Molecular Psychiatry (2015) 20, 585–593

www.nature.com/mp

ORIGINAL ARTICLE

Vascular hippocampal plasticity after aerobic exercise in older adults

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Aerobic exercise in young adults can induce vascular plasticity in the hippocampus, a critical region for recall and recognition memory. In a mechanistic proof-of-concept intervention over 3 months, we investigated whether healthy older adults (60–77 years) also show such plasticity. Regional cerebral blood flow (rCBF) and volume (rCBV) were measured with gadolinium-based perfusion imaging (3 Tesla magnetic resonance image (MRI)). Hippocampal volumes were assessed by high-resolution 7 Tesla MRI. Fitness improvement correlated with changes in hippocampal perfusion and hippocampal head volume. Perfusion tended to increase in younger, but to decrease in older individuals. The changes in fitness, hippocampal perfusion and volume were positively related to changes in recognition memory and early recall for complex spatial objects. Path analyses indicated that fitness-related changes in complex object recognition were modulated by hippocampal perfusion. These findings indicate a preserved capacity of the aging human hippocampus for functionally relevant vascular plasticity, which decreases with progressing age.

Molecular Psychiatry (2015) **20,** 585–593; doi:10.1038/mp.2014.114; published online 14 October 2014

INTRODUCTION

Physical exercise in old age is a promising lifestyle intervention[1–3] to slow age-associated decline of human long-term memory.[4,5] The hippocampus has been in the focus of research into the effects of aerobic exercise on memory because of its potential for plasticity and its role in age-related long-term memory decline.[5,6] Converging evidence from research in rodents and young/middle-aged humans indicates that a hallmark effect of aerobic exercise is to increase perfusion in the hippocampus.[7] In humans, this increase has been associated with improved recall of a recently learned word list.[7] It is unclear whether aerobic exercise in older humans can also improve hippocampal perfusion. Consequently, it remains unknown through which physiological mechanisms exercise may enhance hippocampal function in older individuals. In addition, there is ongoing uncertainty to what extent the memory effects of exercise in old age depend on modality (verbal versus spatial), retrieval demands (recall versus recognition) and delay (early versus late).

Aerobic exercise in mice induces neurogenesis in the dentate gyrus (DG) subfield of the hippocampus.[8,9] This neurogenesis is associated with improved pattern separation, a process that allows forming non-overlapping neural representations for similar memories.[9] A related finding has been recently observed in young human adults, where aerobic exercise also improved performance in a memory test that poses high demands on pattern separation.[10] In this way, aerobic exercise may improve memory by minimizing interference between highly similar inputs due to an increase in DG neurogenesis.[10]

Exercise-related neurogenesis is associated with specific functional circuits that may influence pattern separation for certain types of information.[11] Newly born granular cells in the DG seem to predominantly receive projections from perirhinal cortex (PRC) and lateral entorhinal cortex (LEC).[12] Interestingly, these regions convey novel object information to the hippocampus and are required for visual discrimination and object recognition memory.[13,14] PRC lesions have been found to impair visual discrimination between similar complex stimuli[15–17] and aging comes with deficits in PRC-dependent pattern separation in rodents and monkeys.[18] In contrast, the parahippocampal cortex and the medial entorhinal cortex convey information about spatial layouts and presumably aid context recall.[14,19] If effects of aerobic exercise are specific to a PRC-LEC network linked to the hippocampus, one would expect differential improvement in object-recognition tests that pose high demands on pattern separation.[10] However, an effect of exercise on object recognition memory in older adults has not yet been demonstrated.

Aerobic exercise in old age is effective in preventing the human hippocampus from atrophy over a period of 1 year.[20] This raises the question of whether the positive effects of exercise on hippocampal volume are related to vascular plasticity, that is, increased perfusion. A preliminary observation, using only post-intervention measures (of arterial spin labeling) in a small group of six individuals, provided encouraging results in this direction.[21]

In this mechanistic proof-of-concept study, healthy older humans (60–77 years) were pseudo-randomly assigned to either an aerobic exercise group (indoor treadmill, $N_T = 21$) or to a control group (indoor progressive-muscle relaxation/stretching, $N_C = 19$). Fitness levels were determined before and after each type of intervention using spiroergometry (oxygen consumption at ventilatory anaerobic threshold, $VO_{2\ VAT}$). Gadolinium contrast-

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Received 14 January 2014; revised 7 August 2014; accepted 21 August 2014; published online 14 October 2014

based perfusion imaging (3 Tesla) was used to measure changes in regional cerebral blood flow and volume (rCBF/ rCBV). Changes in hippocampal volumes were assessed by high-resolution structural magnetic resonance imaging (MRI) at 7 Tesla. On the basis of previous studies suggesting a relationship of exercise-related brain changes to verbal memory in young/middle-aged adults[7] and to spatial memory in older adults,[20] we also used verbal (Verbal Learning and Memory Test, VLMT) and spatial object memory (Complex Figure Test, CF) as primary outcome measures of long-term memory effects. The CF Test allows assessments of free recall (both early and late) as well as recognition, the latter test posing high demands on pattern separation and hippocampal integrity.[10,22] Similarly, we also measured early recall, late recall and recognition memory in the VLMT. Path analysis (structural equation modeling, SEM) was used to determine whether memory gains as a function of increased fitness were accounted for by mediation through hippocampal volume changes or were also compatible with a direct effect of perfusion on memory. Finally, we obtained serum cortisol levels, because cortisol is known to have a negative effect on verbal long-term memory in old age[23] and therefore might modify potential exercise effects on verbal memory.

