Exercise training increases size of hippocampus and improves memory

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Edited* by Fred Gage, Salk Institute, San Diego, CA, and approved December 30, 2010 (received for review October 23, 2010)

The hippocampus shrinks in late adulthood, leading to impaired memory and increased risk for dementia. Hippocampal and medial temporal lobe volumes are larger in higher-fit adults, and physical activity training increases hippocampal perfusion, but the extent to which aerobic exercise training can modify hippocampal volume in late adulthood remains unknown. Here we show, in a randomized controlled trial with 120 older adults, that aerobic exercise training increases the size of the anterior hippocampus, leading to improvements in spatial memory. Exercise training increased hippocampal volume by 2%, effectively reversing age-related loss in volume by 1 to 2 y. We also demonstrate that increased hippocampal volume is associated with greater serum levels of BDNF, a mediator of neurogenesis in the dentate gyrus. Hippocampal volume declined in the control group, but higher preintervention fitness partially attenuated the decline, suggesting that fitness protects against volume loss. Caudate nucleus and thalamus volumes were unaffected by the intervention. These theoretically important findings indicate that aerobic exercise training is effective at reversing hippocampal volume loss in late adulthood, which is accompanied by improved memory function.

aging | brain | cognition | plasticity | MRI

Deterioration of the hippocampus precedes and leads to memory impairment in late adulthood (1, 2). Strategies to fight hippocampal loss and protect against the development of memory impairment has become an important topic in recent years from both scientific and public health perspectives. Physical activity, such as aerobic exercise, has emerged as a promising low-cost treatment to improve neurocognitive function that is accessible to most adults and is not plagued by intolerable side effects often found with pharmaceutical treatments (3). Exercise enhances learning and improves retention, which is accompanied by increased cell proliferation and survival in the hippocampus of rodents (4–6); effects that are mediated, in part, by increased production and secretion of BDNF and its receptor tyrosine kinase trkB (7, 8).

Aerobic exercise training increases gray and white matter volume in the prefrontal cortex (9) of older adults and increases the functioning of key nodes in the executive control network (10, 11). Greater amounts of physical activity are associated with sparing of prefrontal and temporal brain regions over a 9-y period, which reduces the risk for cognitive impairment (12). Further, hippocampal and medial temporal lobe volumes are larger in higher-fit older adults (13, 14), and larger hippocampal volumes mediate improvements in spatial memory (13). Exercise training increases cerebral blood volume (15) and perfusion of the hippocampus (16), but the extent to which exercise can modify the size of the hippocampus in late adulthood remains unknown.

To evaluate whether exercise training increases the size of the hippocampus and improves spatial memory, we designed a single-blind, randomized controlled trial in which adults were randomly assigned to receive either moderate-intensity aerobic exercise 3 d/wk or stretching and toning exercises that served as a control. We predicted that 1 y of moderate-intensity exercise would increase the size of the hippocampus and that change in hippocampal volume would be associated with increased serum BDNF and improved memory function.

Results

Aerobic Exercise Training Selectively Increases Hippocampal Volume. One hundred twenty older adults without dementia (Table 1) were randomly assigned to an aerobic exercise group ($n = 60$) or to a stretching control group ($n = 60$). Magnetic resonance images were collected before the intervention, after 6 mo, and again after the completion of the program. The groups did not differ at baseline in hippocampal volume or attendance rates (Table 2 and *SI Results*). We found that the exercise intervention was effective at increasing the size of the hippocampus. That is, the aerobic exercise group demonstrated an increase in volume of the left and right hippocampus by 2.12% and 1.97%, respectively, over the 1-y period, whereas the stretching control group displayed a 1.40% and 1.43% decline over this same interval (Fig. 1*A*). The moderating effect of aerobic exercise on hippocampal volume loss was confirmed by a significant Time × Group interaction for both the left [$F(2,114) = 8.25$; $P < 0.001$; $\eta_p^2 = 0.12$] and right [$F(2,114) = 10.41$; $P < 0.001$; $\eta_p^2 = 0.15$] hippocampus (see Table 2 for all means and SDs).

As can be seen in Fig. 2, we found that aerobic exercise selectively increased the volume of the anterior hippocampus that included the dentate gyrus, where cell proliferation occurs (4, 6, 8), as well as subiculum and CA1 subfields, but had a minimal effect on the volume of the posterior section. Cells in the anterior hippocampus mediate acquisition of spatial memory (17) and show more age-related atrophy compared with the tail of the hippocampus (18, 19). The selective effect of aerobic exercise on the anterior hippocampus was confirmed by a significant Time × Group × Region interaction for both the left [$F(2,114) = 4.05$; $P < 0.02$; $\eta_p^2 = 0.06$] and right [$F(2,114) = 4.67$; $P < 0.01$; $\eta_p^2 = 0.07$] hippocampus. As revealed by t tests, the aerobic exercise group showed an increase in anterior hippocampus volume from baseline to after intervention [left: $t(2,58) = 3.38$; $P < 0.001$; right: $t(2,58) = 4.33$; $P < 0.001$] but demonstrated no change in the volume of the posterior hippocampus (both $P > 0.10$). In contrast,

Author contributions: K.I.E., M.W.V., R.S.P., C.B., J.A.W., E. McAuley, and A.F.K. designed research; K.I.E., M.W.V., R.S.P., A.S., L.C., J.S.K., S.H., H.A., S.M.W., T.R.W., E. Mailey, V.J.V., S.A.M., B.D.P., E. McAuley, and A.F.K. performed research; K.I.E., M.W.V., and R.S.P. analyzed data; and K.I.E., M.W.V., R.S.P., and A.F.K. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1015950108/-/DCSupplemental.

