

# Cardiovascular fitness modulates brain activation associated with spatial learning

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## ABSTRACT

Aerobic exercise has beneficial effects on cognitive functioning in aging humans, especially on executive functions associated with frontal brain regions. In rodents, exercise has been shown to induce structural and neurophysiological changes especially in the hippocampus and to improve spatial learning. The present study investigated the relationship between cardiovascular fitness, spatial learning and associated patterns of brain activation cross-sectionally and longitudinally in a sample of middle-aged men and women (40–55 years) that took part in a six-month exercise intervention and an additional spatial training. Spatial learning capacities before and after the interventions were measured with a virtual maze task. During this task, participants were repeatedly moved through a virtual town and were instructed to infer the spatial layout of the environment. Brain activations during encoding of the virtual town were assessed with functional magnetic resonance imaging (fMRI). The fMRI data revealed that brain activations during successful spatial learning were modulated by the individual fitness level in a neural network, comprising the hippocampus, retrosplenial cortex, cuneus, precuneus, parahippocampal gyrus, caudate nucleus, insula, putamen, and further frontal, temporal, occipital and cingulate regions. Moreover, physical exercising induced changes in cardiovascular fitness that correlated positively with changes in brain activations in the medial frontal gyrus and the cuneus. However, overall spatial learning performance did not vary with cardiovascular fitness. These data suggest that cardiovascular fitness has an impact on brain regions associated with spatial learning in humans and hence, could be a potent intervention to prevent age-related cognitive decline.

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## Introduction

In recent years, a growing body of research examined the associations between physical activity and cognitive functions in both humans and animals (Hillman et al., 2008). Whereas in humans physical activity seems to improve executive functions and affect associated frontal brain regions in particular, research in rodents has repeatedly shown an effect of physical activity on the hippocampus and associated spatial abilities (van Praag, 2009).

In humans, research on physical activity and cognition has mostly focused on sedentary adults older than 55 years of age (Hillman et al., 2008). Positive associations of physical activity with cognitive functions have been demonstrated for attention (Barnes et al., 2003; Hawkins et al., 1992), simple choice reaction time tasks (Dustman et al., 1984), memory (Ruscheweyh et al., in press), and mental flexibility (Dustman et al., 1984; Gordon et al., 2008). The most consistent finding, however, is a positive association between aerobic exercise or cardiovascular fitness and executive functions (e.g. Barnes et al., 2003; Colcombe and Kramer, 2003; Dustman et al., 1984; Gordon et

al., 2008; Themanson et al., 2006). Colcombe et al. (2004), for example, reported less interference in an Erickson flanker paradigm in participants with higher cardiovascular fitness compared to participants with lower cardiovascular fitness and after an aerobic fitness training compared to a non-aerobic stretching training. Functional magnetic resonance imaging (fMRI) data suggested a more efficient neuronal processing in prefrontal and parietal areas as well as in the anterior cingulate cortex in these high-fit participants as compared to low-fit participants (Colcombe et al., 2004). Moreover, structural MRI studies showed that cardiovascular fitness moderated the age-related decline of gray and white matter in frontal, parietal and temporal regions associated with executive functions (Colcombe et al., 2003, 2006; Gordon et al., 2008; Marks et al., 2007).

Although many interventional studies focused on the effects of cardiovascular fitness on executive functions, recent results point to the impact of cardiovascular fitness on memory as well. In a cross-sectional study, positive associations between physical activity and levels of neurotrophins and memory encoding have been reported (Flöel et al., 2010). There are only a few interventional studies assessing memory functions after aerobic exercise training in humans. Stroth et al. (2009) found a significant improvement of visual-spatial memory in a sample of young adults after a six-week running training. Ruscheweyh et al. (in press) reported a correlation between the increase of total physical activity and better episodic memory in

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elderly humans after both a low and medium intense physical training. Moreover, an increase in hippocampal volume after an aerobic exercise training has been reported for older adults (Erickson et al., 2011). Verbal memory performance was improved after a three-month aerobic exercise training in a study by Pereira et al. (2007). This improvement of verbal memory performance correlated positively with the improvement of the individual cardiovascular fitness level and with the cerebral blood volume (CBV) in the dentate gyrus of the hippocampus. The latter is especially interesting as in rodents an exercise induced increase of CBV in the dentate gyrus correlated positively with neurogenesis (Pereira et al., 2007).

Numerous studies in rodents have demonstrated that wheel-running, which is assumed to be the equivalent for human physical activity, enhanced cell proliferation (Uysal et al., 2005; van Praag et al., 1999a, 1999b, 2005). Physical activity also accelerated the integration of newborn neurons into functional neuronal networks in mice (Snyder et al., 2009). The exact functional significance of new neurons in the adult brain, however, is still unknown. It has been suggested that they play an important role in learning and memory (Kempermann, 2008). On the behavioral level, exercise induced hippocampal neurogenesis in rodents co-occurs with improved spatial learning (Uysal et al., 2005; van Praag et al., 1999a, 2005). Thus, an increase in neurogenesis could be the underlying mechanism of behavioral improvements after exercising. Besides neurogenesis, other physiological and anatomical correlates of neuronal plasticity were affected by physical exercise, like long-term potentiation (van Praag et al., 1999a), the release of nerve growth factors (Cotman and Berchtold, 2002; Vaynman et al., 2004) and dendritic spine density (Stranahan et al., 2007). Taken together, the results of animal studies point to an effect of exercise on neuronal plasticity, especially in the hippocampus and this may result in better spatial learning and memory.

The hippocampus has been recognized as an important structure for spatial cognition at least since the discovery of place cells (Ekstrom et al., 2003; O'Keefe and Dostrovsky, 1971). It is supposed to be a critical structure for the construction of allocentric representations (Parslow et al., 2004). Allocentric representations – often termed cognitive maps – are viewer-independent representations of an environment in which spatial relationships (i.e. directions and distances between objects) can be inferred from any perspective. The hippocampus is part of a broad network comprising regions within and outside the medial temporal lobe that supports spatial cognition and spatial navigation. Within this network, the parahippocampal cortex seems to be important for the construction of the spatial layout of indoor and outdoor scenes (Ekstrom et al., 2003; Epstein, 2008). The retrosplenial cortex and the posterior parietal cortex are hypothesized to be important for transforming ego- into allocentric representations and vice versa (Burgess et al., 2002; Epstein, 2008; Iaria et al., 2007; Maguire, 2001) while the caudate nucleus is supposed to be important for egocentric route learning (Hartley et al., 2003; Maguire et al., 1998). Moreover, cuneus and precuneus are also often described to be part of this navigational network (e.g. Shelton and Gabrieli, 2002; Wolbers et al., 2008), as well as medial frontal regions that have been suggested to play an important role in spatial short-term memory processes (Shelton and Gabrieli, 2002).

Whereas the structures that are likely to be activated during spatial navigation are quite well described in the literature, it is less clear how differences in spatial navigation are reflected in functional activations. Moreover, changes of activation patterns due to training have less often been investigated. Iaria et al. (2003), for example, showed that practice of a spatial navigation task resulted in a switch of strategies in some of the participants. In these participants, activity in the hippocampus decreased and activity in the caudate nucleus increased with practice. Further studies found a reorganization of activation after practice of navigation tasks (e.g. Jordan et al., 2004; Ohnishi et al., 2006). Other evidence has suggested that improved navigational performance may result in increasing or decreasing

activation of the initially activated areas (e.g. Orban et al., 2006; Rauchs et al., 2008). It seems to depend on the task and the instruction used, whether or not improved performance resulted in a change of strategies and whether this was accompanied by an increase, decrease or a reorganization of brain activation.