MATERIALS AND METHODS

Participants and experimental design

This study was designed as a controlled 3-month intervention trial with 40 sedentary healthy older participants (mean age = 68.4 ± 4.3 years, 55% female; for a detailed description of subject recruitment, see Supplementary Methods). They signed written informed consent and received monetary compensation for participation. The study was approved by the ethics committee of the University of Magdeburg. After completion of the initial comprehensive cardiological examination, neuropsychological assessment and MRI sessions, participants were pseudo-randomly assigned to either the aerobic exercise group ($N_T = 21$) or the control group ($N_C = 19$). The groups were matched on age, gender, body mass index, activity level (self-reported mean endurance per week) and verbal memory recall (VLMT long delay; see Table 1 for group characteristics) to prevent differences in fitness or cognitive abilities between groups at baseline. There was no drop-out during the intervention.

Fitness assessments

For determination of aerobic fitness, oxygen consumption at the ventilatory threshold ($VO_{2\ VAT}$) was assessed by graded maximal exercise testing on a recumbent cycle ergometer. To avoid any cardiovascular risk, some participants did not perform up to maximal exhaustion, and thus did not exceed a respiratory exchange ratio greater than 1.1.[24] Therefore, $VO_{2\ VAT}$ was calculated (instead of $VO_{2\ MAX}$) as a reasonably accurate predictor of changes in cardiovascular fitness.[25] Additionally, MET scores (metabolic equivalent of task; 1 MET = 1 kcal kg^{-1} h^{-1} or 3.5 ml kg^{-1} min^{-1}) were calculated to assess training-related metabolic rates as well as self-reported 'lifestyle-related' activity levels (see Supplementary Methods for details about the determination of fitness levels and metabolic rates).

Table 1. Characteristics of the training and control group

Variables	*Aerobic exercise group*	*Relax/stretching group*
N	21	19
Age (years)	68.8 (4.5)	67.9 (4.1)
Gender (% female)	52%	58%
BMI	25.0 (2.9)	25.2 (3.0)
Verbal learning (delayed recall)	9.6 (3.1)	9.7 (2.7)
Self-reported activity (MET)	2414 (1851)	2295 (1087)
MMSE	28.95 (0.92)	28.95 (0.91)

Means (s.d.) shown for all participants enrolled in the study ($N = 40$). Variables for both groups were matched at baseline. Self-reported level of exercise was assessed using the Freiburg Questionnaire of Physical Activities. Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; MMSE, Mini-Mental State Examination.

Intervention

Physical exercise (training group). The aerobic endurance training was carried out on stationary treadmills (H/P/Cosmos Mercury Medical, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany). Participants received individually optimized 30-min interval training three times per week for 12 weeks, plus 5-min warm up and 5-min stretching at the end of each training session. Sport scientists and a medical doctor specialized in sport medicine (DA) supervised the intervention. Training intensity was determined by target heart rate (Karvonen method[26]), 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[27]) 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 came in twice a week and received 45 min of supervised progressive muscle relaxation/stretching training.[28] 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 while 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.

Cognitive functioning

Each participant completed the 'Mini Mental State Examination'[29] and the 'Beck Depression Inventory' (BDI-II[30]). Cognitive testing before and after the intervention included the following neuropsychological tests: The 'VLMT'[31] (adapted German version of the 'Rey Auditory Verbal Learning Test' (RAVLT)[32]), 'CF Test'[32,33], 'Digit Span Test' (forward and backward[34]) and 'Multiple-Choice Word Test' (MWT-B[35]). 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 Table 2). Outcomes of the other cognitive measures are summarized in the Supplementary Table S3.

Complex figure test. The CF Test was used to assess spatial object recall (early, late) and recognition memory. The Rey-Osterrieth Complex Figure[32] was applied pre-intervention, whereas the Modified Taylor Complex Figure[33] served as the post-intervention measure. The Modified Taylor Complex Figure was developed as a comparable measure to the Rey-Osterrieth Complex Figure with similar accuracy scores[36] and is a valid alternative for testing visual long-term memory avoiding implicit learning that can occur when the same version of the Rey-Osterrieth Complex Figure is used for repeated testing sessions.[37] The recognition memory test of the CF requires discrimination between similarly looking complex objects and thus poses high demands on accurate memory representations and pattern separation (see Supplementary Methods for details).

Verbal learning and memory test. The VLMT assesses learning of 15 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. Here we focused on early recall, late recall and recognition memory, in line with the memory measures collected in the CF Test. For a detailed description of the testing procedure, see Supplementary Methods.

3 Tesla MRI and perfusion imaging

Gadolinium contrast-based perfusion imaging at 3T (Siemens Magnetom, Verio (Erlangen, Germany), 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}, 1.6 mm in-plane resolution, 3 mm slice thickness, 20 slices with 10% gap; see Supplementary Figure S2) and quantitative perfusion maps for rCBF and rCBV were calculated. Mean

Table 2. Group means (s.d.) for fitness, perfusion, volume and memory pre and post intervention

	Aerobic exercise group		*Relax/stretching group*	
Variables	*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.	0.99 $(.27)_{15}$	0.97 $(.26)_{15}$	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 $(83)_{19}$

Abbreviations: CF, Complex Figure Test; rCBF, regional cerebral blood flow; rCBV, regional cerebral blood volume; VLMT, Verbal Learning and Memory Test. $VO_{2\ VAT}$ was measured in ml kg^{-1} min^{-1}. Bilateral rCBF and rCBV were measured in ml 100 g^{-1} min^{-1} and ml 100 g^{-1} * 10, respectively. Bilateral hippocampal volumes were measured in cm^3. Serum cortisol levels denote nmol l^{-1}. Subscripts indicate number of available data (see also Supplementary Table S1 for missing data).

perfusion values were determined for bilateral hippocampus, hippocampal subsections (head, body, tail) and hippocampal subfields (only body of the hippocampus). Additionally, general (non-hippocampal) gray matter (GM) perfusion was calculated. For a detailed description of the perfusion analyses, see Supplementary Methods.