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Table 1. Characteristics for the aerobic exercise and stretching control groups

Characteristic	Aerobic exercise	Stretching control
n	60	60
Age (y), mean (SD)	67.6 (5.81)	65.5 (5.44)
Sex (% female)	73	60
Attendance (%), mean (SD)	79.5 (13.70)	78.6 (13.61)
Fitness improvement (%), mean (SD)	7.78 (12.7)	1.11 (13.9)

the stretching control group demonstrated a selective decline in volume from baseline to after intervention for the anterior hippocampus [left: $t(2,58) = -3.07$; $P < 0.003$; right: $t(2,58) = -2.45$; $P < 0.01$] but no significant change in volume for the posterior hippocampus (both $P > 0.20$).

The regional specificity of the intervention was investigated further by examining two regions that served as control: thalamus and caudate nucleus. The volume of the thalamus increased for both the aerobic exercise and stretching groups (Fig. 1*C*), but this increase was not significant [$F(2,114) = 0.65$; $P < 0.52$]. Aerobic exercise did not moderate the increase in thalamic volume, as demonstrated by a nonsignificant Time × Group interaction [$F(2,114) = 0.24$; $P < 0.80$]. The volume of both the left and right caudate nucleus declined (Fig. 1*B*), but only for the stretching group. Aerobic exercise attenuated the loss of volume, although the Time × Group interaction was not significant for either the left [$F(2,114) = 2.25$; $P < 0.11$; $\eta_p^2 = 0.03$] or right [$F(2,114) = 1.63$; $P < 0.19$; $\eta_p^2 = 0.02$] hemispheres.

Our results demonstrate that the size of the hippocampus is modifiable in late adulthood and that moderate-intensity aerobic exercise is effective at reversing volume loss. Increased volume with exercise occurred in a selective fashion, influencing the anterior hippocampus but not the posterior hippocampus or the thalamus or caudate nucleus.

Changes in Fitness Are Associated with Increased Hippocampal Volume. The intervention was effective at increasing aerobic fitness levels. The aerobic exercise group showed a 7.78% improvement in maximal oxygen consumption (VO_2 max) after the intervention, whereas the stretching control group showed a 1.11% improvement in VO_2 max (Table 1). This difference between the groups was confirmed by a Time × Group interaction [$F(2,111) = 4.42$; $P < 0.01$; $\eta_p^2 = 0.07$]. We examined whether improvements in fitness levels were associated with the magnitude of the change in hippocampal volume. To test this, we ran correlations between change in aerobic fitness levels and change in hippocampal volume, collapsing across both groups of participants. We found that greater improvements in aerobic fitness level over the 1-y interval were associated with greater increases in hippocampal volume for the left ($r = 0.37$; $P < 0.001$) and right ($r = 0.40$; $P < 0.001$) hemispheres, suggesting that larger changes in fitness translate to larger changes in volume (Fig. 3 *A* and *B*). This result is consistent with several rodent studies of exercise on neurogenesis and BDNF (20, 21). Improvements in VO_2 max were correlated with increases in both anterior (left: $r = 0.28$; $P < 0.001$; right: $r = 0.51$; $P < 0.001$) and posterior (left: $r = 0.32$; $P < 0.001$; right: $r = 0.39$; $P < 0.001$) hippocampal regions, indicating that changes in aerobic fitness have a global influence on hippocampal volume. Correlations between changes in VO_2 max and change in caudate nucleus and thalamic volumes were not significant (all $r < 0.14$; $P > 0.10$).

We reasoned that if higher physical fitness is protective against the loss of brain tissue, then higher fitness levels at baseline would be predictive of less volume loss over the 1-y period. We examined the participants that declined in volume in the stretching group to test this hypothesis, because the stretching group, and not the aerobic exercise group, showed a decline in hippocampal volume over the 1-y interval. We found results partially consistent with this prediction. That is, higher fitness levels at baseline were associated with less hippocampal volume loss over the 1-y interval, for the right ($r = 0.50$; $P < 0.002$) but not for the left ($r = 0.17$; $P < 0.30$) hippocampus. Further, consistent with our expectations, it was only the right anterior hippocampus ($r = 0.48$; $P < 0.003$) that was protected by higher fitness levels at baseline; the posterior hippocampus was not affected by baseline fitness ($r = 0.21$; $P > 0.20$).

BDNF Is Associated with Changes in Hippocampal Volume. Exercise increases levels of BDNF in the hippocampus (5, 7, 20), which, along with the trkB receptor, is considered to be a partial mediator of the enhancing effect of exercise on learning and memory (7, 8). BDNF can be measured in serum, and higher serum levels of BDNF are associated with both better memory function and larger hippocampal volumes (22). Here, we examined whether 1 y of aerobic exercise would change circulating levels of BDNF and whether increased hippocampal volume would be correlated with changes in BDNF. The aerobic exercise group did not demonstrate greater changes in serum BDNF levels compared with the stretching group, as indicated by a nonsignificant Time × Group interaction [$F(1,97) = 1.42$; $P < 0.23$; $\eta_p^2 = 0.01$]. We reasoned, however, that because BDNF mediates cell proliferation in the dentate gyrus of the hippocampus, increased hippocampal vol-