In the present study, we asked whether cardiovascular fitness is a mediating factor that can influence spatial learning and memory. As outlined above, animal studies have shown that the medial temporal lobe is especially sensitive to exercise induced functional and structural changes. Moreover, cognitive stimulation as in enriched environment paradigms has been shown to induce neuronal plasticity in the mice' hippocampus and enhanced spatial learning (van Praag et al., 2000). In animal models, the mechanisms induced by exercise and environmental enrichment can be separated at the cellular level. The results of these studies suggest that physical exercise stimulates the proliferation of precursor cells while environmental enrichment promotes the long-term survival of new neurons (Kempermann et al., 2010). Interestingly, the effects of exercising and cognitive stimulation seem to be additive: physical activity before cognitive stimulation led to increased neurogenesis compared to both exercise and enrichment alone (Fabel et al., 2009). It is not possible to measure activity-dependent neurogenesis in humans. Nevertheless, it could be hypothesized that increasing physical fitness boosts effects of complex cognitive stimulation in humans as well and thus, results in better spatial learning and modulates brain activity in the spatial navigation network.

In the present study, previously sedentary middle-aged adults were randomly assigned to either an aerobic endurance training or a non-endurance training. Only the former was intended to increase cardiovascular fitness. Moreover, half of the participants of each exercising group received a training of spatial abilities while the other half of each group took part in a perceptual training. Before and after the interventions, all participants performed a maze learning task in the fMRI scanner that has previously been shown to activate structures of the spatial navigation network (Wolbers and Büchel, 2005).

We hypothesized that higher cardiovascular fitness is associated with better spatial learning performance in the maze task cross-sectionally as well as longitudinally. The latter means that higher gains in cardiovascular fitness by physical exercising are positively correlated to improvements in spatial learning performance from pre- to posttest. Higher cardiovascular fitness is hypothesized to correlate with functional brain activations associated with spatial learning. Furthermore, we hypothesized that the correlation between cardiovascular fitness and functional brain activations during spatial learning would be largest for those participants that received an additional spatial training.

## Materials and methods

### Participants

Participants were recruited in the greater Hamburg (Germany) area through announcements in the local newspaper and on local radio stations, through flyers in local stores, public libraries, and cultural centers and through local companies' emailing-lists. This study was part of a larger controlled interventional study that comprised  $n=106$  participants. Forty-seven of them took part in the fMRI study reported here. Inclusion criteria were an age between 40 and 55 years, no history of neurological or psychiatric disorders and a rather sedentary lifestyle for the past five years (i.e. less than two exercise sessions per month). A prescreening by phone guaranteed that all participants invited for the medical check met these criteria.

None of the participants showed signs of clinically relevant depression in the German Version of the Center for Epidemiological Studies Depression Scale (Allgemeine Depressionsskala, ADS; 213

Hautzinger and Bailer, 1993). An extensive medical examination (physical examination of the major organs and musculoskeletal system, complete blood count) before the study confirmed that all participants were in appropriate medical constitution to take part in regular exercise. The major aim of this examination was to identify and exclude participants for whom vigorous exercising would have been hazardous (e.g. unstable cardiovascular diseases, untreated metabolic diseases, acute musculoskeletal disorders). One participant was excluded from participation after this examination due to a congenital heart defect. Of the participants included in the analysis, two were diagnosed with high blood pressure (one participated in the stretching/spatial training, one in the cycling/spatial training) and one was diagnosed with diabetes type II (cycling/perceptual training). All three, however, were on stable medication at pre- and post-test and were therefore not excluded from the analyses. Thus,  $n = 46$  participants were randomly assigned to either an aerobic endurance training (cycling) or a non-endurance training (stretching and coordination, abbreviated as “stretching” in the following). Half of each exercise group was randomly assigned to one of two cognitive interventions, either a spatial training or a perceptual discrimination training. Eleven participants dropped out during the course of the study ( $n = 4$  cycling;  $n = 7$  stretching). Two fMRI-datasets were incomplete due to technical problems during data acquisition. Hence data analysis in this report is based on  $n = 33$  participants. Demographical data for the four resulting subgroups are presented in Table 1.

The four subgroups did not differ with respect to mean age ( $F(3, 29) = 2.22$ ;  $p = .107$ ), mean verbal IQ ( $F(3, 29) = 1.02$ ;  $p = .400$ ), spatial reasoning ( $F(3, 29) = 0.429$ ;  $p = .734$ ), and were balanced in terms of sex. All participants were right-handed and had normal or corrected-to-normal vision. All participants signed written informed consent before entering the study. They received monetary compensation for participation. The study was approved by the ethics committee of the German Psychological Society (Deutsche Gesellschaft für Psychologie; DGPs).

## 248 Study design

249 This study was a controlled longitudinal interventional study. Par-  
250 ticipants were randomly assigned to either an aerobic endurance  
251 training (cycling) or a non-endurance training (stretching and coordi-  
252 nation). Both groups exercised twice a week for six months. Each  
253 training session had a duration of 60 min. To investigate potential in-  
254 teractions of a physical and a cognitive intervention, one half of the  
255 participants of each exercise group received a spatial training; the  
256 other half participated in a visual perceptual training. Cognitive train-  
257 ings took place in six individual, computerized training sessions dur-  
258 ing the last month of the physical intervention (1–2 training sessions/  
259 week). Each of the cognitive training sessions had a duration of ap-  
260 proximately 40 min. At baseline (pretest/T0) and after finishing the  
261 physical training and the cognitive training (posttest/T1), all partici-  
262 pants took part in a cardiovascular fitness test and an fMRI-examina-  
263 tion. Data of the fitness test were also used to determine the training  
264 intensity for the cycling group.

t1.1 **Table 1**  
t1.2 Demographical data for the subgroups. M = mean, SD = standard deviation.

t1.3		Cycling/spatial	Cycling/perceptual	Stretching/spatial	Stretching/perceptual
t1.4	<i>n</i>	8	8	9	8
t1.5	Age <i>M</i> ( <i>SD</i> )	50.25 (4.20)	49.00 (4.28)	50.22 (2.91)	46.00 (3.89)
t1.6	female/male	4/4	5/3	4/5	4/4
t1.7	verbal IQ <sup>a)</sup> <i>M</i> ( <i>SD</i> )	126.50 (12.35)	121.50 (10.07)	125.00 (13.72)	116.13 (15.26)
t1.8	Spatial reasoning <sup>b)</sup> <i>M</i> ( <i>SD</i> )	52.50 (17.85)	47.25 (20.22)	53.00 (7.92)	47.00 (7.05)

a) Assessed with a German multiple choice vocabulary test (Lehrl, 2005).

t1.9 b) Assessed with subtests eight and nine of the German intelligence test “Leistungsprüfsystem” (Horn, 1983). Both tests are paper-and-pencil tests of spatial reasoning and require  
t1.10 mental rotation and spatial imagination of three-dimensional objects. They were developed to measure the factor “Space” according to the intelligence model of Thurstone (1938).

## Assessments

### Assessment of cardiovascular fitness

265  
266  
267 The maximal oxygen uptake (VO<sub>2</sub>peak) was used as the indicator  
268 of individual cardiovascular fitness. VO<sub>2</sub>peak reflects the maximal  
269 capacity of a person’s body to transport and utilize oxygen during  
270 incremental exercise. VO<sub>2</sub>peak was assessed during a three-minute  
271 incremental exercise test on a cycle ergometer (Lode Excalibur  
272 Sport 1000 W, Lode Medical Technology, Groningen, Netherlands).  
273 Heart rate and respiration were continuously measured with an  
274 ergospirometric measuring station (MetaMax I, Cortex, Leipzig, Ger-  
275 many). Oxygen and carbon dioxide concentrations in the breath  
276 were analyzed and served to determine VO<sub>2</sub>peak (MetaSoft 1.11.5,  
277 Cortex, Leipzig, Germany). The initial workload depended on the  
278 participant’s physical constitution and was 25 W or 50 W. Within  
279 3 min, the workload was increased by 25 W (8.3 W/min) and  
280 50 W (16.7 W/min), respectively. The incremental exercise test  
281 was terminated when subjective exhaustion was reached. Due to  
282 technical problems, VO<sub>2</sub>peak datasets were incomplete for  $n = 3$   
283 participants, hence the following analyses involving VO<sub>2</sub>peak data  
284 were based on  $n = 30$  participants.

