and late verbal recall performance, a relationship that seemed to be independent of fitness, perfusion or their changes over time. These preliminary findings link IGF-I levels to hippocampal volume changes and putatively hippocampus-dependent memory changes that seem to occur over time independently of exercise. We discuss methodological shortcomings of our study and potential differences in the temporal dynamics of how IGF-1, VEGF and BDNF may be affected by exercise and to what extent these differences may have led to the negative findings reported here.



## Introduction

Animal work has highlighted the complementary roles of central and peripheral growth factors in mediating the downstream effects of

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exercise on hippocampal plasticity and related memory benefits (see Cotman et al., 2007; Voss et al., 2013a for reviews). Brain-derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I), and vascular endothelial growth factor (VEGF) are currently considered as key proteins that are up-regulated after exercise (Carro et al., 2001; Fabel et al., 2003; Neeper et al., 1995) and that can promote cell proliferation and growth (growth factors) or neuronal development and functioning (neurotrophic factors; see, e.g., Bibel and Barde, 2000; Park and Poo, 2013 for reviews).

BDNF is a member of the neurotrophin family of factors that supports neural survival, growth, and synaptic plasticity and that is highly concentrated in the hippocampus (e.g., Cowansage et al., 2010; Gottmann et al., 2009; Lipsky and Marini, 2007) and cortex. Although decreased levels of these factors have been associated with age-related hippocampal dysfunction and memory impairment, increasing BDNF by aerobic exercise seems to ameliorate hippocampal deterioration and improve memory function (for reviews, see Erickson et al., 2012; Cotman and Berchtold, 2002). In rodents, exercise has been shown to increase BDNF expression in the hippocampus and cortical regions (e.g., Neeper et al., 1995; Aguiar et al., 2008, 2011; Vaynman et al., 2004; Uysal et al., 2015), and BDNF increase has been related to exercise-induced benefits on hippocampal-dependent memory (Vaynman et al., 2004). Rodent studies have also found significant links between serum and cortical BDNF levels (Karege et al., 2002), suggesting that peripheral serum BDNF might serve as a proxy for cortical concentrations. VEGF is a hypoxia-inducible protein that promotes the formation and growth of blood vessels that has also been associated with improved cognition (Adams and Alitalo, 2007; During and Cao, 2006). VEGF is expressed in multiple cells and tissues including smooth and skeletal muscle, endothelial cells, macrophages and glial cells (e.g., Namiki et al., 1995; K. Tang et al., 2010). The interactive effects of VEGF and IGF-I, which are both increased in the periphery after exercise (Schobersberger et al., 2000; Llorens-Martín et al., 2010) and can cross the blood–brain barrier, are thought to mediate neurogenesis and angiogenesis (for review, see Cotman et al., 2007). Blocking IGF-1 or VEGF peripheral growth factor entry to the brain has been shown to prevent exercise-induced neurogenesis in the hippocampus (Trejo et al., 2001; Fabel et al., 2003). IGF-I, which is mainly known for its role in energy metabolism and homeostasis, is emerging as a key growth factor which also modulates synaptic plasticity, synapse density, neurotransmission, and even adult neurogenesis (e.g., Fernandez and Torres-Alemán, 2012; Trejo et al., 2007). Furthermore, IGF-I is critically involved in vascular maintenance and remodeling (Lopez-Lopez et al., 2004), and age-related reductions in IGF-I have been associated with decreased cerebral vascular density and blood flow (Sonntag et al., 1997). The bioavailability of IGFs is controlled by IGF binding proteins (IGF-BPs), which are a family of structurally related proteins that bind IGFs with similar or even higher affinity than IGF receptors (Fernandez and Torres-Alemán, 2012). Studies in animals revealed that exercise prevents and protects from brain damage through increased uptake of circulating IGF-I by the brain (Carro et al., 2001). Enhanced IGF-I is also thought to mediate the induction of hippocampal BDNF, and together they are considered as the key factors in the effects of exercise on learning and memory (Cotman et al., 2007). Recent evidence has also highlighted platelet derived growth factor-C (PDGF-C) as a potential new player in neurovascular crosstalk (for a review, see Lee et al., 2013). PDGF-C has been shown to promote blood vessel growth and neuronal survival (e.g., Cao et al., 2002; Z. Tang et al., 2010). It is mainly produced by vascular cells and secreted as homodimer (PDGF-CC). PDGF-C is highly expressed in the brain (e.g., Li et al., 2000; Z. Tang et al., 2010), retina and spinal cord (see Lee et al., 2013 for review). To our knowledge, effects of exercise on PDGF-C levels and related changes in the brain have not been examined.

Although animal models have provided converging evidence that BDNF, VEGF and IGF-I are central to benefits of exercise for the brain, their role in exercise-related changes of human brain function, especially in old age, is still not clear. Previous intervention studies in healthy elderly subjects did not provide consistent evidence for enhancement of these growth factors by chronic aerobic exercise (e.g., Erickson et al., 2011; Voss et al., 2013b; for reviews, see Coelho et al., 2013; Vital et al., 2014; Berg and Bang, 2004). A randomized controlled 1-year aerobic exercise intervention (walking) in healthy older adults did not reveal significant effects on serum levels of BDNF, IGF-I or VEGF compared to a control intervention involving muscle stretching (Erickson et al., 2011; Voss et al., 2013b). However, increases in growth factors were found to correlate with increases in functional connectivity between bilateral parahippocampal and bilateral middle temporal gyrus (Voss et al., 2013b). Furthermore, increases in serum BDNF after 1-year aerobic training were correlated with increases in hippocampal volume (exercise group only; $n = 60$) and these volume changes were related to improvements in spatial memory performance (Erickson et al., 2011). This group further studied how changes in serum BDNF after the 1-year walking exercise intervention were related to changes in executive function (i.e. task-switching performance; Leckie et al., 2014). Interestingly, Leckie et al. found an increase in serum BDNF and improvement in task performance in the exercise group that varied by age, with the oldest individuals showing the greatest benefits. Mediational analyses revealed that circulating BDNF levels were related to the effect of exercise on task-switching performance, but only for individuals older than 71 years. These findings suggest an interaction of age and BDNF serum levels for cortical plasticity in aging.

In a 3-month study with forty healthy older adults (60 to 77 years) who either exercised on an indoor treadmill ($n_T = 21$) or performed indoor progressive-muscle relaxation/stretching ($n_C = 19$), we showed that the aged human hippocampus can exhibit fitness-related vascular plasticity that is closely linked to changes in episodic memory function (Maass et al., 2015). Gadolinium contrast-based perfusion imaging (3 T) was used to measure changes in regional cerebral blood flow and volume (rCBF/rCBV), whereas changes in hippocampal volumes were assessed by high-resolution structural magnetic resonance imaging (MRI) at 7 T. Correlational analyses revealed that changes in fitness levels across all participants were positively associated with changes in hippocampal perfusion (and hippocampal head volumes). Hippocampal perfusion was closely linked to the observed volume changes and both were associated with benefits in configural object memory (early recall and recognition performance in the Complex Figure [CF] Test) and verbal memory (recognition performance in the Verbal Learning and Memory Test [VLMT]). Moreover, exercise-related improvements in perfusion among the older participants declined with age, suggesting that the capacity for vascular hippocampal plasticity may be age-dependent.

In the current study we examined how VEGF, BDNF and IGF/IGF-BPs (IGF-I, IGF-II, IGF-BP2, IGF-BP3), putative markers of exercise-induced benefits on brain function, were affected by the 3-month exercise intervention and whether their changes were related to changes in hippocampal volume, perfusion and memory. We also assessed the BDNF Val66Met polymorphism, a single-nucleotide polymorphism that has been shown to affect activity-related BDNF secretion, hippocampal function and memory (for review, see Bath and Lee, 2006). In addition, we assessed changes of PDGF-C that has been shown to promote blood vessel growth and neuronal survival (e.g., Cao et al., 2002; Z. Tang et al., 2010), and appears to be a regulator of neurovascular crosstalk (for a review, see Lee et al., 2013). Changes in these neurotrophic and angiogenic factors were measured peripherally (via blood samples), because CSF sampling was not possible in this study.

Finally, we measured changes in cortisol, a stress hormone that has been negatively associated with cognitive performance (Lupien et al., 2007; Comijs et al., 2010) and thus might modify changes in memory within our exercise study. For instance, Comijs and colleagues showed that high levels of free cortisol were related to poorer performance on verbal learning in younger and older adults. Cortisol may negatively

influence cognitive performance, because it can cross the blood–brain barrier and bind to receptors localized in the hippocampus, amygdala and frontal lobes, known to be involved in learning and memory (Belanoff et al., 2001; Lupien et al., 2007).

## Materials and methods

### *Participants and experimental design*

In a controlled 3-month intervention trial, 40 sedentary healthy older adults (mean age = 68.4 ± 4.3 years, 55% females) were either training on a treadmill ($n_T = 21$) or performing progressive-muscle relaxation/stretching exercise ($n_C = 19$). After completion of the initial comprehensive cardiological examination, neuropsychological assessment, and MRI sessions, participants were pseudo-randomly assigned to one of both groups (for a detailed description of subject recruitment, see Maass et al., 2015). The groups were matched on age, gender, body-mass-index (BMI), activity level (self-reported mean endurance per week), and verbal memory recall (VLMT long delay) to prevent differences in fitness or cognitive abilities between groups at baseline. There was no dropout during the intervention. Subjects signed written informed consent and received monetary compensation for participation. The study was approved by the ethics committee of the University of Magdeburg. An overview of the main variables targeted in this study, including neuropsychological, physiological, MRI and blood data is given in Fig. 1.