Table 2. Means (SD) for both groups at all three time points

	Aerobic exercise group			Stretching control group		
Variable	Baseline	6 mo	After intervention	Baseline	6 mo	After intervention
VO_2 max	21.36 (4.71)	22.25 (4.66)	22.61 (4.84)	21.75 (4.87)	21.87 (5.07)	21.87 (4.93)
L hippocampus	4.89 (0.74)	4.93 (0.71)	4.98 (0.69)	4.90 (0.80)	4.86 (0.80)	4.83 (0.80)
R hippocampus	5.00 (0.67)	5.03 (0.63)	5.09 (0.63)	4.92 (0.80)	4.89 (0.83)	4.86 (0.82)
L anterior hippocampus	2.86 (0.42)	2.88 (0.41)	2.93 (0.40)	2.84 (0.48)	2.82 (0.48)	2.78 (0.46)
R anterior hippocampus	2.90 (0.40)	2.93 (0.38)	2.99 (0.38)	2.88 (0.48)	2.87 (0.48)	2.84 (0.49)
L posterior hippocampus	2.03 (0.34)	2.04 (0.31)	2.05 (0.30)	2.05 (0.33)	2.03 (0.34)	2.03 (0.37)
R posterior hippocampus	2.05 (0.30)	2.09 (0.27)	2.09 (0.27)	2.03 (0.35)	2.02 (0.37)	2.01 (0.34)
L caudate nucleus	4.65 (0.57)	4.68 (0.57)	4.67 (0.57)	4.66 (0.57)	4.63 (0.51)	4.63 (0.51)
R caudate nucleus	5.04 (0.54)	5.04 (0.52)	5.05 (0.56)	5.06 (0.56)	5.02 (0.57)	5.02 (0.56)
Thalamus	14.11 (1.28)	14.20 (1.32)	14.16 (1.36)	14.22 (1.41)	14.33 (1.36)	14.26 (1.41)
BDNF	21.32 (9.32)	—	23.77 (8.04)	23.41 (9.67)	—	24.04 (10.83)
Accuracy (%)	85.9 (8.2)	84.1 (17.1)	88.2 (7.1)	82.3 (9.9)	82.5 (15.8)	86.0 (8.2)

VO_2 max was measured as ml/kg per min. Brain volumes were measured as cm^3. BDNF was measured as pg/mL. L, left; R, right.

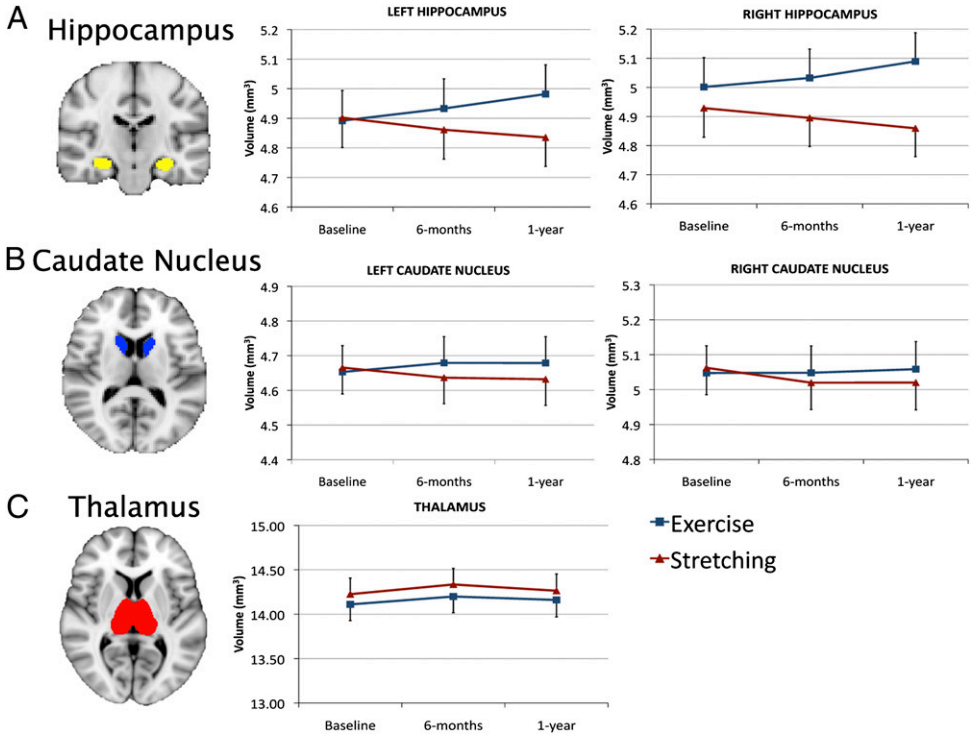

Fig. 1. (*A*) Example of hippocampus segmentation and graphs demonstrating an increase in hippocampus volume for the aerobic exercise group and a decrease in volume for the stretching control group. The Time × Group interaction was significant ($P < 0.001$) for both left and right regions. (*B*) Example of caudate nucleus segmentation and graphs demonstrating the changes in volume for both groups. Although the exercise group showed an attenuation of decline, this did not reach significance (both $P > 0.10$). (*C*) Example of thalamus segmentation and graph demonstrating the change in volume for both groups. None of the changes were significant for the thalamus. Error bars represent SEM.

ume could be associated with increased levels of serum BDNF. Because the aerobic exercise group was the only group to show an increase in volume over the 1-y period, we ran a correlation between change in BDNF and change in hippocampal volume for the aerobic exercise group to test this hypothesis. We found that greater changes in serum BDNF were associated with greater increases in volume for the left ($r = 0.36$; $P < 0.01$) and for the right ($r = 0.37$; $P < 0.01$) hippocampus (Fig. 3 *C* and *D*). Further, these effects were selective for the left ($r = 0.30$; $P < 0.03$) and right anterior hippocampus ($r = 0.27$; $P < 0.04$) and only marginal with the left ($r = 0.25$; $P < 0.06$) and right ($r = 0.22$; $P < 0.08$) posterior hippocampus. There were no associations between changes in serum BDNF and changes in caudate nucleus or thalamus volumes (all $P > 0.50$); nor were there any associations between hippocampal volume and serum BDNF for the stretching control group (all $P > 0.40$). This indicates that exercise-induced increases in BDNF are selectively related to the changes in anterior hippocampal volume resulting from aerobic exercise.