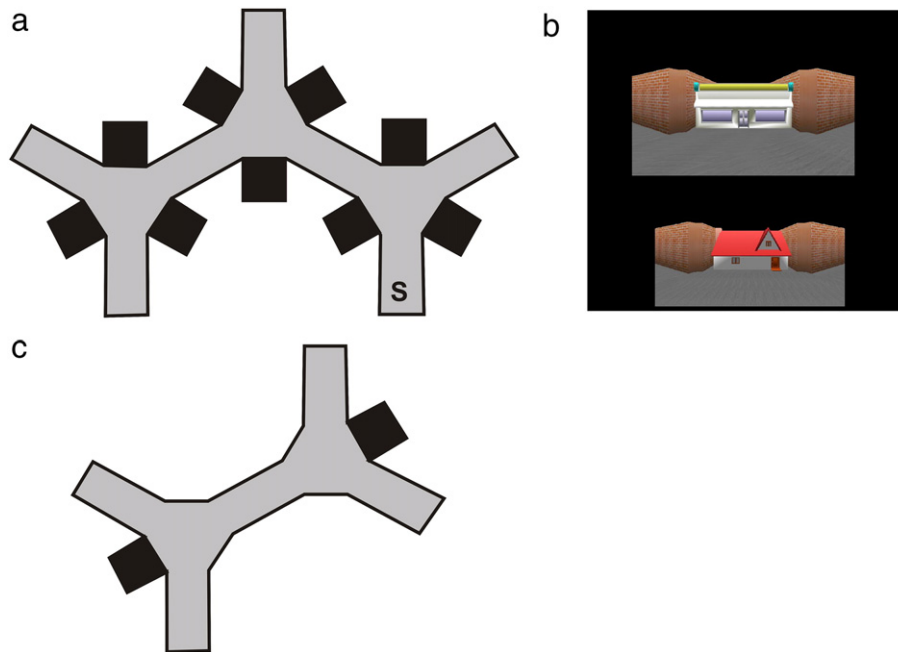
285 Furthermore, a blood sample from the earlobe was taken every mi-  
286 nute during the incremental exercise test to determine the individual  
287 aerobic–anaerobic threshold for each participant (Faude et al., 2009).  
288 The individual heart rate range for the aerobic endurance training  
289 was determined according to this threshold for each participant of  
290 the cycling group: Heart rate at 85% of the workload at the threshold  
291 was appointed the ideal aerobic heart rate. A range of  $\pm 5$  heart-  
292 beats was added to this value.

### Assessment of spatial learning

293  
294 *Experimental stimuli.* For the fMRI assessment of spatial learning  
295 capacities at pre- and posttest, we adapted a virtual maze task from  
296 Wolbers and Büchel (2005) that was slightly modified to create an  
297 easier version for the present middle-aged study population. In this  
298 task, participants were repeatedly and passively moved through a vir-  
299 tual environment (VE) from a first person perspective. Their task was  
300 to generate a mental survey representation of this environment. The  
301 VE contained three intersections (Fig. 1a), and roads were lined by  
302 brick stone walls that restricted the view to the immediate straight  
303 ahead. Nine distinct buildings served as landmarks and were placed  
304 at the intersections. The buildings were hidden behind brick stone  
305 walls. When participants were facing a building, the wall disappeared  
306 and the building was visible.

307 During the retrieval phase 12 pairs of snapshots of the buildings,  
308 which were encountered during the encoding phase, were presented  
309 (Fig. 1b). Pairs always consisted of buildings that were located at ad-  
310 jacent intersections in the learning environment. Participants’ task  
311 was to infer the relative position of the buildings within this environ-  
312 ment. Additionally, a control condition was introduced that was  
313 matched for visual stimulation but did not require spatial learning  
314 abilities. Six experimental and three control sessions were presented





**Fig. 1.** a) Aerial view of the layout of the virtual environment (Version A). Black squares mark positions of buildings. S = starting point. During encoding, participants were moved through the entire environment and encountered all nine buildings. b) Example of a pair of buildings from the retrieval phase. Participants were instructed to imagine standing in front of the top building and to indicate the relative position (left, right, behind) of the bottom building by button press. c) Aerial view of the layout of the control encoding phase. Black squares mark positions of buildings. During the control encoding phase, participants were moved up and down the corridor and saw varying buildings at its end.

inside the scanner. The control sessions took place at the beginning, in the middle and at the end of the experiment.

Four different versions of the experimental environments were constructed (A1, A2, B3, B4) that differed with respect to the arrangement of the intersections (A vs. B) and the buildings used (sets 1–4). To make sure that differences between the experimental sessions and the control sessions were not due to any systematic differences in visual stimulation, the same sets of buildings were used for the control conditions as well (Con1–4). Each of the experimental environments was paired with a control condition comprising a different set of buildings: A1 was paired with Con2, and A2 was paired with Con1 (accordingly for version B). Participants were randomly assigned to one of the four experimental versions at pretest. At posttest, each participant encoded a new layout with new buildings as compared to the pretest. For example, one participant encoded A1 at pretest and B4 at posttest. During the control conditions, he or she then saw building set 2 at pretest and building set 3 at posttest. Thus, each layout and each set of buildings was presented only once to a participant.

### Procedure

**Practice trial.** Each participant underwent extensive practice of the task outside the scanner. They were first asked to learn the names of the buildings they were about to see in the virtual environment. Next, participants were familiarized with the virtual reality paradigm. They passively traveled along the three legs of one intersection and were asked to draw an aerial view map of this intersection afterwards. This procedure was repeated until a correct map was drawn, but no more than three times. The visual appearance of the intersection was identical with the appearance of the virtual environment and was animated in Blitz 3D (Blitz Research, Auckland, New Zealand). In addition, two paper templates of the intersection from an aerial perspective were shown to explain the principle of three legged intersections in more detail. Subsequently, one encoding and one retrieval phase of the experimental and control conditions were shown. During this practice session, the same environments as in the subsequent

fMRI sessions (see below) were used. It was ensured that every participant clearly understood the task before scanning.

**Experimental encoding phase.** Each session started with a fixation cross (20 s), followed by the encoding phase (115 s), another fixation cross (20 s) and the retrieval phase (102 s). A black screen (8 s each) was inserted at the beginning of the experiment and between fixation, encoding and retrieval phases.

In the experimental encoding phase, participants were passively moved through the environment in an ego perspective. As soon as the middle of an intersection was reached, the wall they were facing disappeared with a randomized delay between 0.9 and 1.2 s and the building behind that wall was visible for 4 s. The wall reappeared, the camera performed a 60° turn (at 60°/s) and the tour through the environment continued. After reaching a dead end, the camera made a 180° turn (at 60°/s) and moved on. Traveling from one midpoint to another midpoint took 4 s; the way into a dead end and back to the midpoint of an intersection took 8.94 s. One entire encoding phase lasted 115 s. Each of the nine buildings was visited once per encoding phase.

Before each experimental encoding session, participants were reminded that their task was to establish a mental survey representation of the virtual environment. Furthermore, to encourage the mental construction of a survey map, participants were told that they had to draw a map of the environment after the experiment outside the scanner.

**Experimental retrieval phase.** During the retrieval phase, 12 pairs of buildings were shown in randomized order for 6 s each. Stimuli were presented using Presentation (Version 11.0, Neurobehavioral Systems, Albany, CA, USA). Participants' task was to imagine standing within the environment, facing the top building and to infer the relative position of the bottom building (right, left, behind). Spatial distance between buildings in the virtual environment was held constant across pairs. Responses were given by button press on an MR-compatible button box with index (left), middle (behind) and ring finger (right). The intertrial interval randomly varied between 1.5 and 3 s.