7 Tesla high-resolution MRI

High-resolution structural T1-weighted images (whole-head, magnetization-prepared rapid gradient-echo) were acquired within 1 week pre and post intervention using a 7T 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, segmentation of hippocampal regions (head, body, tail plus subfields (subiculum, CA1 and CA2-3/DG) in the hippocampal body) was performed manually on the 7T high-resolution magnetization-prepared rapid gradient-echo. For a detailed description of the segmentation procedure, see Supplementary Methods and Supplementary Figure S2.

Statistical analysis

Repeated-measures ANOVA. Intervention effects were first examined using repeated-measures analysis of variances (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 (IBM Corp., IBM SPSS Statistics, V 20, Armonk, NY, USA). All dependent variables met criteria for normal distribution. Paired *t*-tests (two-tailed) were performed to assess changes in fitness levels from pre to post intervention. 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 because of different seasonal starting points across subjects with a potential relevance of hydration status on brain structure that has been shown in previous studies (e.g., dehydration-related shrinkage of brain tissue and an associated increase in ventricular volume[38,39]). Although temperature did not significantly affect brain structures in our study, significant negative correlations between changes in temperature and changes in perfusion were found (hippocampal rCBF: $r = -0.38$, $P = 0.027$; GM rCBF: $r = -0.35$, $P = 0.047$).

Correlations of changes. Correlational analyses were performed on percentage change in $VO_{2\ VAT}$, in bilateral hippocampal rCBV/rCBF, bilateral hippocampal volume (head, body, tail) and memory performance. This approach should additionally account for inter-individual differences in hippocampal size. To assess whether exercise-related effects were specific to the hippocampus or unspecific, we further analyzed perfusion and volume changes for overall GM. The correlational analyses performed were planned comparisons, motivated by findings from previous exercise studies,[7,10,20] to determine the extent to which relationships among fitness change, perfusion change and memory change are also present in old age.

For all variables of interest, data were missing occasionally (see Supplementary Methods and Supplementary Table S1 for details about missing data and outlier detection). Little's χ^2 test showed that data were missing completely at random (MCAR; chi-square = 134.07, df = 130, $P = 0.386$). Thus, to conduct correlational analyses including all available variables of interest, we used Full Information Maximum Likelihood (FIML). FIML is a SEM-based missing data estimation approach that yields unbiased parameter estimates and standard errors. This method estimates a likelihood function for each individual based on the variables that are present, such that all available data are used. To test associations for significance, two models are estimated: the H_0 (unrestricted) model, in which variables (percentage change) are correlated and the H_1 (restricted or fixed) model, in which the correlations are set to zero. The difference between the two log-likelihoods is used to derive the chi-square (a value with $P < 0.05$ indicates significant correlations between changes). Reported correlation coefficients (*r*) are standardized parameter estimates: $r_{x,y} = COV_{x,y}/(SD_x * SD_y)$. All dependent variables (percentage changes) were regressed on age, gender and ΔT. Correlations were estimated in Mplus (Version 6.1, 2011). For illustrative purposes, regressions of the residuals were plotted in SPSS after controlling for effects of age, gender and ΔT.

Structural equation modeling. Furthermore, we used path analysis and SEM to test different models for the associations between the measured variables. This technique uses a combination of statistical criteria and qualitative causal assumptions. On the basis of previous research, we defined different models that predict how fitness-related changes in hippocampal perfusion and volume modulate memory. The consistency between our theoretical models and the empirical data was tested in Mplus by means of FIML. Goodness of model fit was evaluated by the chi-squared difference test. The following fit indexes are reported: χ^2/DF, Comparative Fit Index (CFI) and the Root Mean Square Error of Approximation (RMSEA). All dependent variables (percentage changes) were regressed on age, gender and ΔT.

RESULTS

Effects of exercise on fitness-related variables

Physical exercise was effective in increasing aerobic fitness levels (Supplementary Figure S1 and Table 2). A repeated-measures

ANOVA yielded a significant time (pre versus post intervention) × group (training versus control) interaction for oxygen consumption at the ventilatory anaerobic threshold ($VO_{2\ VAT}$, $F(1,34) = 6.49$; $P = 0.016$). Whereas the exercise group improved significantly in $VO_{2\ VAT}$ after the intervention ($\Delta VO_{2\ VAT} = 10.42\%$, $t(19) = 3.78$, $P = 0.001$), the relaxation/stretching group did not change in fitness levels ($\Delta VO_{2\ VAT} = 0.53\%$, $P > 0.80$). The improvement in fitness due to exercise was also confirmed by a significant intervention effect on the power levels that participants could achieve at VAT (Watt $_{VAT}$, $F(1,31) = 5.13$; $P = 0.031$). Independent two-sample *t*-tests between groups also reflected significant differences in overall metabolic rate across the entire intervention period (see Supplementary Methods for details), with significantly higher training-related MET values in the exercise group than the control group ($MET_T = 469$, $MET_C = 148$, $t(20.76) = 23.57$, $P < 0.001$).

Note that the increase in fitness levels in the training group was observed, despite the fact that self-reported 'lifestyle-related' activity levels during the intervention period (e.g., domestic or work-related activities) were higher in the control compared with the training group ($MET_T = 5395$, $MET_C = 9608$, $t(22.5) = -3.18$, $P = 0.013$) as revealed by the IPAC ('International physical activity questionnaire', see Supplementary Methods). Furthermore, fitness levels at baseline were significantly higher in the control than in the training group as revealed by a main effect of group on $VO_{2\ VAT}$ ($F(1,33) = 6.23$; $P = 0.018$) and subsequent *t*-tests ($t(37) = 2.41$, $P = 0.021$). This pattern holds true, although self-reported level of exercise (assessed by the 'Freiburg Questionnaire of Physical Activities'), which was used to match groups in terms of activity levels before intervention, did not differ.