Hippocampal Volume Is Related to Improvements in Spatial Memory. Spatial memory (13, 22) was tested on both exercise and stretching groups at baseline, after 6 mo, and again after the completion of the 1-y intervention to determine whether changes in hippocampal volume translate to improved memory. Both groups showed improvements in memory, as demonstrated by significant increases in accuracy between the first and last testing sessions for the aerobic exercise [$t(2,51) = 2.08$; $P < 0.05$] and the stretching control [$t(2,54) = 4.41$; $P < 0.001$] groups. Response times also became faster for both groups between the baseline and postintervention sessions (all $P < 0.01$), indicating that improvements in accuracy were not caused by changes in speed–accuracy tradeoff. However, the aerobic exercise group did not improve performance above that achieved by the stretching control group, as demonstrated by a nonsignificant Time × Group interaction [$F(1,102) = 0.67$; $P < 0.40$; $\eta_p^2 = 0.007$]. Nonetheless, we found that higher aerobic fitness levels at baseline ($r = 0.31$; $P < 0.001$) and after intervention ($r = 0.28$; $P < 0.004$) were associated with better memory performance on the spatial memory task. Change in aerobic fitness levels from baseline to after intervention, however, was not related to improvements in memory for either the entire sample ($r = 0.15$; $P < 0.12$) or when considering each group separately (both $P > 0.05$). Furthermore, changes in BDNF were not associated with improvements in memory function for either group ($r < 0.15$; $P > 0.20$). On the other hand, larger left and right hippocampi at baseline (both $P < 0.005$) and after intervention (both $P < 0.005$) were associated with better memory performance (12). Therefore, we reasoned that increased hippocampal

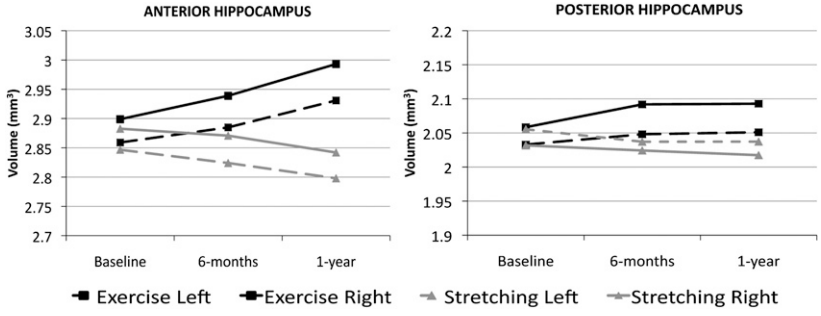

Fig. 2. The exercise group showed a selective increase in the anterior hippocampus and no change in the posterior hippocampus. See Table 2 for Means and SDs.