**Control encoding phase.** For the control encoding phase a virtual environment consisting of two three-legged intersections was constructed (Fig. 1c). Participants were moved up and down the main corridor. Varying buildings were placed behind walls at both ends. As in the experimental encoding phase, the camera stopped at the midpoint of an intersection, the wall disappeared after a randomized delay between 0.9 and 1.2 s, and the building was visible for 4 s. The wall reappeared; the camera performed a 180° turn (at 60°/s) and moved on to the midpoint of the second intersection. Another building was shown, the camera turned, moved back to the first intersection, and so forth, until nine different buildings were presented. A small black cube was placed in front of some of the buildings. Participants were instructed to silently count up the number of cubes seen during the entire encoding phase. The number of cubes varied between three and five between the control sessions.

Experimental and control encoding phases were carefully matched for visual stimulation but the control condition did not require spatial learning abilities. Activation differences between these two conditions could hence be attributed to the learning of the spatial layout.

**Control retrieval phase.** Participants saw 12 pairs of buildings and were asked to indicate by button press if the buildings were identical (index finger) or different (ring finger). Each pair was presented for 6 s followed by a black screen with a randomized intertrial interval between 1.5 and 3 s. Four identical and eight different pairs were presented per retrieval phase in randomized order.

#### fMRI data acquisition

MR scanning was performed on a 3T-MRI scanner (TRIO; Siemens AG, Munich, Germany) using a standard head coil. A T2\*-sensitive echo planar imaging sequence (repetition time, 2420 ms; echotime, 30 ms; field of view, 216 mm × 216 mm) was used to acquire 37 axial slices (voxel size, 3 mm × 3 mm × 3 mm). Subsequent to the functional echo planar imaging sequence, a T1-sensitive standard MPRAGE sequence was used to acquire a structural image.

The stimuli were projected with a video projector onto a screen positioned on top of the head coil. This screen was mirrored by a small mirror (9.5 cm × 11.5 cm) attached to the head coil in a 45° angle, right above participants eyes. Participants lay on their backs and watched the experiment in the mirror. All participants had normal vision or wore MR-compatible correction glasses during scanning. None had difficulties seeing the experimental stimuli. To minimize head movements, participants' heads were stabilized with foam pads.

#### Physical training

##### Cycling training

The aerobic endurance training was an indoor-cycling training on stationary bicycles. Training intensity was based on individual results of the incremental exercise test (see [Assessment of cardiovascular fitness](#)). After a warm-up phase, participants were required to keep their target heart rate within that range for approximately 45 min. Subsequently, a cool-down phase followed. The instructor verbally described an imaginary cycling-tour throughout the session, accompanied by music. This training was supposed to improve cardiovascular fitness. Analysis of the mean heart rate during cycling-sessions revealed a mean of  $M(SD) = 139 (9)$  beats per minute across sessions and participants.

##### Stretching training

The stretching and coordination training was supposed to not affect cardiovascular fitness while holding other variables like social interactions, instructors, schedule as similar as possible compared to the cycling training. Each session started with a short warm-up phase, followed by stretching, strengthening, coordination and

relaxation exercises, accompanied by music. In contrast to the cycling group, participants of this group were not told a target heart rate range that they were supposed to keep up during the training sessions. However, heart rate was recorded as well to make sure that the training intensity was well below that of the cycling training. Analysis of the mean heart rate across sessions revealed a mean of  $M(SD) = 105 (9)$  beats per minute for the stretching group. This was significantly lower than those recorded in the cycling group ( $T(31) = 10.62; p < .001; d = 3.70$ ).

#### Cognitive trainings

##### Spatial training

The spatial training was intended to improve subfunctions of spatial cognition that were required for the formation of an allocentric cognitive map in the spatial maze task used to determine spatial capacities in this study. Therefore, two different tasks were introduced: a viewpoint shift task and a path integration task.

**The viewpoint shift task** involved retrieving objects from different perspectives (i.e. a shifted viewpoint) which has previously been shown to require allocentric memory skills and to depend on the hippocampus (King et al., 2002). During this task, participants looked into a virtual courtyard from a fixed perspective. Different objects were sequentially presented in the courtyard and participants were asked to memorize their positions. The courtyard was surrounded by walls with distinct textures or features. Thus, these walls could be used as landmarks to memorize objects' positions. Objects were either animals, vehicles, furniture, plants, technical devices or home appliances. During learning, participants looked into the courtyard from one of two possible viewpoints, either from 0° or 60° relative to the centroid of the area. The viewpoint was held constant across all learning trials within a day (fixed perspective) and alternated between days. Five objects were sequentially shown for 3 s each with an inter-stimulus interval of 1 s. After five objects were presented, a short distractor phase followed and subsequently participants were asked to retrieve the objects' positions from the same or a different perspective. This learning-retrieval cycle was repeated for 20 min or until all five positions were correctly retrieved. A new set of objects was used in the next training session.

**Path integration** is the ability to keep track of changes in orientation and position during movement through monitoring self-motion (Etienne and Jeffery, 2004; Wolbers et al., 2007). Path integration is a crucial ability for the formation of cognitive maps (Etienne and Jeffery, 2004) and has been shown to be associated with hippocampal function (Whishaw et al., 1997; Wolbers et al., 2007). In the path integration task, participants saw a virtual desert (uniform surface without landmarks) on a computer screen. In ego perspective, they were passively moved straight forward, made a turn, moved further and stopped. Then, they had to point back to their starting location using a joystick. For each trial, the pointing error was determined. This task was repeated for 10 min using different turning directions and angles. Participants significantly improved performance in both of the spatial training tasks across training sessions but improvements did not differ between the cycling and stretching groups. Details of the cognitive training as well as improvements across sessions are beyond the scope of this paper and will be reported elsewhere (Holzschneider et al., submitted for publication).

##### Perceptual training

The cognitive control training was introduced to avoid unspecific effects of a cognitive intervention. It was supposed to not affect spatial abilities while holding variables like duration of individual training sessions, computerized training, etc. comparable to the spatial training. Participants in the perceptual training group practiced visual discrimination of Vernier stimuli. They saw two lines, side by side or one above the other, and indicated by button press, whether the

upper line was moved left or right relative to the lower line (vertical condition) or whether the left line was moved up or down, relative to the right line (horizontal condition). This training was repeated for 30 min. The training of visual discrimination abilities is known to induce plasticity in visual brain areas and to be independent of the medial temporal lobe (Fahle and Daum, 2002). Thus, spatial skills are supposed to be unaffected by this kind of training. Performance in the perceptual training task improved across training sessions; however, there were no differences in improvement between the exercise groups (cycling vs. stretching).

## Data analysis

### Cardiovascular and behavioral data

Cardiovascular and behavioral data were analyzed using SPSS (Version 17.0, SPSS Inc., Chicago, IL, USA). For the analysis of cardiovascular fitness, the  $VO_2$ peak values were entered into a repeated measures analysis of variance (ANOVA), including the factors Time (T0/T1), Physical training (cycling/stretching) and Cognitive training (spatial/perceptual).

For the analysis of behavioral data, the percentage of correct responses in the retrieval phase for each of the six sessions during fMRI scanning and per participant was calculated. Additionally, the mean percentage of correct responses across retrieval phases for the pre- and posttest for each participant were calculated to correlate behavioral data with measurements of cardiovascular fitness.

A repeated measures analysis of variance (ANOVA) with the factors (fMRI-) Session (1–6), Time (T0/T1), Physical training (cycling/stretching) and Cognitive training (spatial/perceptual) was calculated and Huynh–Feldt correction was applied when appropriate. In addition, to determine spatial learning separately for cognitive groups and time points, repeated measures analysis of variance (ANOVA) with the factor Session (1–6) were calculated for each cognitive group and time point separately. To determine a cross-sectional association between spatial learning performance and individual fitness level, the mean percentage of correct responses in the posttest was correlated with the posttest's  $VO_2$ peak value. Since reliable spatial learning was demonstrated in the posttest only (see Cardiovascular fitness) and because there was hardly any variation across participants in the pretest, posttest data were used to calculate cross-sectional associations. Longitudinal associations were determined by correlating the difference between pre- and posttest mean percent correct responses with the difference between pre- and posttest  $VO_2$ peak. Correlations were determined using Pearson's correlation coefficient  $r$ . The significance threshold was set to  $p < .05$  for all analyses.