Fitness-related changes in perfusion, volume and memory

Two types of planned analyses were conducted. First, variables of interest were entered into repeated-measures ANOVAs with time and group as factors (see Table 2 for pre and post values). Second, correlational analyses were performed across all participants (training and control group) taking the percentage change (from pre to post intervention) in $VO_{2\ VAT}$ as an outcome measure of change in fitness (Table 3). The correlational analyses were also performed in the exercise group only and are reported in the Supplementary Table S2. These analyses considered changes in rCBF/rCBV, memory measures (verbal and spatial object recall and recognition memory) and hippocampal volumes (head, body and tail; derived by segmentation on the 7T high-resolution magnetization-prepared rapid gradient-echo). To include all available data and thus account for missing cases, correlations were computed using FIML estimation. However, Pearson's correlations using pairwise deletion for missing data led to similar results.

Repeated-measures ANOVA. ANOVAs with hippocampal rCBF/rCBV, hippocampal volume and memory did not yield any significant time × group interaction (all *P*-values > 0.10). This might be related to the aforementioned findings that the control group showed higher fitness levels at baseline and/or engaged in a more active lifestyle beyond the intervention, which may have reduced the intervention effect due to other experience-dependent effects operating to a greater extent in the control than in the exercise group. Regarding time-related changes, we found a significant main effect of time on hippocampal perfusion (rCBF: $F(1,27) = 6.12$, $P = 0.020$; rCBV: $F(1,27) = 6.61$, $P = 0.016$) with a significant interaction between time and age (rCBF: $F(1,27) = 5.85$, $P = 0.023$; rCBV: $F(1,27) = 6.82$, $P = 0.015$). More specifically, changes in perfusion were negatively correlated with age ($r_{rCBF} = -0.40$, $P = 0.023$; and $r_{rCBV} = -0.42$, $P = 0.016$, see Figure 1). Follow-up ANOVAs within each group (note that unlike *t*-tests, ANOVAs included age as covariate) confirmed a significant change in hippocampal rCBF and rCBV for the exercise group (rCBF: $F(1,12) = 8.34$, $P = 0.014$; rCBV: $F(1,12) = 5.27$, $P = 0.040$) but not the control group (CBF/CBV: $P > 0.79$). As can be seen in Figure 1, the younger individuals of our sample tended towards perfusion increases whereas the older individuals tended towards decreases. There were no significant main effects of time on recall or recognition performance neither for the VLMT (all *P*-values > 0.20) nor for the CF Test (only a trend for late recall: $P = 0.080$; all other *P*-values > 0.20) and no time × age interactions (all *P*-values > 0.14).

A previous exercise study in older individuals[20] reported an improvement in spatial memory in both the training and control group (with no significant time × group interaction). In this aforementioned study, analyses were performed by means of paired *t*-tests within each group without using age and gender as covariates (as it was carried out in the reported ANOVAs). If we replicate this analysis in our sample, we also find a significant improvement in spatial object recall in both groups (all *P*-values < 0.02).

Correlations of changes. The results of the correlational analyses are depicted in Figure 2 and Table 3. Changes in aerobic fitness

Table 3. Correlation coefficents (*r*) for the relationships of changes in fitness, perfusion and volume to memory across all participants.

Fitness-related changes in perfusion and volume

Variables (percentage change)	*Hippocampal rCBF*	*Gray matter rCBF*	*Hippocampal rCBV*	*Gray matter rCBV*	*Hippocampal head vol.*	*Gray matter vol.*
$VO_{2\ VAT}$	**0.48****	0.58**	0.35*	0.32*	**0.37***	0.23

Correlations between changes in fitness, perfusion and hippocampal volume with changes in memory

Variables (percentage change)	*CF early recall*	*CF late recall*	*CF recognition*	*VLMT early recall*	*VLMT late recall*	*VLMT recognition*
$VO_{2\ VAT}$	0.31*	0.09	**0.39***	0.23	0.19	0.06
Hippocampal rCBF	0.42*	0.01	**0.42***	0.13	0.13	0.43*
Gray matter rCBF	0.36*	−0.06	0.30	−0.03	−0.03	0.24
Hippocampal rCBV	0.16	−0.06	0.50**	−0.14	0.12	0.33
Gray matter rCBV	0.02	−0.18	0.35	−0.30	0	0.20
Hippoc. head vol.	0.34*	0.17	**0.42***	−0.16	0.31	0.37*

Abbreviations: CF, Complex Figure Test; rCBF, regional cerebral blood flow; rCBV, regional cerebral blood volume; VLMT, Verbal Learning and Memory Test. Correlations were tested for significance ($P < 0.05$), using Full Information Maximum Likelihood (FIML), a missing-data estimation approach controlling for possible confounding effects of age, gender and ΔT. Reported correlation coefficients (*r*) are standardized parameter estimates. $VO_{2\ VAT}$ was measured in ml kg^{-1} $min - 1$. Bilateral rCBF and rCBV were measured in ml $100\ g^{-1}\ min^{-1}$ and ml $100\ g^{-1} * 10$, respectively. Bilateral hippocampal volumes were measured in cm^3 (after manual segmentation). Asterisks highlight significant correlations (*$P < 0.05$; **$P < 0.01$). Boldface demarcates correlations shown in Figure 1; $N = 40$.