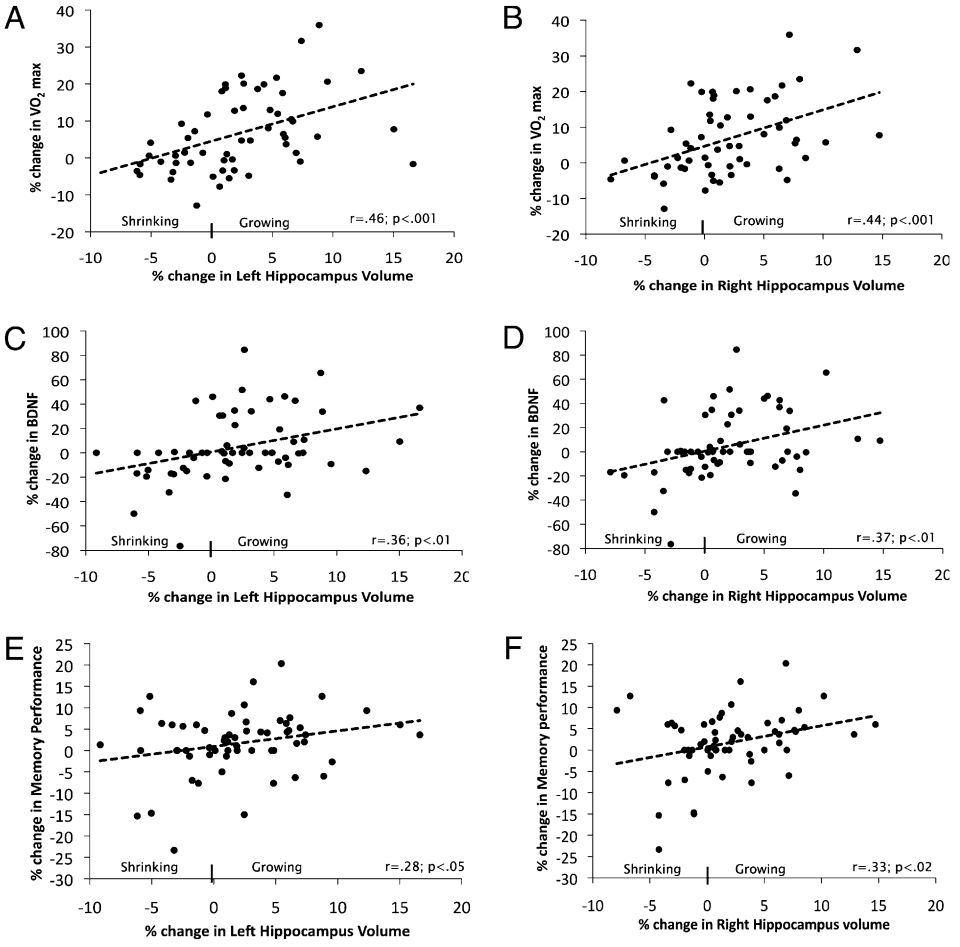

Fig. 3. All scatterplots are of the aerobic exercise group only because it was the only group that showed an increase in volume across the intervention. (*A* and *B*) Scatterplots of the association between percent change in left and right hippocampus volume and percent change in aerobic fitness level from baseline to after intervention. (*C* and *D*) Scatterplots of percent change in left and right hippocampus volume and percent change in BDNF levels. (*E* and *F*) Scatterplots of percent change in left and right hippocampus and percent change in memory performance.

volume after the exercise intervention should translate to improved memory function. In support of this hypothesis, we found that, in the aerobic exercise group, increased hippocampal volume was directly related to improvements in memory performance. The correlation between improvement in memory and hippocampal volume reached significance for left ($r = 0.23$; $P < 0.05$) and right ($r = 0.29$; $P < 0.02$) hemispheres (Fig. 3 *E* and *F*). This indicates that increases in hippocampal volume after 1 y of exercise augments memory function in late adulthood. In contrast, changes in caudate nucleus and thalamus volumes were unrelated to changes in memory performance for either group (all $P > 0.10$).

Discussion

Hippocampal volume shrinks 1–2% annually in older adults without dementia (1), and this loss of volume increases the risk for developing cognitive impairment (2). We find results consistent with this pattern, such that the stretching control group demonstrated a 1.4% decline in volume over the 1-y interval. With escalating health care costs and an increased proportion of people aged >65 y, it is imperative that low-cost, accessible preventions and treatments for brain tissue loss are discovered. In this randomized controlled study of exercise training, we demonstrate that loss of hippocampal volume in late adulthood is not inevitable and can be reversed with moderate-intensity exercise. A 1-y aerobic exercise intervention was effective at increasing hippocampal volume by 2% and offsetting the deterioration associated with aging. Because hippocampal volume shrinks 1–2% annually, a 2% increase in hippocampal volume is equivalent to adding between 1 and 2 y worth of volume to the hippocampus for this age group.

On the basis of the several regions we examined, the effect of exercise was rather selective, influencing only the anterior hippocampus and neither the thalamus nor the caudate nucleus. This indicates that exercise does not influence all brain regions uniformly. In fact, research from human cognitive studies and rodents indicates some specificity, such that exercise influences some brain regions and behaviors but has minimal influence on others (3, 5, 9, 12, 20, 21, 23–25). Such selectivity suggests that there are regionally dependent molecular pathways influenced by exercise. In fact, we found here that changes in serum BDNF levels were associated with changes in anterior hippocampal volume; an important link because the hippocampus is rich in BDNF, and BDNF levels increase with exercise treatments in both rodents (5, 7, 20) and humans (26, 27). BDNF is a putative mediator of neurogenesis and contributes to dendritic expansion (28, 29) and is also critical in memory formation (30–32). Our results suggest that cell proliferation or increased dendritic branching might explain increased hippocampal volume and improvements in memory after exercise; however, increased vascularization (15, 16, 33) and dendritic complexity (34) may also be contributing to increased volume.

Aerobic exercise increased anterior hippocampal volume but had little effect on the posterior hippocampus. Neurons in the anterior hippocampus are selectively associated with spatial memory acquisition (17) and show exacerbated age-related atrophy compared with the posterior hippocampus (18, 19). It is possible that regions demonstrating less age-related decay might also be less amenable to growth. Thus, aerobic exercise might elicit the greatest changes in regions that show the most precipitous decline in late adulthood, such as the anterior hippo-

campus and prefrontal cortex (9). Overall, these data suggest that the anterior hippocampus remains amenable to augmentation.

In sum, we found that the hippocampus remains plastic in late adulthood and that 1 y of aerobic exercise was sufficient for enhancing volume. Increased hippocampal volume translates to improved memory function and higher serum BDNF. We also demonstrate that higher fitness levels are protective against loss of hippocampal volume. These results clearly indicate that aerobic exercise is neuroprotective and that starting an exercise regimen later in life is not futile for either enhancing cognition or augmenting brain volume.

Methods

Participants. Community-dwelling older adults (n = 842) were recruited, and 179 were enrolled. One hundred forty-five participants completed the intervention (81.0% of the participants originally enrolled). Five participants were excluded because they did not attend the 6-mo MRI session, owing to scheduling conflicts; eight participants were excluded because they did not attend the 12-mo follow-up MRI session; and 12 participants were excluded because they had excessive head motion that created inaccurate hippocampal, caudate nucleus, or thalamus segmentations. Therefore, 120 participants had complete MR data from all three sessions (82.7% of the enrolled sample) and were included in the analyses.