### fMRI data

**Preprocessing.** Image preprocessing and statistical analyses were performed using SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5>). Preprocessing was conducted for each participant and time point separately. The first four volumes per session were discarded. The remaining volumes were realigned to the first volume that was included in the analysis. The T1-weighted anatomical image was coregistered to the first included functional volume and segmented subsequently. Then, all functional images were spatially normalized to the MNI template – using the normalization procedure implemented in SPM5 (affine transformation followed by a nonlinear estimation of deformations) with the normalization parameters obtained from the segmentation procedure – and smoothed with an 8 mm full-width at half-maximum Gaussian kernel.

**Statistical analysis.** Data analysis was performed on the individual level (first-level-model) and on the group level (second-level-model). For the first-level-model three conditions per session were modeled (encoding phase, retrieval phase, fixation). The onset of

each condition was modeled as a  $\delta$ -function convolved with a hemodynamic response function (HRF). A high pass filter (256 s) was applied to reduce baseline drifts. The resulting design matrix on the first level comprised nine sessions (six experimental and three control sessions) with three conditions each. For each participant and time point t-contrast images, contrasting experimental and control encoding phases, were calculated and entered into second-level-models. As experimental and control encoding phases were similar in visual stimulation, those contrasts were expected to represent activations specific to spatial learning. Activation differences during spatial learning between groups (cycling vs. stretching, spatial vs. perceptual training) and time points (T0 vs. T1) are reported elsewhere (Holzsneider et al., submitted for publication). This report focuses on the correlation between cardiovascular fitness and brain activations associated with spatial learning.

On the group level, correlational analyses were performed in SPM5 using regression analysis with  $VO_2$ peak values entered as a covariate. To determine cross-sectional associations between brain activation during spatial learning and the cardiovascular fitness level, t-contrast images for the posttests' encoding phase (experimental > control) were entered into the regression analysis, posttests'  $VO_2$ peak values were entered as a covariate. For the longitudinal analysis the pretests' (T0) t-contrast (experimental > control) was subtracted from the respective posttests' (T1) contrast to calculate an image that included activation changes from pre- to posttest for each participant. These difference images were entered into the regression analysis. The difference between  $VO_2$ peak T1 and T0 values was entered as a covariate.

The main analyses were performed on an FDR-corrected threshold ( $p < .05$ ), corrected for the whole volume. Moreover, small volume correction was applied for predefined regions of interest (ROI) in the medial frontal gyrus, the inferior parietal cortex, the superior parietal cortex, the cuneus, the retrosplenial cortex, the parahippocampal gyrus, hippocampus and the caudate nucleus. ROIs were selected on the basis of former studies using a similar spatial learning paradigm (Wolbers and Büchel, 2005; Wolbers et al., 2004). In this analysis, an FDR-corrected threshold ( $p < .05$ ), corrected for the respective region was applied. An additional analysis comprising the first three learning sessions only was conducted to compare groups with regard to fMRI activations at a time when behavioral performance did not differ yet. For this additional analysis, the significance threshold was set to  $p < .001$  (uncorrected). For all analyses, only significantly activated clusters comprising at least four voxels were reported.

Except for the retrosplenial cortex, all regions were determined using the SPM5 toolbox WFU pickatlas, Version 2.4 (Lancaster et al., 1997, 2000). Within this toolbox, the automatic anatomic labeling (AAL) atlas (Tzourio-Mayer et al., 2002) and the Talairach daemon (TD) atlas (Lancaster et al., 1997) were used. The retrosplenial cortex was defined as a spherical search volume with a 15 mm radius, positioned at the MNI coordinates  $\pm 15, -45, 9$  (Talairach coordinates  $\pm 8, -44, 11$ ) (see Brede database; Nielsen, 2003). Small volume corrected analyses for the ROIs were performed using the SPM5 toolbox WFU pickatlas, Version 2.4. Left- and right hemispheres were tested together.

## Results

### Cardiovascular fitness

From pre- to posttest, the members of the cycling group significantly improved their  $VO_2$ peak by 17.71% ( $SD = 19.71\%$ ;  $T(15) = 3.59$ ;  $p = .003$ ;  $d = .90$ ) from 29.97 ml/min/kg ( $SD = 4.81$ ) to 34.83 ml/min/kg ( $SD = 5.75$ ), whereas the stretching group did not significantly improve in  $VO_2$ peak ( $M = 5.59\%$ ;  $SD = 15.78\%$ ;  $T(13) = 1.08$ ;  $p = .30$ ;  $d = .29$ ; pretest:  $M = 30.72$  ml/min/kg;  $SD = 3.84$ ; posttest:  $M = 32.09$  ml/min/kg;  $SD = 3.95$ ; Time  $\times$  Physical training:  $F(1, 26) = 3.60$ ;  $p = .069$ ;  $\eta^2 = .121$ ).



As expected, the cognitive training did not influence cardiovascular fitness (Time×Cognitive training:  $F(1, 26) = 1.13$ ;  $p = .298$ ;  $\eta^2 = .042$ ), and there were no interactions between the physical and the cognitive training in the enhancement of  $VO_2$ peak (all  $p > 0.7$ ).

### Behavioral data

Behavioral data did not differ between the cycling and the stretching group, neither at pretest nor at posttest. Thus, data collapsed across physical training groups will be presented in the following. There was a trend from pre- to posttest, for the spatial training group to improve performance in the virtual reality task more than the perceptual training group (Time×Cognitive training:  $F(1, 31) = 3.07$ ;  $p = .090$ ;  $\eta^2 = .090$ ; see also marginally significant main effect of Cognitive training:  $F(1, 31) = 3.32$ ;  $p = .078$ ;  $\eta^2 = .097$ , Fig. 2). Separate ANOVAs for the pre- and posttest revealed that only at posttest, participants showed reliable spatial learning of the virtual environment, indicated by a gradual increase of correct responses from sessions 1 to 6 (main effect of Session at T1:  $F(5, 155) = 5.03$ ;  $p < .001$ ;  $\eta^2 = .140$ ). The spatial training group showed better performance compared to the perceptual training group at the posttest (main effect of Cognitive training at T1:  $F(1, 31) = 4.25$ ;  $p = .048$ ;  $\eta^2 = .120$ ; Cognitive Training×Session  $F(5, 155) = 1.94$ ,  $p = 0.096$ ,  $\eta^2 = 0.059$ ).

Since we had a predefined hypothesis regarding the influence of the cognitive training, separate ANOVAs for each time point and each cognitive training group were performed as well. Results of these analyses emphasized that only the spatial training group (Fig. 2 right, black/solid line) showed reliable spatial learning in the posttest (main effect of Session:  $F(5, 80) = 6.41$ ;  $p < .001$ ;  $\eta^2 = .286$ ), whereas in the perceptual training group the percentage of correct responses remained at chance level throughout the experiment (main effect of Session:  $F(5, 75) = 1.46$ ;  $p = .231$ ;  $\eta^2 = .089$ ). At the pretest, neither the spatial training group (main effect of Session:  $F(5, 80) = 1.19$ ;  $p = .322$ ;  $\eta^2 = .069$ ) nor the perceptual training group (main effect of Session:  $F(5, 75) = 1.34$ ;  $p = .258$ ;  $\eta^2 = .082$ ) showed a significant increase in the percentage of correct responses over sessions (Fig. 2 left).

Correlations between cardiovascular fitness and cognitive performance were performed for the entire group and the spatial training and perceptual training groups separately. The correlational analysis revealed no significant associations between the mean percent correct responses across learning sessions and the  $VO_2$ peak, neither cross-sectionally ( $r = .209$ ;  $p = .268$ ) nor longitudinally ( $r = -.192$ ;  $p = .308$ ). Significant correlations were not found, neither in separate analyses for the spatial training group (cross-sectionally:  $r = .077$ ;  $p = .776$ ; longitudinally:  $r = -.428$ ;  $p = .098$ ) nor for the perceptual training group (cross-sectionally  $r = .392$ ;  $p = .166$ ; longitudinally:  $r = .195$ ;  $p = .503$ ).