### *Intervention*

The effects of the intervention on fitness and cognition have already been reported in (Maass et al., 2015). We summarize the relevant methods here again for convenience. We have not included any new analyses on fitness or cognition. As outlined in the introduction, we focus on the relationship between the intervention and growth factors.

#### *Assessment of fitness levels*

For the assessment of aerobic fitness, oxygen consumption was measured by graded maximal exercise testing on a recumbent cycle ergometer. $VO_{2\ VAT}$ (oxygen consumption at ventilatory anaerobic threshold) was calculated (instead of $VO_{2\ MAX}$) as a reasonably accurate predictor of changes in cardiovascular fitness (Noonan and Dean, 2000) as some participants did not perform up to maximal exhaustion (see also Maass et al., 2015).

#### *Physical exercise (training group)*

Participants in the aerobic exercise group received individually optimized 30 min interval training on stationary treadmills (HP Cosmos) 3 times/week, plus 5-min warm up and 5-min stretching at the end of each training session. Training intensity was determined by target heart rate (Karvonen et al., 1957), starting at 65% and increasing by 5% in steps for 4 weeks (accomplished by adjusting pace and/or the steepness of the treadmills). The target heart rate during training was based on the maximum heart rate during $VO_{2\ VAT}$ assessment and the felt exertion (CR10 Scale; see Borg, 1998) during the training sessions. Walking/running interval duration was increased from 5 min, with slow walking breaks of 2 min at the beginning of the training period, up to 30 min continuous walking/running periods at the end of the aerobic intervention.

#### *Progressive muscle relaxation/stretching (control group)*

Participants in the control group received 45 min of supervised progressive muscle relaxation/stretching training (Jacobson, 1938) twice a week. Progressive muscle relaxation was chosen as control intervention to hold variables like social interactions, schedule, and motivation as similar as possible to the training group whilst not affecting cardiovascular fitness. Here, participants were asked to tense and then relax specific muscle groups with closed eyes in supine position, following the instructions of a course leader. Total duration of training per week (=90 min) was matched between both groups.

### *Measurement of growth factor levels and cortisol*

For all subjects, fasting serum was obtained in the morning of the cardiological examination pre-intervention. Post-intervention, serum was obtained either in the morning of the cardiological examination (within one week after the last training/control session; n = 17) or on

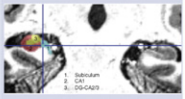
| Target | Method | Parameter (pre and post training) | |
|---|---|---|---|
| Fitness Level | Spiroergometry | Consumption of oxygen at Ventilatory Anaerobic threshold (**$VO_2$VAT**) |  |
| Perfusion | 3T Gadolinium contrast-based perfusion imaging | **rCBF**, **rCBV** in bilateral hippocampus & gray matter [ml/100g/min] |  |
| Hippocampal Structure | 7T MRI (Manual segmentation on T1-weighted images) | **Hippocampal Volume** [$cm^3$] (head, body, tail, subfields) |  |
| Episodic Memory | Complex Figure Test (CF)<br>Verbal learning and Memory Test (VLMT) | **Early & Late Recall**<br>**Recognition Memory**<br>(CF: high demands on pattern separation) |  <br>Rey Figure |
| Growth factors | Blood sample (ELISA) | Concentration in serum [pg/ml]<br>**VEGF** (Vascular endothelial growth factor)<br>**PDGF** (Platelet-derived growth factor)<br>**BDNF** (Brain-derived neurotrophic factor)<br>**IGF** (Insulin-like growth factor) |  |

**Fig. 1.** Summary of main parameters assessed in the training study. In a controlled 3-month intervention study with 40 healthy older adults, a variety of physiological and psychological parameters were measured before and after the intervention. Fitness levels were assed via spiroergometry (oxygen consumption at ventilatory anaerobic threshold, $VO_{2\ VAT}$). Gadolinium contrast-based perfusion imaging (3 T) was used to calculate changes in regional cerebral blood flow and volume (rCBF/rCBV) in the hippocampus and gray matter. Changes in hippocampal volumes (including subregions) were assessed using high-resolution structural MRI at 7 T. Hippocampus-dependent memory was tested by means of a visuospatial (configural) object and a verbal memory task. Blood samples were drawn to assess levels in angiogenic and neurogenic growth factors.

the last training day (n = 23). The cooled (~4 °C) samples were centrifuged, aliquoted and stored at minus 80 °C. These blood samples were used to determine levels of VEGF, PDGF, IGF/IGF-BPs and cortisol.

Cortisol levels were determined using IMMULITE 2000 Cortisol (Siemens Healthcare). VEGF and PDGF-CC (homodimer of PDGF-C) levels were quantified using enzyme-linked immunosorbent assays (ELISAs) following the manufacturer's instructions (R & D Systems, Minneapolis, MN; DCC00 for PDGF-CC and DVE00 for VEGF). PDGF-C is abundantly expressed in vascular cells, such as endothelial cells, vascular smooth muscle cells, pericytes, and vascular fibroblasts (for a review, see Lee et al., 2013). Pre- and post-intervention levels of IGF-I and IGF-II were also quantitatively analyzed via ELISAs (E 20 and E 30: Mediagnost GmbH, Reutlingen, Germany). IGF-BP-3 and IGF-BP-2 levels were determined using a quantitative Western ligand blot analysis. IGF and IGF-BP levels were determined by Ligandis GbR (Gülzow-Prüzen, Germany).

For pre- and post-intervention acquisition of BDNF levels, fasting blood samples were collected in the morning of the first training day and on average 1 week after the last training session. Moreover, six additional blood samples were drawn during the intervention period in order to assess the time course of BDNF serum and plasma level changes in both groups (see Supplementary Fig. S1). Serum and plasma concentrations of BDNF were determined by use of sandwich ELISAs (BDNF DuoSets; R&D Systems, Wiesbaden, Germany).

In addition, we assessed the BDNF Val66Met polymorphism. Genomic DNA was extracted from venous blood sample using Gen Elute Blood Genomic DNA-Kit (Sigma, St. Louis, MO) following the manufacturer's instructions. The BDNF Val66Met genotype (rs6265) was determined using PCR amplification and PCR-based restriction fragment length polymorphism (RFLP) analysis. Amplification reaction was performed in a total volume of 192 μl using 1.6 μl Taq DNA polymerase (Qiagen, Venlo, Netherlands), 40 μl Q-Solution, 16 μl dNTPs, 12 μl $MgCl_2$, 20 μl PCR buffer, and 20 μl of each primer. The amplified fragments were digested with the restriction enzyme Eco72I (PmlI).

### Cognitive measures

All participants completed the *Mini Mental Status Examination* (MMSE; Folstein et al., 1983; Rovner and Folstein, 1987), the *Beck Depression Inventory* (BDI-II; Beck et al., 1996) and the *Multiple-Choice Word Test* (MWT-B; Lehrl, 2005) before the intervention. Cognitive testing before and after the intervention included the following neuropsychological tests: the *Verbal Learning and Memory Test* (VLMT; Helmstaedter and Durwen, 1990), adapted German version of the *Rey Auditory Verbal Learning Test* (RAVLT), *Complex Figure Test* (CF Test; Strauss et al., 2006) and the Digit Span Test (forward and backward; Wechsler, 1997). For the purpose of the current study, only results from the CF Test and the VLMT will be reported (early recall, late recall, and recognition; see Fig. 1).

#### Complex Figure Test (CF Test)

The CF Test was used to assess spatial object recall (early, late) and recognition memory. The *Rey-Osterrieth Complex Figure* (ROCF) was used pre-intervention, whereas the *Modified Taylor Complex Figure* (MTCF (Strauss et al., 2006)) served as the post-intervention measure. The MTCF was developed as a comparable measure to the ROCF with similar accuracy scores (Hubley, 2010) and is a valid alternative for testing visual long-term memory avoiding implicit learning that can occur when the same version of the ROCF is used for repeated testing sessions (Casarotti et al., 2014). The CF consists of 18 different geometric patterns and first has to be copied (copy trial). After a 3-min (early recall) and a 30-min (late recall) delay interval, participants are asked to reproduce the figure as accurately as possible from memory (see example trial in Fig. 1). In the recognition trial (RT), subjects have to decide whether single elements were part of the original or are lures. The recognition trial was given after the late recall and requires discrimination between highly similar objects, thus posing high demands on pattern separation.

#### Verbal Learning and Memory Test (VLMT)

The VLMT assesses learning of words including early recall, an interference list after five learning trials, free recall tests directly after interference and 30 min later (late recall), and a final recognition test (Helmstaedter and Durwen, 1990). Here we focused on early recall, late recall, and recognition memory, in line with the memory measures collected in the CF Test.

### Magnetic resonance imaging (MRI)

The methodology of MRI, including contrast-based perfusion imaging, is reported in Maass et al. (2015). We shortly summarize the relevant methods here. Although we have not included any new analyses on the perfusion data, we are now reporting high-resolution region-of-interest (ROI)-based and voxel-based morphometry (VBM) data (at 7 T) for the hippocampus. As outlined in the introduction, we now focus on the relationship between the MRI measures and growth factors.