Eligible participants had to (*i*) demonstrate strong right handedness (35), (*ii*) be between the ages of 55 and 80 y, (*iii*) score ≥51 on the modified Mini-Mental Status Examination (36), (*iv*), score <3 on the Geriatric Depression Scale to rule out possible depression (37), (*v*) have normal color vision, (*vi*) have a corrected visual acuity of at least 20/40, (*vii*) have no history of neurological diseases or infarcts, including Parkinson's disease, Alzheimer's disease, multiple sclerosis, or stroke, (*viii*) have no history of major vasculature problems, including cardiovascular disease or diabetes, (*ix*) obtain consent from their personal physician, and (*x*) sign an informed consent form approved by the University of Illinois. In addition, all participants had to report being currently sedentary, defined as being physically active for 30 min or less in the last 6 mo. Participants were compensated for their participation.

After completion of the initial blood draw, MR session, and fitness assessment, participants were randomized to an aerobic walking group (n = 60) or a stretching control group (n = 60) (Fig. 4).

Fitness Assessments. Participants were required to obtain consent from their personal physician before cardiorespiratory fitness testing was conducted. Aerobic fitness (VO_2 max) was assessed by graded maximal exercise testing on a motor-driven treadmill. The participant walked at a speed slightly faster than their normal walking pace (≈30–100 m/min), with increasing grade increments of 2% every 2 min. A cardiologist and nurse continuously monitored oxygen uptake, heart rate, and blood pressure (see *SI Methods* for more detail).

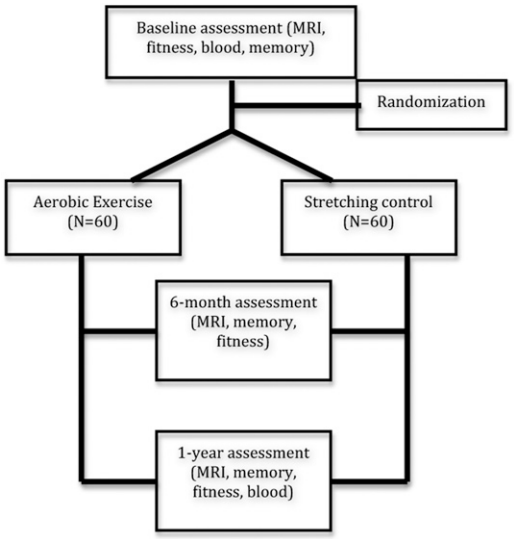

Fig. 4. Flow diagram for the randomization and assessment sessions for both exercise and stretching control groups.

MRI Parameters and Segmentation Algorithm. MR images were collected on all participants within 1 mo of the start of the intervention, after 6 mo, and within 2 wk after the completion of the intervention. High-resolution (1.3 mm × 1.3 mm × 1.3 mm) T1-weighted brain images were acquired using a 3D magnetization-prepared rapid gradient echo imaging protocol with 144 contiguous slices collected in an ascending fashion.

For segmentation and volumetric analysis of the left and right hippocampus, caudate nucleus, and thalamus we used the Oxford Centre for Functional MRI of the Brain (FMRIB)'s Integrated Registration and Segmentation Tool in FMRIB's Software Library version 4.1 (38–40) (see *SI Methods* for more detail).

Training Protocol. ***Aerobic exercise condition.*** For the aerobic exercise program, a trained exercise leader supervised all sessions. Participants started by walking for 10 min and increased walking duration weekly by 5-min increments until a duration of 40 min was achieved at week 7. Participants walked for 40 min per session for the remainder of the program. All walking sessions started and ended with approximately 5 min of stretching for the purpose of warming up and cooling down. Participants wore heart rate monitors and were encouraged to walk in their target heart rate zone, which was calculated using the Karvonen method (41) according to the resting and maximum heart rates achieved during the baseline maximal graded exercise test. The target heart rate zone was 50–60% of the maximum heart rate reserve for weeks 1 to 7 and 60–75% for the remainder of the program. Participants in the walking group completed an exercise log at each exercise session. Every 4 wk, participants received written feedback forms that summarized the data from their logs. Participants with low attendance and/or exercise heart rate were encouraged to improve their performance in the following month.

Stretching and toning control condition. For the stretching and toning control program, all sessions were led and monitored by trained exercise leaders. All classes started and ended with warm-up and cool-down stretching. During each class, participants engaged in four muscle-toning exercises using dumbbells or resistance bands, two exercises designed to improve balance, one yoga sequence, and one exercise of their choice. To maintain interest, a new group of exercises was introduced every 3 wk. During the first week, participants focused on becoming familiar with the new exercises, and during the second and third weeks they were encouraged to increase the intensity by using more weight or adding more repetitions. Participants in the stretching and toning control group also completed exercise logs at each exercise session and received monthly feedback forms. They were encouraged to exercise at an appropriate intensity of 13–15 on the Borg Rating of Perceived Exertion scale (42) and to attend as many classes as possible.

Spatial Memory Paradigm. To test memory function, all participants completed a computerized spatial memory task at baseline, after 6 mo, and again after completion of the intervention (13, 22, 43).