As sex has repeatedly been shown to have an impact on spatial navigation tasks (Wolbers and Hegarty, 2010), possible sex differences were assessed for the virtual maze task. At baseline, spatial learning performance did not differ between female and male participants (main effect of Sex:  $F(1, 31) = .37$ ;  $p = .548$ ;  $\eta^2 = .012$ ). At posttest, there was a trend for male participants to outperform female participants (main effect of Sex:  $F(1, 31) = .363$ ;  $p = .066$ ;  $\eta^2 = .105$ ) and male participants tended to improve performance more than female participants in the maze task (Time×Sex:  $F(1, 31) = 3.33$ ;  $p = .078$ ;  $\eta^2 = .097$ ).

### fMRI data

Since the analysis of the behavioral data revealed spatial learning in the spatial training group at posttest only, correlations between  $VO_2$ peak and brain activations during spatial learning were calculated separately for the spatial and perceptual training groups. Brain activations associated with spatial learning were defined by contrasting the mean activity during the six experimental encoding sessions of the maze task with those during the control encoding sessions (main effect experimental > control condition). Both cross-sectionally and longitudinally, positive associations between brain activation during spatial learning and the individual cardiovascular fitness level were found for participants who took part in the spatial training program. In this group, brain activations and individual  $VO_2$ peak values correlated positively in a large network of regions cross-sectionally: higher activations in the hippocampus, retrosplenial cortex, cuneus and parahippocampal gyrus and further frontal, temporal, occipital and cingulate regions were associated with a higher individual fitness level at posttest (see Table 2 and Fig. 3). The longitudinal analysis revealed that the change in brain activation from pre- to posttest correlated positively with the change in  $VO_2$ peak in the medial frontal gyrus ( $r = .85$ ;  $T = 6.03$ ) and the cuneus ( $r = .81$ ;  $T = 5.14$ ) (see Table 3 and Fig. 4). Negative correlations were not found, neither cross-sectionally nor longitudinally.

By contrast, in the perceptual training group no correlations between brain activation and individual cardiovascular fitness level were found, neither cross-sectionally or in the longitudinal analysis. This finding emphasizes that the positive correlation between brain activation and  $VO_2$ peak in the spatial training group was not due to an unspecific increase of brain activity at posttest, but rather depend on spatial navigation abilities. In addition, to further exclude the possibility of an unspecific increase of activation, contrast images showing higher activations in the control encoding sessions as compared to the experimental encoding sessions were calculated and correlated with the individual  $VO_2$ peak values. No significant correlations between cardiovascular fitness and activation during the control condition were found, neither cross-

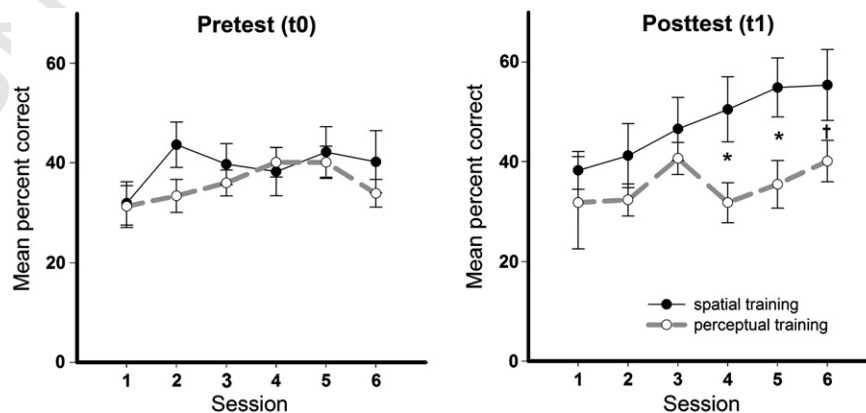


Fig. 2. Mean percent correct responses in the pretest (T0/left) and the posttest (T1/right) separately for the spatial training (solid/black line) and perceptual training (dashed/gray line) groups. Since three response alternatives were given, the chance level for correct responses was 0.33. Error bars depict  $\pm 1$  standard error. \*  $p < 0.05$ , †  $p < 0.1$ .

**Table 2**  
Spatial training group: local activation maxima for the correlation between VO<sub>2</sub>peak/T1 and activation during spatial learning at T1 (p<.05; FDR-corrected; >4 voxel per cluster).

Region	Coordinates (x, y, z in mm)		Voxel per cluster	Voxel-level	
	Right hemisphere	Left hemisphere		T	r
<i>Small volume correction</i>					
Hippocampus	18, -36, 0	-24, -18, -21	137	5.95	.85
Retrosplenial Cortex	18, -42, 0		180	6.34	.86
Cuneus	18, -81, 25		137	5.35	.81
Parahippocampal gyrus	24, -42, -4		29	6.40	.85
		-18, -24, -25	14	4.07	.73
<i>Whole volume analysis</i>					
Parahippocampal gyrus		-33, 0, -25	14	4.03	.72
	24, -9, -14		18	4.48	.77
Precuneus	15, -51, 56		10	4.35	.76
Caudate nucleus		-15, 3, 18	14	4.27	.73
Middle frontal gyrus	42, 36, -14		36	6.37	.86
	24, 42, 28		70	4.23	.74
		-33, 18, 46	5	3.91	.72
Inferior frontal gyrus		-42, -18, -4	12	5.62	.81
		-45, 33, -11	8	4.60	.77
Anterior cingulate		-9, 21, 25	21	5.00	.78
Posterior cingulate	6, -57, 4		8	4.01	.73
Cingulate gyrus	15, 6, 46		15	4.23	.74
Insula	39, 6, 4		31	4.81	.76
Putamen	30, 6, -11		23	4.96	.77
	30, -21, 4		1528	9.20	.91
		-27, 12, -7	12	4.45	.77
		-24, 3, 0	11	4.33	.76
	27, -45, 11		23	4.37	.76
Superior temporal gyrus		-51, -3, 0	55	5.51	.83
		-51, 3, -7	7	4.35	.76
	60, -51, 14		33	3.95	.73
		-42, 12, -28	12	3.84	.72
Middle occipital gyrus	30, -78, 25		18	4.37	.72
Vermis	3, -45, -7		13	4.52	.77

**Table 3**  
Spatial training group: local activation maxima for the correlation between the change in VO<sub>2</sub>peak and the change in activation during spatial learning (p<.05; FDR-corrected; >4 voxel per cluster, small volume corrected).

Region	Coordinates (x, y, z in mm)		Voxel per cluster	Voxel-level	
	Right hemisphere	Left hemisphere		T	r
Medial frontal gyrus	12, 36, 32		89	6.03	.85
Cuneus	12, -81, 35		323	5.14	.81

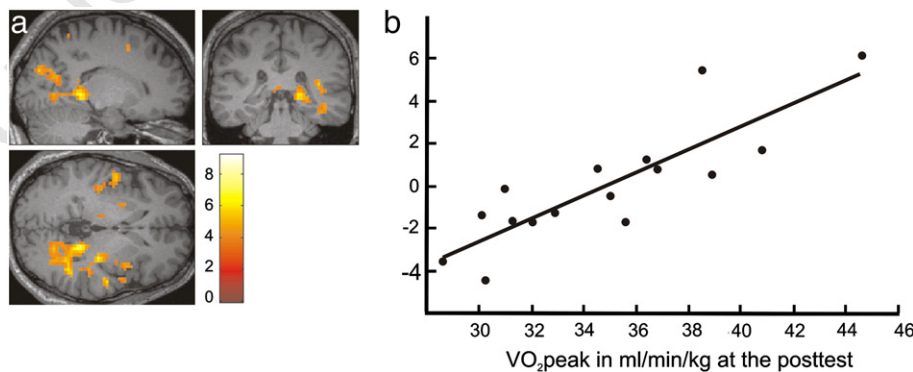
affected the results, we performed additional analyses: Brain activity during the first three experimental encoding phases (vs. control encoding phases) were correlated with cardiovascular fitness. Since the two groups did not differ behaviorally during the first three encoding phases, no confound existed. Confirming the results of the main analysis, we found positive associations between cardiovascular fitness and brain activations in the spatial training group only. In the cross-sectional analysis, brain activations during the first three sessions correlated positively with cardiovascular fitness in the inferior frontal gyrus, the cingulate gyrus, the superior temporal gyrus, the cuneus, precuneus and cerebellum (Table 4). The longitudinal analysis revealed that the change in brain activation during the first three sessions correlated positively with a change in VO<sub>2</sub>peak in frontal regions, the cingulate gyrus, the insula and the parahippocampal gyrus (Table 5). As in the main analysis, we did not find any significant correlation between brain activation of the first three encoding phases and VO<sub>2</sub>peak in the perceptual training group, neither cross-sectionally nor in the longitudinal analysis.