#### 3 T MRI and perfusion imaging

Gadolinium contrast-based perfusion imaging at 3 T (Siemens Magnetom, Verio, 32-channel head coil) was used to measure changes in rCBV and rCBF. High-resolution (partial) perfusion-weighted images were acquired with slice alignment parallel to the hippocampal main axis (TR/TE = 1500/30 ms, 1.6 mm in-plane resolution, 3 mm slice thickness, 20 slices with 10% gap) and quantitative perfusion maps for rCBF and rCBV were calculated. Mean perfusion values are reported for bilateral hippocampus. Additionally, general (non-hippocampal) gray-matter perfusion was calculated. The perfusion analyses are described in detail in Maass et al., 2015.

#### 7 T high-resolution MRI

High-resolution structural T1-weighted images (whole head Magnetization Prepared Rapid Gradient Echo [MPRAGE]) were acquired within one week pre- and post-intervention using a 7 T MR system (Siemens, Erlangen, Germany; 32-channel head coil) with a resolution of 0.6 mm isometric voxels (TE = 2.8 ms, TR = 2500 ms, TI = 1050 ms, flip angle = 5°, scanning duration = 14 min). To measure changes in hippocampal volume and assess their relation to changes in neurotrophic or angiogenic factors, segmentation of hippocampal regions was performed manually on the 7 T high-resolution MPRAGEs. The hippocampus was segmented into head (HH), body (HB) and tail (HT). Moreover, hippocampal subfields were labeled in the body (subiculum, CA1, and CA2–3/dentate gyrus [CA2–3/DG]). For a detailed description of the segmentation procedure, see Maass et al., 2015. Subregion/subfield-specific volume changes were derived by determining the voxels in every ROI for every subject pre- and post-intervention.

Inter-rater reliability for hippocampal segmentation was tested by a second manual segmentation of 10 randomly chosen subjects (five scans pre- and five scans post-intervention) by a different rater (see Maass et al., 2015). Intra-class correlation coefficients (ICCs, two-way mixed, single measure) for left hippocampus ($ICC_{left\ HC}$ = .973, $ICC_{head}$ = .982; $ICC_{body}$ = .906; $ICC_{tail}$ = .944) and for right hippocampus ($ICC_{right\ HC}$ = .920, $ICC_{head}$ = .969; $ICC_{body}$ = .902, $ICC_{tail}$ = .861) demonstrated high-to-excellent consistency for all subregions.

#### Longitudinal VBM

In addition to the ROI-based assessment of volumetric changes in the hippocampus, we also assessed longitudinal patterns of change by means of a novel automatic voxel-based technique in order to carry out correlational analyses. Regarding the longitudinal VBM analysis, pre- and post-exercise MPRAGEs (T1-weighted images from 7 T) were submitted to a pairwise longitudinal registration (Ashburner and

Ridgway, 2012) in SPM12 (*Statistical Parametric Mapping*; Wellcome Department of Cognitive Neurology, London, England). Default settings were applied and the mid-point average (avg) images as well as the Jacobian-difference (jd) images (representing the longitudinal change) were saved.

The segmentation of the resulting average T1-images into gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF) was done by using the segment algorithm (called "New Segment" in SPM8; see Ashburner and Friston, 2005; 30 mm cutoff; very light bias regularization = 0.0001). Within the segmentation, native and DARTEL-imported segments were saved. The probabilistic segmentations for GM, WM and CSF were summed to create an intracranial mask for each subject. GM-weighted Jacobian-difference maps were calculated by multiplying each jd-image with the corresponding GM-segment (the so called c1-image).

The DARTEL-imported GM- and WM-segments were than used to create a study-specific DARTEL template. The resulting flow fields served to finally normalize the GM-masked jd-images and the avg-images to MNI space (preserve concentration, resolution: $0.6 \times 0.6 \times 0.6\ mm^3$). In order to meet our interest in local (subregion specific) changes in the hippocampus, we applied a low smoothing kernel of 3 mm.

The normalized GM-masked jd-images were finally entered into a multiple regression analysis in SPM12. Age, gender and ΔT were included as covariates of no interest. The resulting SPM-T maps were converted to correlation coefficient R-maps using the VBM8 toolbox (http://www.neuro.uni-jena.de/vbm/) similar to the approach reported by La Joie et al. (2010) and masked with a mean hippocampus map (mean of all normalized hippocampal ROIs derived from manual segmentation; thresholded at 0.1). Hippocampal R-maps were superimposed onto the mean T1-image (mean of all normalized avg-images).

### Statistical analysis

#### Missing data and outlier detection

The outlier-labeling rule (Hoaglin et al., 1986) was applied to define outliers in measures of percentage change. This rule declares observations that lie more than 2.2 times the interquartile range away from the nearest quartile as outliers and is resistant to extreme values. A summary of missing cases and outliers is given in Supplementary Table S1.

For three subjects, IGF-BP2 levels could not be estimated because values were below detection limits of the assays. Furthermore, one outlier was detected for change in IGF-BP2 and one outlier for change in IGF-BP3 levels, respectively. There were also three outliers detected within the percentage change in VEGF levels. Complete pre- and post-data were available for 39 subjects regarding IGF-BP3, 36 subjects regarding IGF-BP2 and 37 subjects regarding VEGF. Full data were available for PDGF, BDNF (serum and plasma), IGF-I and IGF-II levels. A detailed description of reasons for all missing data (including cognitive and MRI variables) is reported Maass et al., 2015.

#### Repeated-measures ANOVA

Intervention effects were first examined using repeated-measures ANOVAs with *group* (aerobic exercise, stretching control) as a between-subjects factor and *time* (pre, post) as a within-subjects factor. ANOVAs were run in SPSS (*PASW Statistics* V.20). All dependent variables met criteria for normal distribution. Age, gender and differences in outside temperature on the day of measurement ($\Delta T = T_{post} - T_{pre}$) were included as covariates of no interest in all analyses. The difference in temperature was included to account for possible confounding effects of (seasonal) differences in hydration status on MRI and perfusion scans ($\Delta T_T = -2.7 \pm 2.6$ K, $\Delta T_C = -7.0 \pm 2.1$ K). This was deemed necessary due to different seasonal starting points across subjects with potential relevance of hydration status on brain structure as has been shown in previous studies (e.g. dehydration-related shrinkage of brain tissue and an associated increase in ventricular volume) (Kempton et al., 2011; Streitbürger et al., 2012). Although temperature did not significantly affect brain structures in this study, significant negative correlations between changes in temperature and changes in perfusion were found (hippocampal rCBF: $r = -0.38$, $p = .027$; GM rCBF: $r = -0.35$, $p = .047$). To test whether BDNF plasma or serum levels differed between BDNF genotypes (Val/Val vs. any Met carrier) at baseline or across all measurements, we performed univariate one-way ANOVAs (blood levels at baseline) and repeated-measures ANOVAs with *BDNF genotype* (Val/Val vs. any Met) as a between-subjects factor and *time* (8 timepoints of blood sampling) as within-subjects factor. Finally, we also assessed whether memory performance or its changes were affected by BDNF genotype by means of repeated-measures ANOVAs on performance in the VLMT and CF Test. Age and gender were included as covariates in all the aforementioned analyses.

We also report effects sizes $\eta^2$ (Eta-squared: .02 = small, .13 = medium, .26 = large effect) and observed power (Cohen, 1988).

#### Correlations of changes

Correlational analyses were performed between percentage change as well as pre-intervention levels of growth factors and percentage change in $VO_{2\ VAT}$, in bilateral hippocampal rCBV/rCBF, bilateral hippocampal volume (including head, body, tail), and episodic memory performance. To assess whether effects of growth factors were specific to the hippocampus, perfusion and volume changes for overall gray matter were additionally included in the analyses. The correlational analyses were motivated by findings from animal studies and studies in young adults, which have shown that exercise can increase levels of BDNF, IGF-I and VEGF and that these growth factors may modulate hippocampal vascular and structural plasticity as well as memory function (Vaynman et al., 2004; Cotman and Berchtold, 2002; Carro et al., 2001; Llorens-Martín et al., 2010; Fabel et al., 2003; Uysal et al., 2015). The specific hypotheses that were tested by the correlational analyses are described in the beginning of each subsection of the results section. For completeness, we also report all other correlation coefficients and the corresponding (uncorrected, two-tailed) *p*-values, although these were not part of our hypotheses.

For all variables of interest, data were missing occasionally (see Supplementary Table S1). Little's chi-square test showed that data were missing completely at random (MCAR; chi-square = 134.07, df = 130, $p = .386$). To perform correlational analyses including all available variables of interest, Full Information Maximum Likelihood (FIML) was used. FIML is a SEM-based missing data estimation approach that yields unbiased parameter estimates and standard errors (see also Maass et al., 2015). The method minimizes the −2 log-likelihood function for each individual based on the variables that are present and, in this sense, makes optimal use of all available data. Reported correlation coefficients (r) are standardized parameter estimates: $r_{x,y} = COV_{x,y}/(SD_x \times SD_y)$. Correlations were estimated using structural equation modeling (SEM) in *Mplus* (Version 6.1, 2011). Within each model, all dependent variables (percentage changes) were regressed on age, gender and ΔT. For illustrative purposes, regressions of the residuals were plotted in SPSS after controlling for effects of age, gender, and ΔT.