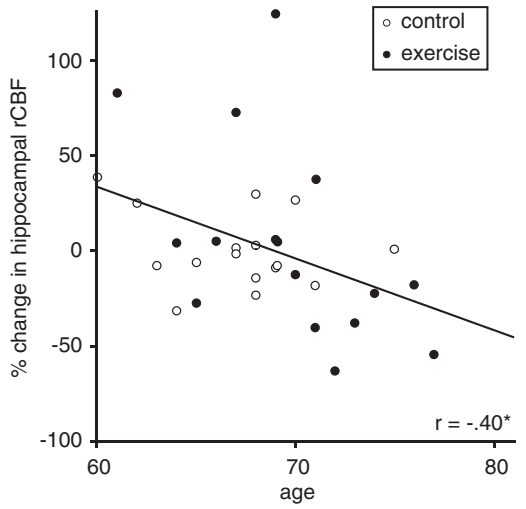

Figure 1. Relationship between changes in perfusion and age. Changes in hippocampal blood flow (rCBF) and blood volume (rCBV; not shown) over a 3-month period were negatively correlated with age (*$P < 0.05$). While the younger individuals tended towards perfusion increases as a result of exercise, older individuals tended towards decreases. Note that for this correlation plot no covariates are included. Perfusion changes refer to bilateral hippocampus.

levels were positively associated with changes in hippocampal rCBF ($r = 0.48$, $P = 0.003$; see Figure 2a) and rCBV ($r = 0.35$, $P = 0.031$). Fitness-related changes in perfusion were not restricted to the hippocampus, but also positively related to changes in non-hippocampal cortical rCBF ($r = 0.58$, $P < 0.001$) and rCBV ($r = 0.39$, $P = 0.048$).

Regarding hippocampal structure, significant positive correlations were found between changes in fitness level and in hippocampal head volumes ($r = 0.37$, $P = 0.032$; see Figure 2a), with no significant effect on body or tail volumes (all *P*-values > 0.20). These fitness-related changes in hippocampal head volume showed strong correlations with changes in hippocampal rCBF ($r = 0.66$, $P < 0.001$; see Figure 2a) and rCBV ($r = 0.74$, $P < 0.001$). This relationship was specific to the hippocampus, as whole-brain GM volume was not reliably correlated with changes in fitness ($P > 0.20$). Although hippocampal head volumes did not increase overall in the exercise group, the majority of individuals showing an increase in hippocampal perfusion from pre to post intervention also showed increased volume (see Figure 2a).

To examine whether fitness-related changes in hippocampal perfusion, volume or both were specifically associated with spatial object memory performance, we conducted correlational analyses on changes in early recall, late recall and recognition in the CF Test. Additionally, verbal list learning was assessed using the VLMT, which has been used in an exercise study with young/middle-aged individuals.[7]