A fixation crosshair appeared for 1 s, and participants were instructed to keep their eyes on the crosshair. After the fixation, one, two, or three black dots appeared at random locations on the screen for 500 ms. The dots were removed from the display for 3 s. During this time, participants were instructed to try and remember the locations of the previously presented black dots. At the end of the 3-s delay, a red dot appeared on the screen in either one of the same locations as the target dots (match condition) or at a different location (nonmatch condition). Participants had 2 s to respond to the red dot by pressing one of two keys on a standard keyboard—the "x" key for a nonmatch trial and the "m" key for a match trial (Fig. 5). Forty trials

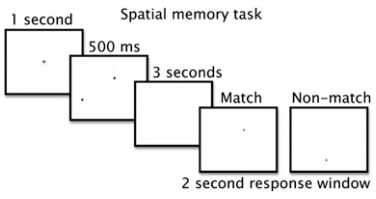

Fig. 5. Display of the spatial memory task used in this study. The spatial memory task load was parametrically manipulated between one, two, or three items (two-item condition shown here). Participants were asked to remember the locations of one, two, or three black dots. After a brief delay, a red dot appeared, and participants were asked to respond whether the location of the red dot matched or did not match one of the locations of the previously shown black dots. This task was administered to all participants at baseline, after 6 mo, and again after completion of the intervention.

were presented for each set size (one, two, or three locations), with 20 trials as match trials and 20 trials as nonmatch trials. Participants were instructed to respond as quickly and accurately as possible. Several practice trials were performed before the task began to acquaint the participants with the task instructions and responses (see *SI Methods* for more detail).

Serum BDNF Assay. Blood was collected at baseline before the intervention and again immediately after the completion of the program. Blood sampling for BDNF analysis was performed approximately 2 wk before the MR sessions. Fasted subjects reported to the laboratory at 0800 hours, at which time blood from the antecubital vein was collected in sterile serum separator tubes (Becton Dickinson). The blood samples were kept at room temperature for 15 min to allow for clotting, after which the samples were centrifuged at $1,100 \times g$ at 4 °C for 15 min. Serum was then harvested, aliquoted, and stored at −80 °C until analysis. Serum BDNF was quantified using an enzyme-linked immunosorbant assay (Human BDNF Quantikine Immunoassay, DBD00, R & D Systems) according to the manufacturer's instructions (see *SI Methods* for more detail).

Analyses. All dependent variables were tested and met criteria for normality and skew before general linear model and Pearson correlations were conducted. Effects of the intervention on VO_2, BDNF, and the volume of the hippocampus, caudate nucleus, and thalamus were examined using an ANOVA with repeated measures with Group (aerobic exercise, stretching control) as a between-subjects factor and Time (baseline, 6 mo, and 1 y) as a within-subject factor. Because the distribution of men and women was slightly different between the two groups (Table 1) we included sex as a covariate in all analyses. In addition, as a safeguard against any residual effects of height or head size, we included intracranial volume (ICV) as a covariate of no interest. Finally, age was slightly different between the two groups, so we also included age as a covariate of no interest in all models.

Correlations were calculated using percent change in VO_2 max, percent change in left and right hippocampal volumes, percent change in BDNF, and percent change in memory performance. We also ran correlations between absolute difference scores while controlling for variation in baseline values. These results were identical, so the correlations from the percent change scores are included in this report. For all correlations, we used a partial correlation approach to control for the possible confounding effects of age, sex, and ICV.

ACKNOWLEDGMENTS. We thank Susan Herrel, Edward Malkowski, Dawn Epstein, Zuha Warraich, Nancy Dodge, and Holly Tracy for help with data collection. This work was supported by National Institute on Aging, National Institutes of Health Grants RO1 AG25667 and RO1 AG25032. K.I.E. was supported by a Junior Scholar Award (P30 AG024827) from the Pittsburgh Claude D. Pepper Older Americans Independence Center and a seed grant (P50 AG005133) awarded through the University of Pittsburgh Alzheimer's Disease Research Center.

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Supporting Information

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SI Text

SI Methods. ***Fitness assessment.*** Oxygen uptake (VO_2) was measured from expired air samples taken at 30-s intervals until a maximal VO_2 (VO_2 max) was attained or to the point of test termination due to symptom limitation and/or volitional exhaustion. VO_2 max was defined as the highest recorded VO_2 value when two of three criteria were satisfied: (*i*) a plateau in VO_2 peak between two or more workloads; (*ii*) a respiratory exchange ratio >1.00; and (*iii*) a heart rate equivalent to their age-predicted maximum (i.e., 220 − age). Because of scheduling conflicts, four participants from the sample of 120 did not have fitness assessments at the postintervention session (three participants in the exercise group and one participant in the stretching control group). These participants were removed from the analyses when conducting correlations using fitness measures but were otherwise included for all other analyses. All other participants (n = 116) had fitness assessments at baseline, after 6 mo, and again after the completion of the 1-y intervention.

MR analysis. All brain images were collected on a 3T Siemens Allegra scanner with an echo time = 3.87 ms, repetition time = 1800 ms, field of view = 256 mm, an acquisition matrix of 192 mm × 192 mm, and a flip angle of 8°.

For segmentation and volumetric analysis of the left and right hippocampus, caudate nucleus, and thalamus, we used the Oxford Centre for Functional MRI of the Brain (FMRIB)'s Integrated Registration and Segmentation Tool (FIRST) in FMRIB's Software Library (FSL) version 4.1. FIRST is a semiautomated model-based subcortical segmentation tool using a Bayesian framework from shape and appearance models obtained from manually segmented images from the Center for Morphometric Analysis, Massachusetts General Hospital, Boston.