#### Structural equation modeling (SEM)

Furthermore, SEM was used to test different stepwise regression models for the associations between the hippocampal volume changes (outcome variable) and changes in hippocampal perfusion (rCBF) and IGF-I levels (predictor variables). We tested how much variance of hippocampal volume changes was captured by IGF-I over and above perfusion changes. In each model, first the covariates (age, gender, and ΔT), second hippocampal perfusion (rCBF) changes, and third the percentage changes in IGF-I were entered as predictors. SEMs were set up for whole hippocampal (HC) and HH volume changes because these were correlated with both rCBF and IGF-I changes.

Goodness of model fit was evaluated by the chi-squared difference test. The following fit indexes are reported: $\Delta\chi^2$/degrees of freedom (df), $R^2$ and $R^2$ change ($\Delta R^2$).

## Results

Exercise-induced increases in peripheral neurotrophic and angiogenic factors are thought to be critical to the effects of aerobic exercise on hippocampal plasticity and related improvements in memory (Cotman and Berchtold, 2007; Voss et al., 2013a). Based on these previous findings, we investigated the effects of BDNF (plasma and serum levels), VEGF, PDGF (PDGF-CC) and IGF/IGF-BPs (IGF-I, IGF-II, IGF-BP2, IGF-BP3) on fitness-related hippocampal vascular and structural plasticity. Furthermore, the relationship between growth-factor changes and memory benefits was tested. Finally, we analyzed whether baseline levels or changes of these factors were related to age, as we found that the potential for vascular hippocampal plasticity was reduced with increasing age (Maass et al., 2015). A summary of pre- and post-intervention levels for the measured variables in each group is given in Table 1.

### *Effects of exercise on growth factor levels*

As reported by Maass et al. (2015), the exercise intervention was effective in increasing aerobic fitness levels ($VO_{2\ VAT}$, $F(1,34) = 6.49$, $p = .016$, $\eta^2 = 0.16$, observed power = .70). Repeated-measures ANOVAs on VEGF, PDGF, IGF/IGF-BPs and BDNF did not yield any significant time (pre vs. post) × group (exercise vs. control) interactions (all $p$-values > .12). There was only a main effect of time on IGF-II levels ($F(1,35) = 6.94$, $p = .012$) and a significant age × IGF-II interaction ($F(1,35) = 7.52$, $p = .010$; see negative correlation with age below). IGF-II levels were significantly decreased after the 3-month intervention ($t(39) = 2.25$, $p = .030$).

There was also no significant correlation between changes in fitness and changes in VEGF, PDGF, IGF/IGF-BPs or BDNF levels (after regressing out age, gender and ΔT) as summarized in Table 2. The only trend for a relationship with fitness changes was found for changes of IGF-BP2 ($r = -0.32$, $p = .056$; all other $p$-values > .10).

We also assessed how changes in growth factor levels after the 3-month intervention were related among each other. We found a strong positive correlation between PDGF and VEGF changes ($r = 0.56$, $p < .001$), which are both signaling factors that promote or regulate angiogenesis (Adams and Alitalo, 2007). In addition, changes in IGF-I and IGF-II levels were positively correlated with changes in IGF-BP3 (IGF-I: $r = .53$ and IGF-II: $r = 0.56$, $p < .001$) and negatively correlated with changes in IGF-BP2 (IGF-I: $r = -0.34$, $p = .043$). Furthermore, IGF-BP2 increases were related to IGF-BP3 decreases ($r = -0.37$, $p = .025$). IGF-BPs bind IGFs with high affinity and thus control their bioavailability. There was no further significant relationship between changes of the aforementioned factors, despite a positive correlation between PDGF and IGF-I level changes that was driven by the exercise group ($r = 0.31$, $p = .038$; $r_T = 0.47$, $p = .03$; $r_C = 0.11$, $p > .90$).

### *Relation between growth factor levels and hippocampal plasticity*

Although our previous analyses did not reveal any significant increase in VEGF, PDGF, IGF/IGF-BPs or BDNF due to exercise, baseline levels or changes of these growth factors might modulate vascular and structural hippocampal plasticity.

As reported previously (Maass et al., 2015) changes in fitness were strongly related to changes in hippocampal and GM perfusion (rCBF/rCBV; $r_T > 0.50$). Although repeated-measures ANOVAs did not yield significant time × group interaction for hippocampal perfusion (despite a significant interaction effect on GM perfusion with $F(1,27) = 5.69$, $p = .024$; $\eta^2 = 0.17$, observed power = 0.63), hippocampal perfusion significantly changed in the exercise group (rCBF: $F(1,12) = 8.34$, $p = 0.014$, $\eta^2 = 0.41$, observed power = 0.75; rCBV: $F(1,12) = 5.27$, $p = .040$, $\eta^2 = 0.30$, observed power = 0.56), but not in the control group (CBF/CBV: $p > .70$, $F < 1$, $\eta^2 < 0.01$) and these changes were negatively related to age (see Maass et al., 2015). Moreover, hippocampal perfusion changes largely accounted for hippocampal volume changes ($r = 0.63$, $p = .001$). We hypothesized that increased hippocampal perfusion (e.g. due to increased vascularization) after the intervention might be

**Table 1**
Group means (SD) for all measured variables pre- and post-intervention.

| Variables | Aerobic exercise group | | Relax/stretching group | |
|---|---|---|---|---|
| | Pre (baseline) | Post (3 months) | Pre (baseline) | Post (3 months) |
| $VO_{2\ VAT}$* | $18.04\ (3.55)_{20}$ | $19.92\ (3.33)_{20}$ | $21.03\ (4.16)_{19}$ | $21.14\ (3.87)_{19}$ |
| Hippocampal rCBF | $104.0\ (38.8)_{16}$ | $97.5\ (37.4)_{16}$ | $102.2\ (22.3)_{16}$ | $101.1\ (23.2)_{16}$ |
| Hippocampal rCBV | $58.2\ (29.9)_{16}$ | $63.1\ (51.0)_{16}$ | $54.1\ (20.6)_{16}$ | $53.9\ (24.4)_{16}$ |
| Gray matter rCBF* | $57.9\ (16.6)_{16}$ | $60.1\ (19.9)_{16}$ | $60.9\ (14.2)_{16}$ | $57.0\ (14.0)_{16}$ |
| Gray matter rCBV | $28.8\ (11.7)_{16}$ | $31.6\ (19.5)_{16}$ | $30.8\ (9.8)_{16}$ | $30.4\ (15.6)_{16}$ |
| Hippocampal head vol. | $3.24\ (.64)_{15}$ | $3.17\ (.58)_{15}$ | $3.30\ (.73)_{17}$ | $3.22\ (.73)_{17}$ |
| Hippocampal body vol. | $2.09\ (.30)_{15}$ | $2.05\ (.32)_{15}$ | $2.08\ (.37)_{17}$ | $2.00\ (.34)_{17}$ |
| Hippocampal tail vol. | $.99\ (.27)_{15}$ | $.97\ (.26)_{15}$ | $1.03\ (.26)_{17}$ | $1.01\ (.24)_{17}$ |
| CF early recall | $17.3\ (5.6)_{18}$ | $21.9\ (6.4)_{18}$ | $18.2\ (5.6)_{19}$ | $21.7\ (6.0)_{19}$ |
| CF late recall | $17.6\ (5.1)_{18}$ | $22.3\ (6.5)_{18}$ | $16.9\ (6.1)_{19}$ | $21.5\ (6.0)_{19}$ |
| CF recognition | $19.5\ (1.7)_{20}$ | $18.0\ (2.1)_{20}$ | $19.2\ (2.0)_{19}$ | $18.1\ (2.3)_{19}$ |
| VLMT early recall | $5.1\ (1.7)_{21}$ | $5.1\ (1.3)_{21}$ | $5.3\ (1.6)_{18}$ | $5.8\ (1.4)_{18}$ |
| VLMT late recall | $9.6\ (3.1)_{21}$ | $8.5\ (2.5)_{21}$ | $10.1\ (2.2)_{18}$ | $9.2\ (2.6)_{18}$ |
| VLMT recognition | $11.5\ (2.9)_{20}$ | $8.4\ (4.2)_{20}$ | $12.1\ (2.7)_{16}$ | $10.4\ (3.4)_{16}$ |
| Cortisol | $386\ (95)_{18}$ | $395\ (70)_{18}$ | $361\ (107)_{19}$ | $354\ (83)_{19}$ |
| VEGF | $351.8\ (183.2)_{18}$ | $351.4\ (225)_{21}$ | $297.2\ (144)_{19}$ | $276.9\ (131)_{19}$ |
| PDGF | $825.5\ (194.4)_{21}$ | $893.3\ (182.9)_{21}$ | $802.9\ (211.9)_{19}$ | $782.8\ (169.9)_{19}$ |
| $BDNF_{serum}$ | $17{,}635.9\ (5754.5)_{21}$ | $16{,}917.8\ (6958.6)_{20}$ | $18{,}468.1\ (6616.2)_{19}$ | $18{,}471.2\ (7282.9)_{19}$ |
| $BDNF_{plasma}$ | $840.2\ (373.7)_{21}$ | $1100.8\ (565.4)_{21}$ | $1091.3\ (726.1)_{19}$ | $1189.3\ (829.8)_{19}$ |
| IGF-I | $152.0\ (39.8)_{21}$ | $148.8\ (46.5)_{21}$ | $160.3\ (45.9)_{19}$ | $155.1\ (47.9)_{19}$ |
| IG-II | $880.6\ (188.6)_{21}$ | $816.2\ (134.2)_{21}$ | $881.98\ (168.1)_{19}$ | $868.0\ (181.2)_{19}$ |
| IGF-BP2 | $449.9\ (159.8)_{20}$ | $526.4\ (286.9)_{21}$ | $422.3\ (243.4)_{17}$ | $517.6\ (294.7)_{18}$ |
| IGF-BP3 | $3677.2\ (728.5)_{20}$ | $3641.8\ (798.7)_{21}$ | $4140.1\ (787.8)_{19}$ | $3959.1\ (793.1)_{19}$ |

$VO_{2\ VAT}$ was measured in ml/kg per min. Bilateral rCBF and rCBV in hippocampus and gray matter were measured in ml/100 g/min and ml/100 g × 10, respectively. Bilateral hippocampal volumes were measured in $cm^3$. Serum levels of cortisol, BDNF (also measured in plasma), VEGF and PDGF denote ngl/ml; IGF and IGF-BP levels denote pg/ml. CF: Complex Figure Test. VLMT: Verbal Learning and Memory Test. Subscripts indicate number of available data (see also Supplementary Table S1 for missing data).