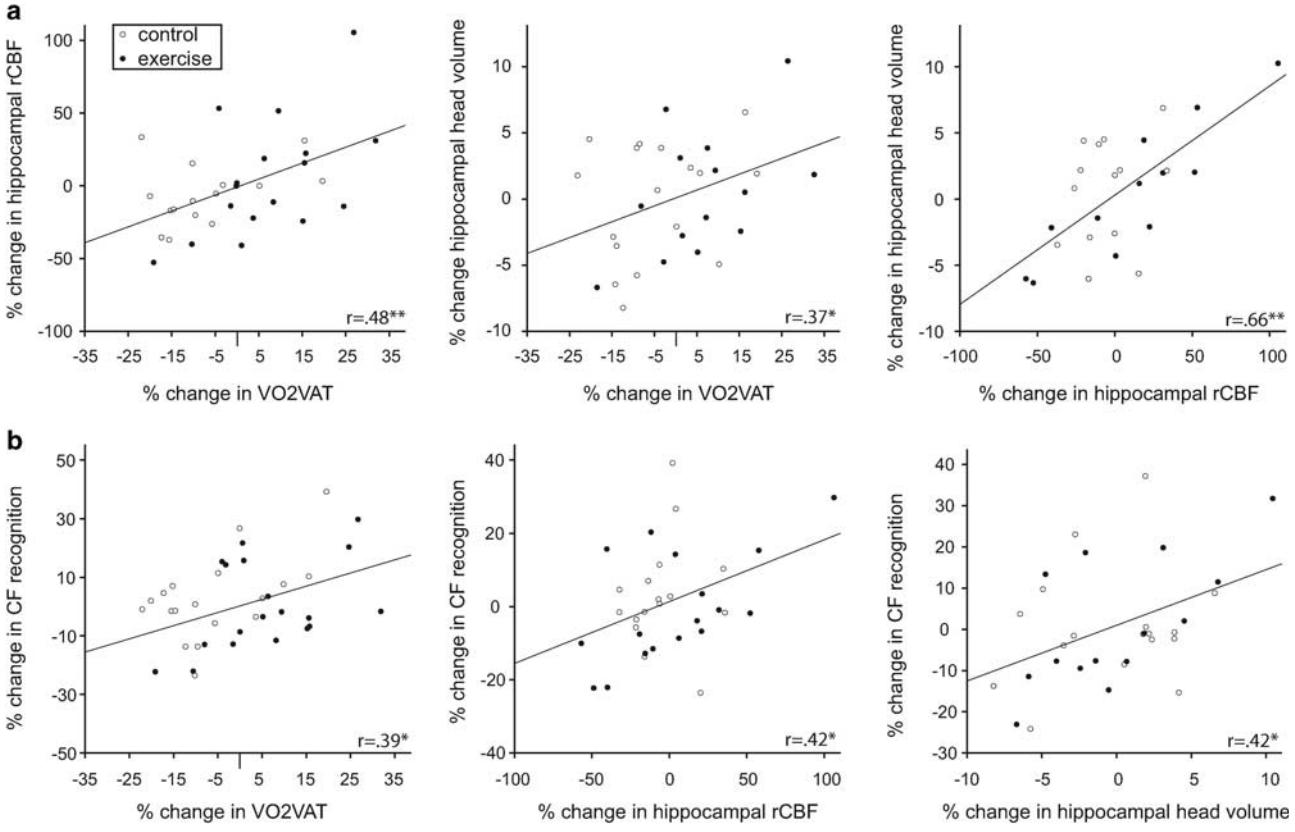

Figure 2. Relationships of changes in fitness, hippocampal perfusion and volume to memory. (**a**) Changes in aerobic fitness levels ($VO_{2\ VAT}$) over a 3-month period were positively correlated in hippocampal blood flow (rCBF), blood volume (rCBV; not shown) and hippocampal head volume. Changes in hippocampal perfusion and volume were closely related. (**b**) Fitness-related changes in hippocampal perfusion and head volume were associated with changes in recognition memory for complex spatial objects in the CF Test. The same relationships were found for early, but not for late, CF recall (see Results and Table 3 for details). Correlations were tested for significance ($P < 0.05$), using Full Information Maximum Likelihood (FIML), a missing-data estimation approach. Plots display partial residuals after controlling for age, gender and ΔT (temperature changes from pre to post intervention). Volumes and perfusion changes refer to bilateral hippocampus. Asterisks highlight significant correlations (*$P < 0.05$; **$P < 0.01$).

Changes in early recall and recognition memory in the CF Test were positively correlated with changes in $VO_{2\ VAT}$ (early recall: $r = 0.31$, $P = 0.050$; recognition: $r = 0.39$, $P = 0.012$; see Figure 2b), hippocampal rCBF (early recall: $r = 0.42$, $P = 0.013$; recognition: $r = 0.42$, $P = 0.015$; see Figure 2b), hippocampal rCBV (recognition: $r = 0.50$, $P = 0.003$) and hippocampal head volume (early recall: $r = 0.34$, $P = 0.037$; recognition: $r = 0.42$, $P = 0.013$; see Figure 2b). There were no significant correlations between changes in CF late recall and fitness, perfusion or volume (all *P*-values > 0.30). Similar to the CF Test, significant correlations were also found between changes in recognition memory in the VLMT and changes in hippocampal rCBF ($r = 0.43$, $P = 0.017$) as well as hippocampal head volume ($r = 0.37$, $P = 0.042$). For VLMT early and late recall, no significant associations were found ($P > 0.30$).

Additionally, we investigated whether correlations of changes in fitness, perfusion or head volume with early recall and recognition were significantly higher than correlations with late recall. Differences between correlation coefficients (using Fischer's Z-Test as described in ref. 40), showed that correlations of changes in perfusion (rCBF/rCBV) with early recall and recognition memory were significantly higher than the corresponding correlations for late recall performance in the CF Test ($P < 0.05$).

Finally, we tested whether memory benefits were linked to perfusion in specific hippocampal subfields (subiculum, CA1 and CA2/3-DG). These subfield-specific analyses showed that correlations among hippocampal perfusion, fitness changes and changes in CF memory (early recall and recognition) were most consistent when the entire hippocampus was taken into account (see Supplementary Results for more details).

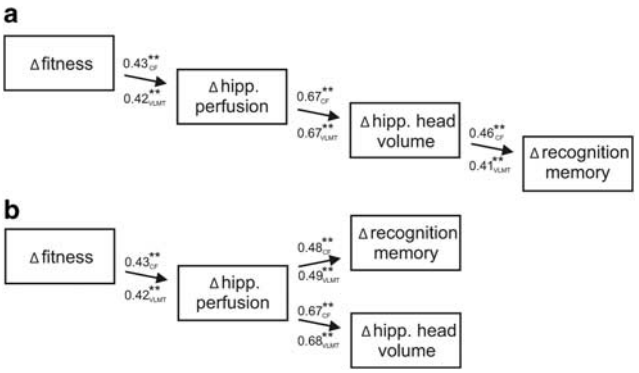

Figure 3. Estimated path models predicting the relationships of changes in fitness, hippocampal perfusion and volume to recognition memory. (**a**) The initial Model A predicts that fitness-related memory improvements are modulated by hippocampal volume changes (see also Supplementary Figure S3 for an alternative Model A′ that includes additional paths from fitness and perfusion to all other variables) (**b**). Model B predicts that memory benefits are directly linked to hippocampal perfusion. All models showed good fit to the data (CFI > 0.98, RMSEA < 0.06). Model B yielded slightly better fit than Model A, suggesting that memory changes can be well accounted for by perfusion changes, with no strong additional contribution of hippocampal volume. Note that the same model (B), in which hippocampal perfusion and volumes are replaced by non-hippocampal cortical perfusion and whole-brain gray matter, volumes did not fit the data well (CFI < 0.90, RMSEA > 0.08). Numbers are standardized path coefficients. Asterisks highlight significant paths (**$P < 0.01$). CF, Complex Figure Test; VLMT, Verbal Learning and Memory Test.

Structural equation modeling

Our correlational analyses revealed associations between fitness-related changes in hippocampal perfusion, hippocampal head volumes and recognition memory in both the CF Test and the VLMT. Next, we used path analysis and SEM to test different models in which effects of changes in physical fitness on memory are accounted for by hippocampal volume and/or perfusion changes. In the following analyses, we focused on rCBF as a measure of perfusion (rCBF and rCBV changes were strongly correlated: $r = 0.69$, $P < 0.001$).

First, we defined Model A, which is illustrated in Figure 3a. According to Model A, increases in fitness lead to higher hippocampal perfusion, leading to an increase in hippocampal head volume, which in turn results in improved recognition memory. Model A is based on the assumption that volume changes in the hippocampus are the result of different types of plasticity including vascular plasticity, synaptogenesis and neurogenesis. Accordingly, volume changes should be the best predictor for cognitive changes. Changes in perfusion, on the other hand, should directly relate to changes in hippocampal volume,[41] but not necessarily to cognition independently from hippocampal volume.