Structural and landmark information were obtained from 317 manually segmented and labeled T1-weighted images of the brain from normal children, adults, and pathological populations (including schizophrenia and Alzheimer's disease) and were modeled as a point distribution model in which the geometry and variation of the shape of the structure are submitted as priors. Volumetric labels are parameterized by a 3D deformation of a surface model based on multivariate Gaussian assumptions. FIRST then searches through linear combinations of shape modes of variation for the most probable shape given the intensity distribution in the T1-weighted image (1). Previous studies have successfully used this technique in elderly populations (2, 3).

This method first runs a two-stage affine registration to a standard space template (Montreal Neurological Institute space) with 1-mm resolution using 12 degrees of freedom and a subcortical mask to exclude voxels outside the subcortical regions. Second, the left and right hippocampus, caudate nucleus, and thalamus were segmented with 30, 30, and 40 modes of variation, respectively. Modes of variation were optimized according to leave-one-out cross-validation on the training set to increase the robustness and reliability of the results (1). Finally, boundary correction takes place for each structure that classifies the boundary voxels as belonging to the structure or not according to a statistical probability (z score >3.00; $P < 0.001$). The hippocampus volume comprised the dentate gyrus, the ammonic subfields (CA1-4), the prosubiculum, and the subiculum. The caudate nucleus comprised both the head and tail of the region. The thalamus consisted of the entire region for both left and right hemispheres. Segmentations from each participant and at each time point were visibly checked for any error that could have occurred during the segmentation process. Because of excessive motion artifact or poor image quality at one of the three time points, 12 participants (from the enrolled sample of 145) were not included in the analysis.

Intracranial volume (ICV) is frequently used to adjust the regional volumes for sex and for height (2–4). Here, we calculated ICV as the sum of gray, white, and cerebrospinal fluid and adjusted the volume of each region by this measure using FMRIB's automated segmentation tool in FSL version 4.1 (5, 6). In accordance with other volumetric analyses, adjustment was performed for each region by an analysis of covariance approach: adjusted volume = raw volume − $b \times$ (ICV − mean ICV), where b is the slope of a regression of a region of interest volume on ICV (2–4). Adjusted volume was used for all analyses described in this article.

Anterior and posterior sections of the hippocampus were calculated by determining the center of gravity for both the left and right hippocampus for each subject. The y coordinate from the center-of-gravity calculation was used to divide the region into anterior and posterior sections.

Spatial memory task. The main outcome measure of interest is accuracy (percent correct) for both the stretching and exercise groups. Correlations among all three set sizes ranged from 0.70 to 0.87 at baseline, after 6 mo, and after 1 y, suggesting that collapsing across set sizes would both reduce the number of multiple comparisons and simplify interpretations. Average accuracy rates for each of the time points (baseline, 6 mo, 1 y) were calculated by collapsing across the three set sizes. Percent change in accuracy from baseline to after intervention was calculated for each subject, and correlations with hippocampal volume were done using the percent change measure. Data from 14 of the 120 participants were not included in the analysis because of scheduling conflicts, experimenter error, computer failure, or lack of subject compliance (e.g., less than chance performance). All analyses with memory performance were conducted on the remaining 106 participants (n = 49 for aerobic exercise group, n = 57 for stretching control group).

Serum BDNF. Serum BDNF was quantified using an enzyme-linked immunosorbant assay (Human BDNF Quantikine Immunoassay, DBD00, R & D Systems) according to the manufacturer's instructions. The intra- and interassay coefficients of variation were 5% and 9%, respectively. Briefly, serum samples were diluted 1:80 in the supplied sample diluent and assayed against a standard curve with a 500 $pg{\cdot}mL^{-1}$ highest concentration. The supplied mouse anti-human BDNF–biotin primary antibody and strepavidin–HRP secondary antibody were used at a dilution factor of 1:1,000. After incubation with the provided substrate solution, the reaction was stopped with the addition of stop solution, and the plate was read at 450 nm using a spectrophotometric plate reader (Multiskan Plus, Thermo Labsystems). Sixteen participants (nine from the exercise group, seven from the stretching control group) did not have baseline blood draws because of scheduling conflicts or errors in blood storage. An additional six participants (four from the exercise group, two from the stretching control group) did not have postintervention blood draws because of scheduling conflicts. All correlations and analyses with BDNF were done without including these 22 participants.

SI Results. We examined whether variation in attendance could account for our results. Attendance to the exercise training sessions did not differ between the aerobic exercise and stretching control groups, as demonstrated by nonsignificant t test results [$t(2,118) = 0.37$]. Attendance across the 1-y period was 79.5% for the aerobic exercise group and 78.6% for the stretching

control group. These results indicate that both groups received an equivalent amount of social interaction and attended the same number of exercise sessions.

In addition, independent group t tests demonstrated that the aerobic exercise and stretching control groups did not differ in baseline fitness levels [$t(2,118) = 0.45$], mean years of education [$t(2,118) = 1.21$], baseline levels of serum BDNF [$t(2,102) = 1.19$], or sex [$t(2,118) = 1.55$]. The walking and stretching groups also did not differ at baseline in terms of left [$t(2,118) = -0.07$] or right [$t(2,118) = 0.53$] hippocampal volumes, left [$t(2,118) = -0.12$] or right [$t(2,118) = -0.14$] caudate nucleus volumes, or thalamus [$t(2,118) = -0.46$] volume (all $P > 0.20$). The two groups also did not differ in baseline spatial memory performance in terms of either response time or accuracy [all $t(2,114) < 1.09$]. The aerobic exercise group was slightly older [$t(2,118) = 2.00$; $P < 0.05$] and consisted of more women than the stretching control group. Age and sex were included as covariates in all ANOVA and correlation analyses reported above.

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