* Denote variables showing significant time × group interactions ($p < .05$).

**Table 2**
Correlation coefficients (r) for the relationships of changes in growth factor levels and fitness, perfusion, volume, and memory across all participants.

| Variables (percentage change) | BDNF (serum) | BDNF (plasma) | VEGF | PDGF | IGF-I | IGF-II | IGF-BP2 | IGF-BP3 |
|---|---|---|---|---|---|---|---|---|
| $VO_{2\ VAT}$ | −.01 | .15 | .16 | .26 | .14 | −.20 | −.32° | .01 |
| Hippocampal rCBF | .15 | −.06 | −.01 | .10 | .08 | .15 | −.10 | .07 |
| Gray matter rCBF | .02 | −.01 | −.04 | .07 | .04 | .04 | −.02 | .13 |
| Hippocampal vol. | .25 | −.01 | −.04 | .26 | **.56**** | −.03 | **−.42*** | .20 |
| Hippocampal head vol. | .11 | .19 | .03 | .24 | **.53**** | .15 | **−.41*** | **.35*** |
| Gray matter vol. | −.03 | −.15 | .14 | .19 | .24 | .02 | −.09 | .03 |
| CF early recall | −.04 | .19 | −.27 | −.12 | .18 | .32° | −.28 | .25 |
| CF late recall | −.02 | .23 | −.27 | −.16 | .08 | .04 | −.25 | .08 |
| CF recognition | .12 | −.16 | .03 | .00 | .02 | .09 | **−.31*** | .08 |
| VLMT early recall | .26 | −.26 | −.26 | −.07 | −.09 | .20 | −.20 | −.02 |
| VLMT late recall | −.00 | .31° | −.10 | .15 | **.42**** | −.21 | −.20 | −.03 |
| VLMT recognition | .06 | −.28 | −.01 | .14 | .10 | .23 | −.06 | −.07 |

Partial correlations of percentage changes across all participants were calculated using Full Information Maximum Likelihood (FIML), a missing-data estimation approach controlling for possible confounding effects of age, gender and ΔT. BDNF was measured in serum and plasma; VEGF, PDGF and IGF/IGF-BP (binding protein) were measured in serum before and after the intervention. Bilateral hippocampal volumes were assessed by manual segmentation on high-resolution T1-weighted images. CF: Complex Figure Test. VLMT: Verbal Learning and Memory Test. Asterisks denote p-values of correlations (*$p < .05$; **$p < .01$; °$p < .06$, n.s.; $p$-values are uncorrected and two-tailed), with boldface correlation coefficients being significant at $p < .05$.

related to increases in VEGF, PDGF and IGF-I (Sonntag et al., 1997; Adams and Alitalo, 2007; Lee et al., 2013), which all have been shown to have angiogenic effects. Furthermore, we hypothesized that increased levels in IGF-I and BDNF might be positively related to hippocampal volume changes (Erickson et al., 2010, 2011; Trejo et al., 2001). However, correlational analyses did not yield any significant relation between changes in VEGF, PDGF, IGF/IGF-BPs or BDNF (after regressing out age, gender and ΔT) and changes in perfusion (all $p$-values > .14). The absence of any relationship between changes in perfusion and VEGF is striking, as VEGF baseline levels were positively related to baseline GM and hippocampal perfusion (GM rCBF: $r = 0.43$, $p = .024$; hippocampal rCBF: $r = 0.37$, $p = .045$).

With regard to changes in hippocampal volume, there was a positive correlation with changes in IGF-I (HC: $r = 0.50$; $p = .004$; HH: $r = 0.48$, $p = .006$; see Fig. 2) but with no other growth factor (all $p$-values > .15). Interestingly, this correlation was strong in the control group but not significant in the training group (HC: $r_C = 0.65$, $p = .005$; $r_T = 0.34$, $p = .22$) and thus likely to be independent of exercise. Partial correlational analyses further supported this possibility, showing that the positive relationship between HC volume changes and IGF-I level changes was independent of changes in fitness and perfusion, as it remained significant after regressing out $VO_{2\ VAT}$ ($r = 0.46$, $p = .010$) and hippocampal rCBF ($r = 0.57$, $p = .003$). A trend for a similar positive relationship with hippocampal head volume changes was also found for changes in IGF-BP3 (HH: $r = 0.34$. $p = .058$), whereas IGF-BP2 changes showed a negative correlation (HC: $r = -0.42$, $p = .022$; HH: $r = -0.42$, $p = .029$). Moreover, these relationships were specific to the hippocampus, as whole-brain GM-volume changes were unrelated to all of the aforementioned factors (all $p$-values > .26).

*Linear regression model for hippocampal volume changes*

The correlational analyses revealed a significant relationship between hippocampal volume changes and IGF-I level changes as well as hippocampal perfusion changes. Although IGF-I is known to mediate angiogenesis and vessel remodeling (e.g., Lopez-Lopez et al., 2004), which could be a potential cause of increased brain perfusion, IGF-I changes were unrelated to perfusion changes. On the other hand, increased blood flow (e.g. in response to neural activation) has been shown to increase localized transfer of serum IGF-I into the brain (Nishijima et al., 2010). Thus, increased hippocampal blood flow might also promote IGF-I transport to the hippocampus (despite constant peripheral serum IGF-I levels) and thereby positively modulate neurotrophic actions of IGF-I on hippocampal structure. Finally, IGF-I could positively influence neurogenesis in the dentate gyrus independent from any vascular factors (e.g., Trejo et al., 2008). In a stepwise multiple regression analysis (using FIML), we tested which model would best explain hippocampal volume changes (see Table 3). In the baseline model, paths from hippocampal rCBF and IGF-1 were set to zero and hippocampal volume changes were only regressed on age, gender and ΔT (HC: $R^2 = 0.19$; HH: $R^2 = 0.14$; see Model 1 in Table 3). Adding hippocampal perfusion changes as predictor for hippocampal volume changes to the model significantly increased model fit (HC: $\Delta R^2 = 0.30$, $p < .001$; HH: $\Delta R^2 = 0.37$; $p < .001$; see Model 2 in Table 3), which replicates the findings from the correlational analyses reported above. In the next step, IGF-1 changes were added to Model 2. For both changes in whole hippocampal volume and hippocampal head volumes, IGF-I accounted for a reliable portion of additional variance over and above perfusion changes (HC: $\Delta R^2 = 0.10$, $p = .006$; HH: $\Delta R^2 = 0.05$; $p = .015$; see Model 3 in Table 3). We further tested whether adding a direct path from hippocampal perfusion on IGF-I changes would significantly improve our model. This is based on the assumption that increased hippocampal blood flow might positively modulate the effects of IGF-I on hippocampal volume (e.g. due to increased IGF-I transport to the hippocampus; Nishijima et al., 2010). However, adding a path from perfusion on IGF-I did not result in statistically significant improvement in fit over our initial regression model (HC: $\Delta R^2 = 0.01$, $p > .60$; HH: $\Delta R^2 = 0.03$, $p > .40$; not shown in Table 3) and moreover, the path from rCBF on IGF-I was not significant (HC: $r = 0.10$, $p > .60$; HH: $r = .13$, $p > .40$). Thus, a model in which IGF-I and hippocampal rCBF changes independently predict hippocampal volume changes best fit our data and together hippocampal perfusion and IGF-1 changes accounted for almost 60% of the variance in hippocampal volume changes over the 3-month intervention period (see Fig. 3).

*Subregion-specific correlational analyses*

Finally, we analyzed subregion-specific longitudinal effects of hippocampal perfusion and IGF-I on hippocampal volume using a ROI-based and an automatic VBM-based approach (similar to La Joie et al., 2010).