The fit of the data to Model A was good (spatial object memory: $\chi^2(6) = 6.76$, CFI = 0.997, RMSEA = 0.056; verbal memory: $\chi^2(6) = 5.51$, CFI = 1, RMSEA = 0). All path coefficients in the model differed reliably from zero (see Figure 3a). Thus, a model in which fitness-related improvements in recognition memory are modulated by structural (volume) changes in the hippocampus provides a viable rendition of the observed data, with an estimated path coefficient from volume to memory of 0.46 and 0.41 for spatial object (CF Test) and verbal (VLMT) recognition memory, respectively.

Furthermore, we examined whether adding direct paths from fitness to volume and memory and from perfusion to memory would improve our initial model (see Supplementary Figure S3). This Model A′ accounts for the possibility that fitness can modulate hippocampal volume also via other mechanisms that are orthogonal to perfusion. Additionally, Model A′ assumes that fitness and perfusion changes may have a direct influence on cognitive changes, that is, effects that are not mediated by hippocampal structure or volume. Model A′ did not result in statistically significant improvement in fit over Model A ($\chi^2\text{diff}(3)_{CF} = 4.11$ and $\chi^2\text{diff}(3)_{VLMT} = 2.86$, $P > 0.25$). Consequently, the more parsimonious Model A is favored over Model A′. Furthermore, as highlighted in Supplementary Figure S3, neither the path from perfusion nor the path from volume to memory was reliably different from zero. This is compatible with the possibility that perfusion is a strong mediator of volume changes in the hippocampus (and thus accounts for a large portion of the variance), both likely affecting hippocampal memory functions.

Finally, we tested whether a model in which memory benefits are directly linked to hippocampal perfusion would also provide a good account of our data (see Figure 3b). This alternative Model B predicts that increases in fitness lead to increases in perfusion, which result in improved recognition memory. Furthermore, this model includes a direct path from perfusion to hippocampal volume. According to this model, changes in perfusion are sufficient to account for changes in cognition, because hippocampal volume is predominantly determined by perfusion and does not reflect additional plastic processes that contribute to cognitive change. Model B yielded a good fit (spatial object memory: $\chi^2(6) = 6.13$, CFI = 0.996, RMSEA = 0.023; verbal memory: $\chi^2(6) = 3.43$, CFI = 1, RMSEA = 0). Furthermore, the effect of perfusion on memory in Model B was significant (path coefficient = 0.48 and 0.49 for spatial and verbal recognition memory, respectively; see Figure 3b). Models A and B are not nested, therefore a direct statistical comparison with a chi-square test is not possible. However, adding a direct path from volume to memory in Model B did not significantly improve the model fit ($\chi^2\text{diff}(1)_{CF} = 1.17$ and $\chi^2\text{diff}(1)_{VLMT} = 0.89$, $P > 0.28$). These findings suggest that there is no strong unique contribution of changes in hippocampal volume to changes in memory.

Multiple regression analyses and SEM controlling for non-specific gray-matter changes

To test whether our findings could also be explained by more general, hippocampus-unspecific, changes in GM, we repeated our SEM analyses for Model A and B after replacing hippocampal by non-hippocampal GM perfusion and hippocampal head volumes by whole-brain GM volumes. In addition, we performed multiple regression analyses (using FIML estimation), controlling for non-specific changes in GM.

Path models A and B, in which fitness-related changes in recognition memory are modulated by whole-brain structural and/or perfusion changes, did not yield acceptable fits to the data (CFI < 0.90, RMSEA > 0.08[42]).

Furthermore, multiple regression analyses in which changes in CF or VLMT recognition memory were regressed on hippocampal and GM perfusion (allowing both to correlate) revealed a specific effect of hippocampal rCBF on CF recognition ($r = 0.64$, $P = 0.048$) and VLMT recognition ($r = 0.88$, $P = 0.005$) memory, respectively, with no significant correlation between non-hippocampal GM rCBF and memory ($P > 0.15$). Similarly, we performed multiple regressions in which changes in cognition are explained by changes in hippocampal head and GM volumes (allowing both to correlate). Again, we found a significant relation between hippocampal head volumes and recognition memory in the CF Test ($r = 0.37$, $P = 0.026$) that was also marginally significant for the VLMT ($r = 0.34$, $P = 0.057$). However, in the same regression models whole-brain volumes and memory were not significantly related ($P > 0.08$). These results suggest that the benefits of fitness-related changes in perfusion and volume on recognition memory are mediated by the hippocampus rather than being a whole-brain effect.

DISCUSSION

We observed that increasing fitness levels were positively associated with changes in hippocampal perfusion after a 3-month intervention period. Such a relationship has been previously reported in young/middle-aged adults[7] and our results indicate that it is preserved in old age. Performance changes in early spatial object recall and recognition showed a positive association to fitness and perfusion, whereas delayed recall did not. We also observed that exercise-related improvements in perfusion among our older participants declined with age, suggesting that the capacity for vascular hippocampal plasticity may be age-dependent.

Recent exercise studies in mice suggest that newly born granular cells in the DG preferentially receive projections from PRC and LEC.[11,12] Moreover, findings in animals and humans indicate that object recognition memory and object pattern separation critically depend on PRC and DG, respectively.[13,19,43] Therefore, the relationship of both fitness and perfusion to configural object recognition memory found here is consistent with the neurogenic connectivity observed in mice. Furthermore, it is conceivable that also tests of early recall can be sustained by PRC/LEC and DG, whereas for delayed recall, which was not affected by exercise, other hippocampal subfields and medial temporal regions, such as the parahippocampal cortex, may play a greater role. However, note that angiogenic effects of increased fitness could also be uncoupled from neurogenesis and relate to plasticity of mature neurons.[44] Furthermore, other mechanisms such as hypoxia associated with exercise challenge[45] can also induce angiogenesis independent of neurogenesis in the hippocampus.