**Discussion**

This study examined the association between individual cardiovascular fitness and spatial learning abilities and associated brain activations after a six-month physical exercise intervention and an additional cognitive intervention in healthy middle-aged men and women. We hypothesized that physical exercising augments brain activity associated with spatial learning and resulting spatial performance. We found that only participants that had received a spatial training improved their spatial navigation abilities in a virtual maze task from pre- to posttest and showed reliable spatial learning gains at posttest. In this subgroup, a positive association was found between cardiovascular fitness and brain activation during spatial learning. Cross-sectionally, higher cardiovascular fitness was associated with activity in a large network of areas, including the hippocampus, retrosplenial cortex, cuneus, precuneus, parahippocampal gyrus,

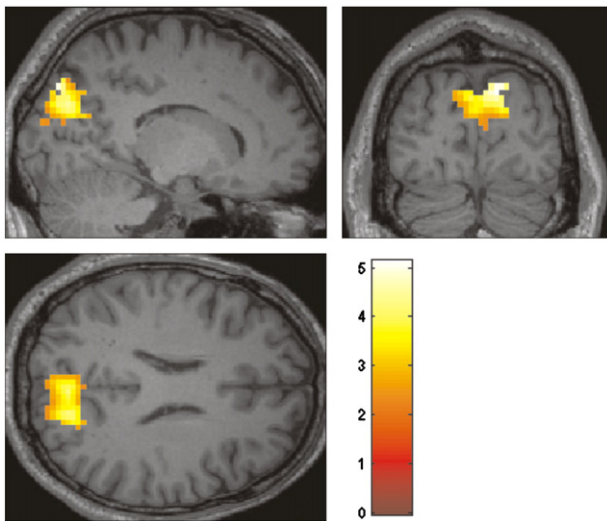
sectionally nor longitudinally and neither in the spatial training group nor in the perceptual training group.

To address the fact that the perceptual and spatial training group differed in behavioral performance which could have potentially



**Fig. 3.** a) Statistical parametric map showing activation during spatial learning at the posttest correlating positively with the VO<sub>2</sub>peak at the posttest in the spatial training subgroup (FDR-corrected, p<.05, >4 voxel per cluster). x = 18, y = -36, z = 0. Activation is superimposed on a normalized T1 image of one participant. Color scale indicates T-scores. b) Fitted regression curve for the correlation between VO<sub>2</sub>peak and brain activation during posttest at a hippocampal peak voxel (x = 18, y = -36, z = 0), spatial training group only. Y-axis in arbitrary units.





**Fig. 4.** Statistical parametric map showing the difference of activation during spatial learning at pre- and posttest correlating positively with the change in  $VO_{2peak}$  from pre- to posttest in the spatial training subgroup (FDR-corrected,  $p < .05$ ,  $> 4$  voxel per cluster), derived from the ROI analysis for the cuneus.  $x = 15$ ,  $y = -81$ ,  $z = 24$ . Activation is superimposed on a normalized T1 image of one participant. Color scale indicates T-scores.

caudate nucleus, insula, putamen as well as in a few subregions of the frontal, temporal, occipital and cingulate cortices. Longitudinal associations between changes in cardiovascular fitness and changes in brain activations were found in the medial frontal gyrus and the cuneus.

Since spatial learning and brain regions associated with spatial learning, especially the hippocampus, have been shown to be affected by physical exercise and cognitive stimulation in rodents (e.g. van Praag et al., 1999a), a spatial learning paradigm (maze task) was used during the fMRI session in the present study. Our results fit well with these animal data as more pronounced activity in the hippocampus and parahippocampal gyrus at posttest was observed in participants with higher cardiovascular fitness who had received an additional spatial training. During spatial learning, the hippocampus is of particular importance for the construction of allocentric representations (Burgess et al., 2002; Parslow et al., 2004), while the parahippocampal gyrus is supposed to be important for representing the spatial layout of scenes (Burgess et al., 2002; Ekstrom et al., 2003). Thus, one might speculate that improved cardiovascular fitness is associated with better functioning of these regions.

**Table 5**  
Spatial training group: Local activation maxima for the correlation between the change in brain activation during the first three sessions from T0 to T1 and the change in  $VO_{2peak}$  from T0 to T1 ( $p < .001$ ; uncorr.;  $> 4$  voxel per cluster).

Region	Coordinates (x, y, z in mm)		Voxel per cluster	Voxel-level	
	Right hemisphere	Left hemisphere		T	r
Medial frontal gyrus	15, 39, 28		10	3.87	.72
	12, 45, 21		5	3.82	.71
Superior frontal gyrus	9, 39, 42		6	4.25	.75
	3, 54, 28		48	4.65	.78
Middle frontal gyrus		-27, 60, 14	16	5.29	.82
Inferior frontal gyrus		-54, 24, 18	10	4.46	.77
Cingulate gyrus		-39, 24, -7	19	4.15	.74
	15, 3, 42	-15, 3, 39	21	5.08	.81
			6	4.13	.74
		-15, -3, 49	12	3.92	.72
Precentral gyrus	63, 0, 32		45	6.06	.85
Insula		-33, 21, 4	23	4.59	.76
Parahippocampal gyrus		-33, -24, -14	6	4.01	.73

**Table 4**  
Spatial training group: local activation maxima for the correlation between brain activation during the first three sessions of the spatial maze task at T1 and  $VO_{2peak}$  at T1 ( $p < .001$ ; uncorr.;  $> 4$  voxel per cluster).

Region	Coordinates (x, y, z in mm)		Voxel per cluster	Voxel-level	
	Right hemisphere	Left hemisphere		T	r
Inferior frontal gyrus		-45, 33, -7	7	3.98	.73
Cingulate gyrus		-15, -45, 25	22	5.28	.82
Superior temporal gyrus	39, -42, 11		46	5.59	.83
Cuneus	21, -84, 7		35	4.64	.78
Precuneus		-18, -48, 46	4	4.05	.73
Cerebellum		-3, -45, -14	4	3.90	.72

A few previous studies in humans have shown that the hippocampus might be influenced by cardiovascular fitness. Pereira et al. (2007), for example, found a very selective increase of the cerebral blood volume (CBV) in the dentate gyrus of the hippocampus after a three-month aerobic fitness training in a sample of young adults. Moreover, structural changes could contribute to the effects observed in the present study as have been shown recently by Erickson et al. (2011). They reported an increase in hippocampal volume after an aerobic exercise training as compared to a control group in a sample of older adults. fMRI data in humans, however, do not allow for defining the precise contribution of vascular, structural and functional changes that contribute to the relationship between brain activations and cardiovascular fitness observed in the present study. Data from animal research, however, have suggested that physical exercise induces a cascade of interdependent functional and structural changes like an increase in angiogenesis (Black et al., 1990), neurogenesis (Kempermann et al., 2010; van Praag et al., 1999b), long-term potentiation (van Praag et al., 1999a) and an increased production of neurotrophic molecules like the brain-derived neurotrophic factor (BDNF, Cotman and Berchtold, 2002) or the insulin-like growth factor-1 (IGF-1, Trejo et al., 2001).