For the ROI-based approach, subregion-specific volume changes were derived by manual segmentation on the high-resolution 7 T MRI data (see 2.5). Although hippocampal perfusion seemed to affect most strongly head and tail volumes (HH: $r = 0.66$, $p < .001$; HB: $r = 0.25$, $p > .20$; HT: $r = 0.59$, $p = .001$), IGF-I was related to changes in head and body volumes (HH: $r = 0.48$, $p = .006$; HB: $r = 0.37$, $p = .039$; HT: $r = 0.25$, $p > .16$). With regard to subfields in the body, both rCBF and IGF-I only showed significant correlations with CA1 volume changes (rCBF: $r = 0.40$, $p = .043$; IGF-I: $r = 0.38$, $p = .030$), whereas CA2-3/DG and subiculum did not show significant correlations (all $p$-values > .11).

We further tested whether an automatic VBM-based approach would yield similar results and whether we could gain more

**Table 3**
Stepwise regression models for changes in hippocampal volumes.

| Dependent variable | Model | Predictors | Coefficients | SE | *p* | $R^2/\Delta R^2$ | Incremental validity** ($\Delta\chi 2$/df/*p*) |
|---|---|---|---|---|---|---|---|
| *Stepwise latent regression models for whole hippocampal volume changes* | | | | | | | |
| HC volume | 1. | Age | −.313 | .156 | .044 | | |
| | | Gender | −.058 | .163 | .724 | .19/– | |
| | | ΔT | .298 | .165 | .072 | | |
| | 2. | Hipp. rCBF | .620 | .131 | <.001 | .49/.30 | 13.7/1/<.001 |
| | 3. | Hipp. rCBF | .506 | .138 | <.001 | .59/.10 | 7.6/1/.006 |
| | | IGF-I | .419 | .137 | .002 | | |
| *Stepwise latent regression models for hippocampal head volume changes* | | | | | | | |
| HH volume | 1. | Age | −.264 | .164 | .108 | | |
| | | Gender | −.069 | .168 | .684 | .14/– | |
| | | ΔT | .250 | .175 | .152 | | |
| | 2. | Hipp. rCBF | .678 | .120 | <.001 | .51/.37 | 15.6/1/<.001 |
| | 3. | Hipp. rCBF | .570 | .138 | <.001 | .56/.05 | 5.9/1/.015 |
| | | IGF-I | .382 | .146 | .009 | | |

In a stepwise multiple regression, we tested which model best explained changes in hippocampal volumes. Specifically, we were interested whether IGF-I changes would account for a reliable amount of variance over and above perfusion changes (rCBF). In a first step (Model 1) volume changes were only regressed on the covariates age, gender and ΔT. In a next step, hippocampal perfusion changes were added as predictor variable (Model 2). Finally, volumes were also regressed on IGF-I changes (Model 3). Regressions were conducted in *Mplus* using FIML to account for missing data. Regression models were compared by chi-squared difference tests. Note that coefficients for age, gender and ΔT are only reported for Model 1. The variables volume, perfusion and IGF-I denote percentage change from pre- to post-intervention across all participants (N = 40). HH: hippocampal head; HC: whole hippocampus.

information about which subfields in head and tail was most strongly affected by perfusion and IGF-I. Therefore, we used the novel longitudinal registration tool in SPM12, which allows good alignment of longitudinal brain data (Ashburner and Ridgway, 2012; Rohrer et al., 2013). In order to keep the highest possible precision, we applied a low smoothing kernel of only 3 mm on our normalized GM-masked Jacobian difference images (and kept the original resolution of 0.6 mm isotropic voxels). Correlations between changes in hippocampal perfusion (rCBF) or IGF-I levels with hippocampal volume were assessed through a voxel-wise multiple regression analysis using the smoothed, normalized GM-masked jd images. Age, gender and ΔT were included as nuisance variables in accordance with the ROI-based analyses. The resultant SPM-T map was then converted to a correlation coefficient (R) map using the VBM8 toolbox (http://www.neuro.uni-jena.de/vbm/).

Fig. 4 depicts the hippocampal correlation maps for regressions including hippocampal rCBF, IGF-I and VEGF changes (as control). Similar to the ROI-based correlational analyses, the strongest correlations of hippocampal rCBF changes were found with the hippocampal head and tail (bilaterally) as illustrated in Fig. 4A. In the hippocampal body, correlations appeared to be strongest in CA1 at the border to the subiculum and more posteriorly in CA1/DG. This pattern suggests that DG volumes might have been positively associated with perfusion changes, but that these effects were more local (posterior body and tail) and thus not detected with a ROI-based approach, in which subfield segmentation was limited to the hippocampal body and volumes were averaged along the longitudinal axis. With respect to IGF-I changes, the strongest correlations were found with voxels in the right hippocampus at the transition from head to body (CA1/subiculum border, see Fig. 4B) and in the posterior body at the transition to the tail (peak in DG/CA3). Correlation coefficients in the left hippocampus were overall lower, with the highest R values in the head. These findings are partially consistent with the ROI-based analyses, which revealed the strongest relations of IGF-I changes to HH and HB volumes, particularly for CA1. Similar to the perfusion changes, also IGF-I showed high correlation values with voxels that presumably correspond to DG in the posterior body and tail.

A regression analysis with VEGF changes did not reveal any correlations higher than 0.4 and most voxels had R-values smaller than 0.2 (Fig. 4C; mean hippocampal R = .18). This is in line with the ROI-based analyses that did not reveal significant correlations with VEGF changes.

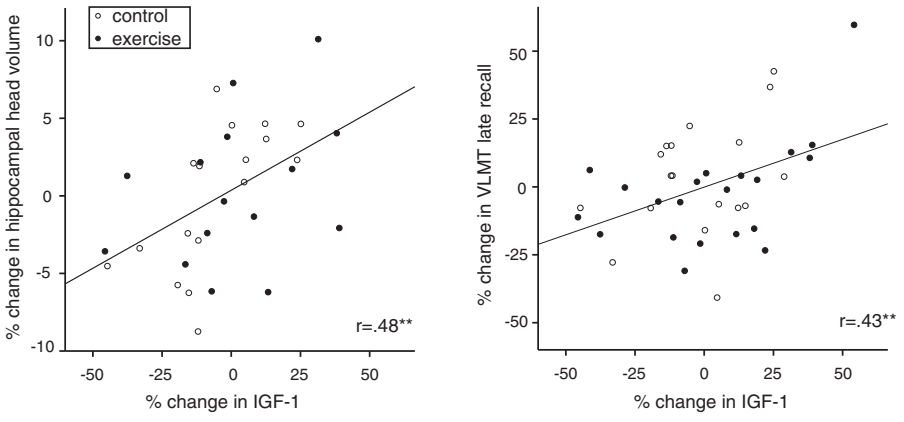



**Fig. 2.** Relationship of changes in IGF-I levels, hippocampal volume and verbal recall. Changes in IGF-I over a 3-month period were positively correlated with changes in hippocampal volume (head and whole hippocampus) measured by manual segmentation on the 7 T high-resolution T1-images and changes in late recall performance in the VLMT. Plots display partial residuals after controlling for age, gender, and ΔT (temperature changes from pre- to post-intervention). Volumes refer to bilateral hippocampus. Asterisks highlight significant correlations (**$p < .01$). VLMT: *Verbal Learning and Memory Test*.

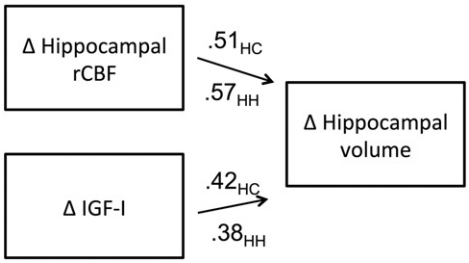



**Fig. 3.** Multiple regression model for changes in hippocampal volume. Stepwise multiple regression analyses revealed that hippocampal volume changes over the 3-month intervention period across all participants (N = 40) were best explained by changes in hippocampal perfusion and IGF-I levels. Notably, effects of perfusion and IGF-I seemed to be independent of each other. Adding a direct path from perfusion to IGF-I changes did not significantly improve the model. Numbers are standardized path coefficients (all *p*-values < .01). HH: hippocampal head; HC: whole hippocampus.

*Effects of growth factors on changes in memory*

Based on findings in animals (e.g., Trejo et al., 2007; Vaynman et al., 2004), we hypothesized that changes in BDNF and IGF-I might be positively related to changes in hippocampus-dependent memory. Indeed, IGF-I was not only related to hippocampal volume changes but changes in IGF-I were also positively related to changes in VLMT late recall performance ($r = 0.42$, $p = .005$; see Fig. 2) across both the exercise and the control group. Regarding BDNF, we found a trend for a similar positive relation between changes in VLMT late recall performance and BDNF plasma levels ($r = 0.31$, $p = .052$). There was no significant positive relationship for any of the other neurotrophic or angiogenic factors with memory changes (despite a trending relationship between IGF-II changes and CF early recall: $r = 0.32$, $p = .068$, all other *p*-values > .06). Regarding IGF-BPs there was also a negative correlation between changes in IGF-BP2 levels and CF recognition memory ($r = -0.31$, $p = .034$).

*Relation of growth factor levels to age and perfusion changes*

Our previous analyses revealed that fitness-related changes in hippocampal perfusion were reduced with age. Specifically, we found that perfusion (rCBF/rCBV) tended to increase in younger, but to decrease in older individuals after the 3-month intervention. We thus tested whether baseline levels of neurotrophic factors (or changes) were related to age or perfusion changes.