The perfusion changes observed were negatively related to age (Figure 1). Whereas the younger individuals (between 60 and 70 years) tended towards perfusion increases as a result of exercise, older individuals tended towards decreases. Studies in old mice report a similar pattern, where the exercise-related potential for neurogenesis and angiogenesis in old age is reduced[6,46] and is absent in very old mice.[46] It remains unclear why the potential for increased perfusion after exercise tends to decline with progressing age. One source of variability contributing to this decline may be amyloid deposition, which can occur in around 20% of apparently healthy older adults[47] and may have a negative impact on neural metabolism and plasticity.[48] Another possibility is that stress-related increases in cortisol levels in older individuals have a negative influence on neurovascular plasticity.[49] However, we found no relationship between cortisol levels at baseline or changes in cortisol and perfusion changes. The age-independent sources of interindividual variability that causes increases in perfusion in some individuals who show increased fitness, but not in others are also unclear (Figure 2a). One factor that needs to be addressed in studies with larger sample sizes is that carriers of the APOE4 allele may show a different benefit from exercise.[2,50] Also, although gadolinium-based measures of perfusion are sensitive to increases in capillaries, and thereby likely to detect effects of angiogenesis, other factors may contribute as well, and should be assessed in future studies. In particular, MRI angiography at high fields may provide estimates of exercise-induced changes in vessel numbers, the radius of vessels, and their tortuosity.[51]

As expected, changes in hippocampal perfusion were closely linked to changes in hippocampal volume.[51] Volumetric increases might be directly linked to perfusion through increased vascularization, or be related to subsequent cell proliferation, synaptogenesis and dendritic branching. Indeed, we observed that fitness-related changes in hippocampal perfusion were apparent in all subfields and not limited to DG, making it likely that increased fitness-related perfusion largely accounted for fitness-related increases in hippocampal volume. We used path analysis to estimate whether the benefit of increased hippocampal perfusion for recognition memory was mediated by increased hippocampal volume or could also be accounted for by a direct influence of perfusion on memory (Figure 3). A model in which perfusion had a direct effect on memory yielded good fit to our data and was statistically parsimonious (Figure 3b). This finding is compatible with the possibility that higher levels in perfusion can directly benefit neural function. Indeed, there are a number of mechanisms through which increased perfusion could be associated with improved neuronal function aside from improving supply of oxygen and nutrients. These include the upregulation of brain-derived neurotrophic factor after physical exercise in the hippocampus[52] and increase of insulin-like growth-factor,[53] which may act through brain-derived neurotrophic factor and also improve neuronal glucose uptake.[54]

We observed a relationship between exercise-related changes in perfusion and early recall for configural spatial object memory, but not for verbal memory. In contrast, a previous study in young adults showed a correlation of hippocampal perfusion and early verbal recall[7] (this study did not have a non-exercise control group). We hypothesized that these different patterns might be related to fluctuations in serum cortisol levels because previous studies of memory in old age have shown a negative correlation between cortisol levels and verbal memory (i.e., ref. 23, 55). We indeed observed that serum cortisol levels at baseline were negatively correlated with early verbal recall across the entire sample (see Supplementary Results). Individuals who had high baseline levels of cortisol showed decreased levels post intervention, and this was associated with improved early verbal recall. Collectively, these data indicate that fluctuations in serum cortisol that are unrelated to the exercise intervention may have a considerable impact on verbal memory and this impact may obscure the effects of (a short-lasting) exercise intervention in older adults.

Developing interventions to promote healthy aging critically depends on a better understanding of the neurobiological mechanisms through which physical activity protects and improves memory functions in the brain.[54] Our results indicate

that exercise-related changes in memory are closely linked to vascular changes in the hippocampus. They also suggest that among older individuals, the capacity for vascular hippocampal plasticity is age-dependent. We observed that, although the cognitive effects of this plasticity were relatively specific, its extent within the hippocampus was not and comprised several subfields of the hippocampal body. It remains to be established whether more long-lasting interventions, particularly when combined with cognitive stimulation such as novelty[56] and navigation,[57] would lead to broader cognitive benefits that extend also to delayed free recall.[58] Although our findings are encouraging towards developing clinical interventions to promote healthy aging and prevent cognitive decline, it is also evident that considerable research efforts are necessary to understand the pathophysiological and genetic causes of individual variability in neurovascular plasticity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank the German Center for Neurodegenerative Diseases and the Otto-von-Guericke University (IKND) for funding this study and the Leibniz Institute for Neurobiology for providing access to their 7 Tesla MR scanner. Furthermore, we thank Urte Schneider for recruitment and neuropsychological testing and Anica Weber for subfield segmentation. We also thank Hartmut Schütze for editing the figures. LB was supported by grants from the Swedish Research Council, the Swedish Research Council for Health, Working Life, and Welfare, Swedish Brain Power, an Alexander von Humboldt Research Award, and a donation from the af Jochnick Foundation.

AUTHOR CONTRIBUTIONS

ED, NM and ML conceived the project and designed the experiments. SD, MG, US, AB and AM performed the experiments and supervised the project. DA and RB-D performed the cardiological examination. SD, MG, US, AB, KN, ED, AM and ML analyzed the data and/or contributed to analyzing tools. ED, LB, UL, ML, SD and AM wrote the manuscript. All authors discussed the results and implications and commented on the manuscript.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)