In the present study, associations between cardiovascular fitness and brain activations were not only seen in the medial temporal lobe, but in a larger network of areas that have repeatedly been demonstrated to be activated during spatial tasks, including the retrosplenial cortex (Epstein, 2008; Maguire, 2001), the caudate nucleus (Maguire et al., 1998; Moffat et al., 2007), the cuneus and precuneus (Frings et al., 2006; Shelton and Gabrieli, 2002; Wolbers and Büchel, 2005; Wolbers et al., 2007, 2008). Moreover, positive correlations between cardiovascular fitness and activity in subregions of areas not specifically associated with spatial learning were found, for example in frontal gyri and the anterior cingulate cortex. Interestingly, both regions seem to be especially vulnerable to accelerated age-related structural decline (Good et al., 2001) and to age-related functional changes, i.e. older individuals showed less effective neuronal processing in these areas as compared to younger adults (Grady et al., 2006; Milham et al., 2002; Moffat et al., 2006). The anterior cingulate cortex is known to be involved in executive tasks and associations between decreased anterior cingulate activity during an executive task and higher cardiovascular fitness have previously been reported for the elderly (Colcombe et al., 2004). In the present study, however, higher cardiovascular fitness level was positively associated with increased activity in the ACC.

In contrast to previous investigations that found positive associations between cardiovascular fitness, behavioral performance and neuronal structure or function (Colcombe et al., 2004; Pereira et al., 2007), behavioral performance in the present study did not correlate with cardiovascular fitness, neither cross-sectionally nor longitudinally. Thus, the behavioral relevance of the increased activity in high-fit individuals remains to be determined. One explanation for the non-significant correlation of individual cardiovascular fitness

with the behavioral data could be the low variance in the number of correct responses, most probably due to the rather difficult task. Another explanation might be that effects of cardiovascular fitness on behavioral performance will not show up until an older age (Colcombe and Kramer, 2003). It could be possible that cardiovascular fitness modulates functional and structural aspects of the brain to a relatively low degree, at least for short term intervention intervals as used in the present study. The resulting changes in brain activity might thus not be sufficient yet to cause a change in behavior. However, after brain degenerative processes have further progressed, as in elderly people, and, thus, when performance is at an overall lower level, the brain might be more sensitive to positive effects of cardiovascular fitness and changes might be more likely to result in higher cognitive functioning.

An unexpected finding of the present study was the low performance of our participants in the virtual maze task (compared to the performance of the participants of Wolbers and Büchel, 2005): only participants of the spatial training group showed successful learning of the layout at posttest. Thus, one might ask whether the positive correlation between cardiovascular fitness and brain activations in the spatial training group and, the lack of a corresponding correlation in the perceptual training group, were possibly due to differences in behavioral performance between the groups. However, since the same correlation turned out significant when only the first three phases of the fMRI session were included in the correlation analyses, this account of our data seems unlikely. Thus, results of our main analyses were most likely not confounded by differences in behavioral performance, but rather reflect a specific interaction between cardiovascular fitness and a spatial training.

To fully exclude an influence of task performance and possible strategy differences between the spatial and the perceptual learning group, future studies should use an easier task or should implement a trails-to-criterion procedure.

Given the lack of a clear relationship between the behavioral data and the fMRI data in the present study, one might wonder whether higher cardiovascular fitness led to higher overall brain activity. One could argue that overall cerebral blood flow might differ between high- and low-fit participants and thus the correlations observed might not be linked to spatial abilities at all, but rather reflect unspecific changes throughout the brain. However, this account seems rather unlikely since effects of cardiovascular fitness on brain activations were only found in the sample that had received an additional spatial training and thus, showed reliable spatial learning during the posttest. By contrast, similar effects were found neither at pretest nor in the perceptual training group at posttest. Moreover, brain activations during the control condition did not correlate with cardiovascular fitness, what would have to be expected if they were unspecific consequences of cardiovascular training. Thus, cardiovascular fitness specifically modulates brain activations linked to spatial learning in the present maze task. Based on the animal literature we had decided to investigate spatial functions. Future research has to address whether a similar modulation by cardiovascular fitness occurs for other cognitive domains.

A positive correlation between brain activation and cardiovascular fitness does not allow for causal interpretations. In the present study, participants were randomly assigned to either the cycling training or a stretching/coordination training. Only the cycling training was hypothesized to improve cardiovascular fitness. Although this is what we observed at the group level, there was considerable variance among participants in the changes in cardiovascular fitness in both groups. While some participants of the cycling group did not gain in fitness from pre- to posttest, some participants of the stretching group did. Thus, correlations between brain activations and fitness level were calculated across both exercise groups. We think that the variance in changes of fitness within groups and the rather small sample size might explain why the two physical exercise groups did

not differ significantly in spatial learning. Thus, future randomized studies with larger sample sizes and a more intense and/or longer lasting cardiovascular training are necessary to prove a causal link between gains in cardiovascular fitness and changes of the processing in the spatial and possibly other neural networks. We consider it likely that such links will be demonstrated because of the results of the longitudinal analysis of the present study: Activation differences from pre- to posttest correlated positively with the differences in cardiovascular fitness between pre- and posttest in the medial frontal gyrus and the cuneus. The medial frontal gyrus is supposed to be involved in the transfer of spatial information from short term memory to long term memory systems and is hence regarded as an important part of the spatial network (Parslow et al., 2004; Shelton and Gabrieli, 2002; Wolbers et al., 2007). The cuneus has been shown to be active during spatial navigation as well (e.g. Shelton and Gabrieli, 2002). As a higher-order visual region, it is supposed to be involved in establishing spatial associations (de Rover et al., 2008). As described above, frontal regions seem to be especially susceptible to age-related changes in structure and function (e.g. Good et al., 2001; Moffat et al., 2006) and the cuneus has been reported to be affected by aging processes as well (Scarmeas et al., 2003). Thus, these data fit well with the hypothesis proposed by Kramer et al. (1999) that exercise has beneficial effects especially on brain functions that show a disproportionately high age related decline. They refer to previous studies in elderly humans which focused on executive tasks. The present data extend these findings by showing that brain networks involved in spatial tasks can be modulated by cardiovascular fitness as well.

A cardiovascular training alone, however, does not seem to be sufficient to induce task-related changes in brain activations as a correlation between VO<sub>2</sub>peak and fMRI data were seen only for the group of participants which received an additional spatial training. This finding is in line with results in mice that showed an additive effect of physical exercise and cognitive stimulation (Kempermann et al., 2010) and points to the importance of cognitive trainings in addition to physical exercise to improve cognitive functions in humans. Therefore, this combined treatment might be particularly protective and efficient to counteract age-related neurocognitive decline (Kempermann, 2008).

Since both the spatial training and the maze task employed virtual environments whereas the perceptual training did not, one could hypothesize that the spatial learning improvements in the maze task in the spatial training group might merely be due to an increased familiarity with virtual environments. The spatial training addressed two subfunctions, view point shifting and path integration, important for succeeding in the maze task. The stimuli and tasks used during the training differed, however, clearly from those of the maze task during the fMRI scanning. Thus it is unlikely that the specific gains during the maze task can be accounted for by a mere exposure to a virtual environment. If this were true, one would have expected at least some improvements in the perceptual learning group during the second fMRI session as well, i.e. when they became increasingly familiar with the task setting.

To summarize, cardiovascular fitness was found to modulate patterns of brain activation during spatial learning in a sample of middle-aged men and women that took part in a six-month physical exercise intervention and an additional spatial training. The results suggest that combining physical exercise with cognitive trainings might be a promising approach to enhance neurocognitive functioning and thus prevent age-related cognitive decline.

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