Regarding age, there were significant negative correlations with baseline levels of IGF-II and IGF-BP3 (IGF-II: $r = -0.47$, $p = .002$; IGF-BP3: $r = -0.41$, $p = .009$; all other *p*-values > .10) as well as a significant negative relationship between changes in IGF-II levels and age ($r = -0.39$, $p = .012$; all other *p*-values > .01). However, IGF-II and IGF-BP3 did not show any relation to perfusion changes ($p > .50$). There was only a negative relation between baseline levels in IGF-I

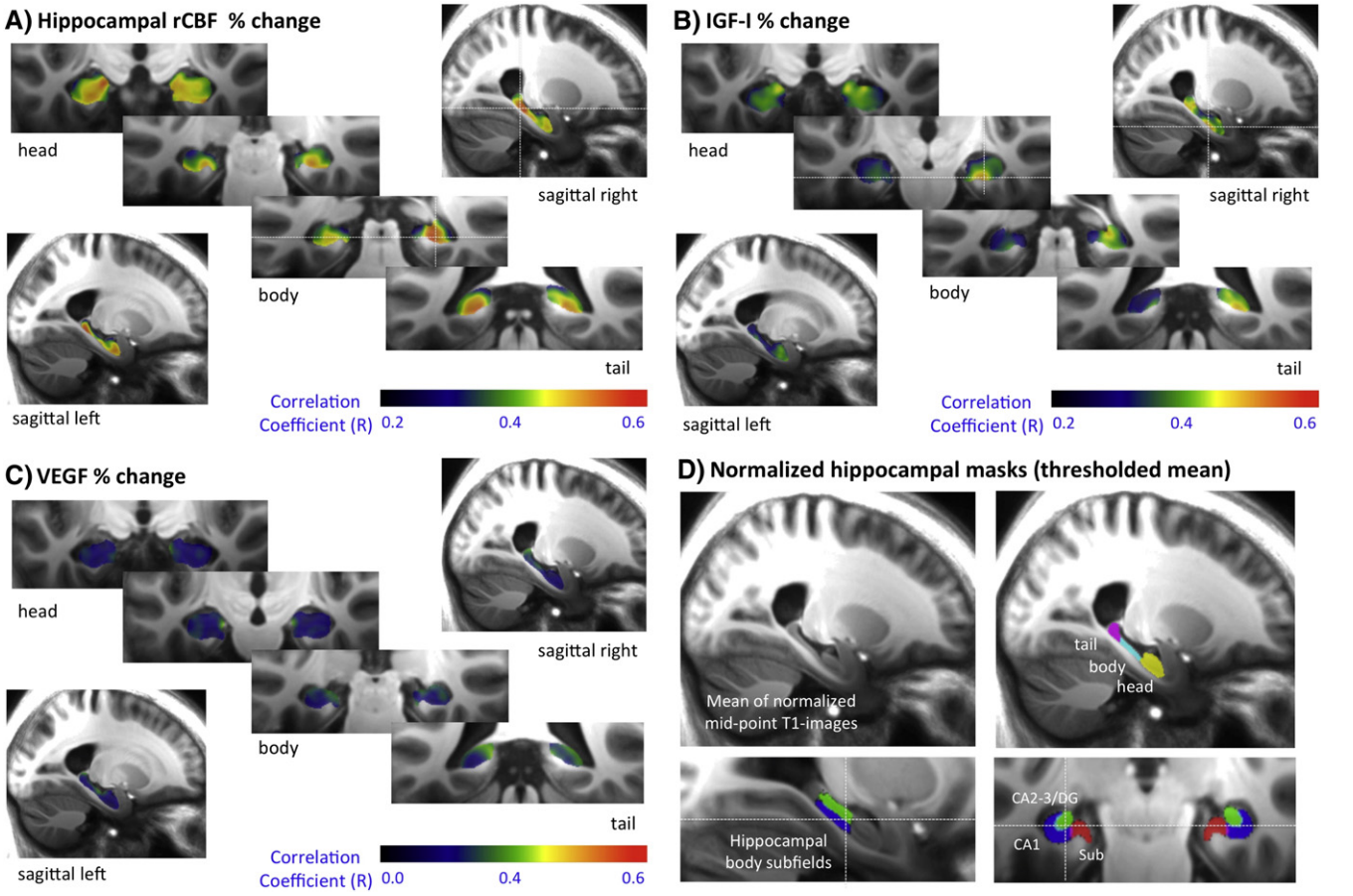



**Fig. 4.** Voxel-based regressions of changes in hippocampal perfusion, IGF-1 and VEGF with hippocampal GM changes. Results of the voxel-based multiple regression analyses between changes in hippocampal rCBF (A), IGF-1 (B) and VEGF (C; used as control) with changes in hippocampal GM density across all subjects. Goal was to localize subregion-specific longitudinal effects and verify the findings from the ROI-based volumetry. Analyses were performed on the normalized and 3 mm-smoothed Jacobian-difference maps. Age, gender and ΔT were included as nuisance regressors. Each SPM-T map was converted to a correlation coefficient R-map and masked by a group hippocampus image (thresholded mean of normalized HC ROIs; see D). R-maps are superimposed onto the group-specific mean T1-image (mean of all normalized "average" MPRAGEs). Warm colors (red) indicate high positive correlations between the covariates and hippocampal GM volume, whereas cold (dark blue) colors indicate low correlation coefficients. The white cross demarcates the location of the peak R. Note that R-values for VEGF are scaled from 0 to 0.6, as most voxels had R-values lower than 0.2.

and changes in GM perfusion ($r = -0.41$, $p = .022$), such that individuals with low baseline levels tended to show increases.

With regard to serum BDNF level changes, Leckie et al. (2014) recently found a significant interaction between age and exercise group on change in serum BDNF. In their study increases in BDNF after 1-year walking were modulated by age such that those subjects in the highest age quartile showed the greatest increase in BDNF after 1 year of moderate intensity walking exercise ($p = .036$). We also tested for interactions between time × age across all participants or the exercise group only with regard to serum BDNF. Repeated-measures ANOVAs revealed no significant interaction between time × age on serum BDNF across all subjects ($F(1,35) = 2.93$, $p = .096$, $\eta^2 = 0.077$, observed power = .38; although a weak trend was observed) or the exercise group only ($F(1,17) < 1$, $p > .90$) including the Val66Met polymorphism as an additional covariate as done by Leckie et al. did not change these results ($p > .10$) either. However, it is possible that significant differences would emerge with a larger sample size.

*Effects of Val66Met polymorphism on BDNF levels and cognition*

Previous work has shown that the Met allele of the Vall66Met polymorphism (carried by about 35% of the population) is related to reduced activity-dependent BDNF secretion in hippocampal neurons and poorer cognitive performance (Egan et al., 2003; for review, see Bath and Lee, 2006), and negative effects might be modulated by vascular risk factors (Raz et al., 2008, 2009). Moreover, the BDNF genotype might also affect experience-dependent hippocampal plasticity (e.g., Lövdén et al., 2011).

Based on these previous findings, we also assessed the BDNF genotype of our subjects and tested whether the Val66MET polymorphism was affecting BDNF serum or plasma levels or memory. Our sample consisted of 23 Val/Val carriers (10 in exercise group), 16 Val/Met carriers (11 in exercise group) and 1 Met/Met carrier (control group).

First we tested whether carriers of the Met allele differed in serum or plasma BDNF levels from Val/Val homozygotes at baseline or across the 8 blood measurements. One-way ANOVAs (incl. age and gender as covariates) did not reveal any effect of genotype neither on serum nor on plasma BDNF baseline levels (all $p$-values > .32). Repeated-measures ANOVAs including all 8 measurements of BDNF blood levels did also not yield any between-subject difference due to the BDNF genotype ($p > .13$). Finally, we also tested whether verbal or visuospatial episodic memory performance and its change during the intervention were affected by genotype (repeated-measures ANOVA with genotype as between-subjects factor). There was no significant time × group interaction (all $p$-values > .28) and no between-subjects difference in memory (all $p$-values > .33). Interactions between time × group (exercise vs. control) × genotype (Val/Val vs. any Met) were not addressed in this paper due to the small sample size (note that there were only 6 carriers of the Met allele in the control group and memory measures were missing for 2 subjects).

## Discussion

Although animal models provide converging evidence for the importance of exercise-induced increases of neurotrophic factors in modulating hippocampal plasticity and memory, the role of these molecules in exercise-related changes of human brain function, especially in old age, remains unknown. We analyzed whether changes in neurotrophic and angiogenic growth factors, measured before and after an exercise and control intervention in healthy elderly subjects, were related to changes in hippocampal vascular and structural plasticity as well as memory. Animal research has highlighted BDNF, IGF-I, and VEGF as key factors that may mediate benefits of exercise on brain and cognition (Cotman et al., 2007; Voss et al., 2013a), although the underlying mechanisms are not fully understood (but see Wrann et al., 2013). However, previous intervention studies in healthy older humans did not provide consistent evidence for an exercise-induced enhancement of these growth factors by aerobic exercise (e.g., Erickson et al., 2011; Voss et al., 2013b; see Coelho et al., 2013; Vital et al., 2014 for reviews).

Although the 3-month intervention performed here significantly increased fitness levels in the elderly participants, there was no effect on BDNF, VEGF, PDGF or IGF-I levels in the blood. Furthermore, fitness-related benefits in hippocampal perfusion and volume were also unrelated to changes of any of the aforementioned growth factors. The negative finding regarding the link between the observed vascular hippocampal plasticity and the growth factors suggest that increased perfusion might have benefited neural function independently of angiogenesis, neurogenesis or synaptogenesis. On the other hand, methodological limitations such as analysis techniques used for determining growth factor levels in blood, limitations of the ELISA kits/protocols used in this study or the restriction to pre- and post-intervention measures, could have obscured effects of growth factor on hippocampal plasticity. Furthermore, our study comprised only 40 subjects that were assigned either to a training or control group. Given the low power of this study (0.7 and 0.6 to detect medium effects of exercise on fitness and GM perfusion, respectively) our negative findings thus need to be treated with caution.

Fitness-related increases in perfusion could be mediated by exercise-induced angiogenesis, and animal studies suggest that angiogenesis is closely linked to neurogenesis (Louissaint et al., 2002; Palmer et al., 2000; Pereira et al., 2007). Supporting evidence for an association between exercise-induced increases in DG perfusion (rCBV) measured by Gadolinium-based perfusion MRI and neurogenesis has been provided in a study with mice (Pereira et al., 2007).

Although we found significant relationships among increases in fitness, hippocampal perfusion and hippocampal head volumes, ROI-based analyses (restricted to the body of the HC, where subfields could be reliably distinguished) did not yield evidence for a specific effect on the combined volume of DG/CA2/CA3, where neurogenesis (if detectable with MRI) would have been expected to occur. In contrast, CA1 volume changes showed significant relations to hippocampal perfusion changes. This hippocampal subfield is most vulnerable to vascular diseases and hypertension (Bender et al., 2013; Sabbatini et al., 2000; Schmidt-Kastner and Freund, 1991). Nevertheless, voxel-based regression analyses yielded peak correlations of changes in perfusion with changes in hippocampal GM density in the posterior body and tail regions that might comprise the DG. This suggests that DG volumes might have been positively associated with perfusion changes, but that these effects were focal (posterior body and tail) and thus not detected with a ROI-based approach that was limited to the hippocampal body and in which volumes were averaged along the longitudinal axis.

Aside from stimulating neurogenesis or angiogenesis, there are a number of other mechanisms through which increased fitness and perfusion could be associated with improved neuronal function. Increased hippocampal perfusion can enhance the supply of oxygen and nutrients to hippocampal neurons. Conversely, increased hippocampal activity could induce increases in local perfusion. Furthermore, benefits of regular exercise on cognition might be related to its anti-inflammatory effects, for example by reducing inflammatory cytokines such as IL1-beta or TNF-alpha that both impair growth factor signaling (for reviews, see Cotman et al., 2007; Petersen and Pedersen, 2005). Mouse models have also proposed that exercise reduces the load of amyloid-beta (Aβ) plaques in the hippocampus and cortex, possibly by modulating processing of the amyloid precursor protein and by enhanced degradation and clearance of Aβ (Adlard et al., 2005). Moreover, exercise-induced increases in IGF-I and VEGF in cooperation with BDNF could improve hippocampus-dependent memory by modulating synaptic plasticity, synapse density and neurotransmission involving mature neurons (see, e.g., Cotman et al., 2007; Fernandez and Torres-Alemán, 2012; Licht et al., 2011). In this respect, increased

cerebral blood flow due to increased neural activity has also been found to support transport of IGF-I into the brain (Nishijima et al., 2010).

Nevertheless, the current data did not support any effect of exercise or increased fitness on IGF-I, VEGF, BDNF or PDGF. Although fitness changes in the elderly participants were strongly correlated with hippocampal perfusion, which seemed to have mediated benefits in hippocampus-dependent memory, there was no relation of fitness or perfusion changes to changes in VEGF or IGF-I. This was the case although baseline VEGF levels were positively associated with perfusion levels. We also did not find any relationship between the intervention and another candidate factor that may regulate angiogenesis and neurovascular coupling, PDGF-C. Recent evidence has highlighted PDGF-C as a player in the neurovascular crosstalk (see Lee et al., 2013 for a review). Interestingly, VEGF and PDFG levels correlated with each other, making it unlikely that methodological problems in determining their levels in the blood caused the negative finding. Correlational analyses did also not reveal any significant association between changes in serum or plasma BDNF neither with fitness or perfusion nor with hippocampal volumes.

Although fitness and perfusion benefits were unrelated to increases in IGF-I, hippocampal volume changes (whole HC, HH and HB) were correlated with IGF-I changes across the entire sample, particularly in the control group. Interestingly, these changes in IGF-I were also positively related to late recall performance in the VLMT. This cognitive measure was also unaffected by increased fitness or fitness-related perfusion benefits, which suggest that the positive relationship between IGF-I and late verbal recall was independent of fitness. A direct relationship between IGF-I and cognition has been previously observed in animal experiments, showing that administration of IGF-I can restore cognitive performance in aged animals (Trejo et al., 2007). The independence of the relationship between IGF-I and hippocampal volume from fitness or perfusion changes is further supported by the stepwise multiple regression analysis. This analysis yielded that changes in perfusion and changes in IGF-I levels independently accounted for variance in hippocampal volume changes (see Fig. 3).

Why would changes in IGF-I levels be associated with changes in hippocampal volumes over time (independent of exercise)? One possibility is that IGF-I levels are influenced by factors of bodily health that are not directly related to exercise, such as nutrition and glucose metabolism (Voss et al., 2013b). This possibility is supported by findings by Amir et al. (2007) that basal levels of IGF-I were lower in older individuals with high fitness than in individuals with low fitness, and that transient IGF-I increases after training were only significant in higher fit individuals (Amir et al., 2007). Furthermore, increases in blood IGF-I have been found to occur only during the training period (Berg and Bang, 2004) and to drop immediately within 10 minutes after training, leaving the possibility open that IGF-I levels change rapidly during and after training, and thus have only transient effects on brain vasculature and neural function in the hippocampus. Future studies will have to monitor such possibilities more closely through repeated sampling of IGF-I levels, nutrition and blood levels pertaining to metabolic status.

Our negative findings regarding BDNF may have different reasons. We measured BDNF serum and plasma levels not only before and after the intervention but also at six additional time points during the intervention period (see Supplementary Fig. S1) and found large fluctuations in both serum and plasma BDNF (see also Knaepen et al., 2010 for discussion). The high background levels in human plasma may explain this high intra-individual variability. Aside from methodological issues, subject-specific peripheral BDNF levels can vary already at a diurnal basis due to sex-related cortisol circadian rhythm or hormonal fluctuations (Begliuomini et al., 2008; Pluchino et al., 2009) and energy balance and nutritional variables might also affect peripheral BDNF concentrations. Previous studies have also reported effects of the BDNF genotype (Val66Met polymorphism) on activity-related BDNF secretion in HC neurons (Egan et al., 2003) and on BDNF serum levels (Lang et al., 2009). However, we did not find any difference in serum or plasma levels between carriers of the Met allele and Val/Val homozygotes. Another factor that has to be considered with regard to our negative findings of exercise-induced increases in BDNF levels is the amount of exercise that the older adults underwent. In rodents, upregulation of hippocampal BDNF occurs when mice run voluntarily about 3000–10,000 m/day in the running wheel (Bjørnebekk et al., 2005; Griesbach et al., 2008; Johnson and Mitchell, 2003). In the absence of a young group who underwent a similar intervention regime, we cannot determine whether the negative findings documented here were caused by aging or reflected insufficient training intensity. Furthermore, a previous exercise study in elderly humans (Leckie et al., 2014) that found a significant increase in serum BDNF in the exercise group (despite a non-significant time × group interaction) as a function of age was based on a much longer training period of one year and had a larger sample size ($n_T = 47$). Finally, BDNF levels were measured peripherally in serum and plasma and not in CSF or directly from the hippocampus. Although rodent studies have found relations between serum and cortical BDNF levels (Karege et al., 2002), exercise might also elevate BDNF centrally without significantly effecting peripheral levels (Knaepen et al., 2010).

A lack of exercise-induced increases in VEGF, BDNF and IGF-I in healthy old adults has been also reported in previous exercise studies (e.g., Erickson et al., 2011; Voss et al., 2013b; Coelho et al., 2013; Vital et al., 2014). For instance, Voss et al. (2013b) did not find any group difference in changes of BDNF, VEGF or IGF-I between the aerobic walking and non-aerobic stretching control group after 1-year training. Similar to their study, the present measures of VEGF and IGF-I are limited to pre- and post-exercise time points. However, effects of exercise on these growth factors might be fast and transient (e.g., Rojas Vega et al., 2006; Knaepen et al., 2010), in contrast to vascular and structural hippocampal changes that might occur later and be more long-lasting. Thus, future studies should consider the time course of exercise-induced plasticity by assessing short-term and long-term measures of growth factors, perfusion, volume and memory. Microdialysis in animal models could further help to unravel how circulating growth factors are related to changes in blood flow, hippocampal plasticity and memory. Furthermore, larger sample sizes are needed to reach sufficient power, in particular in order to detect significant group × time interactions. In this regard, it also important to note that our study was restricted to a sample of cognitively and physically healthy older adults with no history of neurological or cardiovascular disease and it thus remains to be established how generalizable the findings are to the general elderly population.

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## Conflict of interest

The authors declare no conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2015.10.084.

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