Exercise — A Cerebral Anti-aging Cure? Effects of Regular Physical Activity on the Senescent Brain and Cognition

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SUMMARY

Advanced age has been consistently linked to performance deterioration in cognitive tasks targeting the ability to mentally manipulate information. In search of the brain mechanisms underlying these decrements, an ever increasing number of studies has documented adverse effects of normal aging on the majority of measures investigated (e.g., cortical thickness, cerebral perfusion). Contrasting with this pessimistic view on brain aging, a growing body of literature suggests that regular physical exercise alleviates the adverse effects of age and helps to preserve cognitive and cerebral capacities in old age. The present dissertation is based on three papers and investigated associations between changes in fitness and changes in cerebral and cognitive measures within a group of older adults who participated in an exercise intervention. *Paper I* shows that previously reported increases in hippocampal volume can be linked to exercise-induced changes in the underlying tissue microstructure. The participants who improved most in fitness showed most increments in hippocampal tissue density. Changes in tissue density were in turn positively associated with changes in hippocampal volume. This finding suggests that volumetric changes result from an increase in the bulk of cell membranes, potentially via genesis of vasculature, neurons, and/or glia cells, and not from a mere dilation of existing cells. In *Paper II*, changes in fitness were associated with changes in the microstructure of a prefrontal white matter tract, namely the forceps minor. Likewise, changes in forceps minor microstructure were related to changes in a composite score of fluid cognitive abilities. This result indicates that changes in white matter microstructure may contribute to the beneficial effects of exercise on cognition. *Paper III* demonstrates that changes in fitness are positively correlated with changes in neural specificity, presumably an indirect marker of dopaminergic neuromodulation.

In summary, findings from the present dissertation extend the literature on beneficial effects of exercise on age-related deterioration and add knowledge regarding the underlying mechanisms: *Paper I* reveals that hyperplastic as opposed to hypertrophic processes most likely account for the frequently reported changes in hippocampal volume, while *Paper II* suggests that changes in white matter microstructure contribute to often reported improvements in cognitive performance. *Paper III* provides preliminary evidence that exercise preserves dopaminergic neuromodulation, which has been associated with cognitive decline. As a major limitation it is necessary to acknowledge that the evidence about beneficial effects of exercise on brain and behavior contributed by the present dissertation is correlational in nature because comparisons to a no-exercise control group were unavailable. Future research should include effective control groups as well as additional measurement modalities (e.g., positron emission tomography) and use the combined information to disentangle differential from common effects of exercise on prefrontal and hippocampal regions, as well as their interactions.

ZUSAMMENFASSUNG

Fortschreitendes Alter geht häufig mit Leistungsabnahmen in kognitiven Aufgaben einher. Die Suche nach den biologischen Mechanismen, die den Leistungsabnahmen im Gehirn zugrunde liegen, ergab negative Alterseffekte in der Mehrzahl der betrachteten Maße (z.B. Dicke des Cortex, Gehirndurchblutung). Dieser pessimistischen Sichtweise des Alterns steht eine steigende Anzahl Studien gegenüber, die zeigen, dass regelmäßige körperliche Aktivität negativen Alterseffekten entgegenwirken kann und somit zur Erhaltung kognitiver und zerebraler Funktionen im Alter beiträgt. Die vorliegende Dissertation basiert auf drei Publikationen und untersuchte im Rahmen eines Ausdauertrainings die Zusammenhänge zwischen Veränderungen in der körperlichen Fitness und Veränderungen in Gehirn und Verhalten bei älteren Erwachsenen. *Studie I* zeigt, dass zuvor gefundene Vergrößerungen des Hippocampus auf Änderungen der Mikrostruktur des zugrunde liegenden Gewebes zurückgeführt werden können. Die Probanden, die ihre Fitness am meisten verbesserten, zeigten auch die stärkste Verdichtung des Hippocampusgewebes. Die Verdichtung des Gewebes stand wiederum in positivem Zusammenhang mit der Veränderung im Hippocampusvolumen. Diese Ergebnisse weisen darauf hin, dass Veränderungen im Volumen aus einer Vermehrung der Zellmembranen resultieren, vermutlich durch Neubildung von Blutgefäßen, Neuronen und/ oder Gliazellen, und nicht aus der reinen Ausdehnung bereits vorhandener Zellen. In *Studie II* hingen Veränderungen in der Fitness zusammen mit Veränderungen in der Mikrostruktur eines präfrontalen Traktes der weißen Substanz, nämlich dem Forceps minor. Gleichermaßen hingen die Veränderungen in der Mikrostruktur des Forceps minor mit Veränderungen in einem zusammengesetzten Maß fluider kognitiver Fähigkeiten zusammen. Dieses Ergebnis zeigt, dass Veränderungen in der Mikrostruktur der weißen Substanz möglicherweise zu den positiven Auswirkungen von körperlicher Aktivität auf kognitive Fähigkeiten beitragen. *Studie III* zeigt, dass Veränderungen der Fitness positiv mit Veränderungen der neuronalen Spezifität korrelieren, welches als indirektes Maß für dopaminerge Neuromodulation angenommen wird.
Zusammenfassend erweitern die Ergebnisse dieser Dissertation die Literatur über positive Effekte von körperlicher Aktivität auf Alterungsprozesse und stärken den Kenntnisstand über zugrunde liegende Mechanismen: *Studie I* deutet darauf hin, dass hyperplastische Prozesse und nicht hypertrophische Prozesse häufig berichtete Veränderungen im Hippocampusvolumen erklären, während *Studie II* zeigt, dass Veränderungen in der Mikrostruktur der weißen Substanz zu den häufig berichteten Verbesserungen der kognitiven Leistungen beitragen. *Studie III* weist vorläufig darauf hin, dass körperliche Aktivität die dopaminerge Neuromodulation erhält. Leider ist es wegen des Fehlens einer Kontrollgruppe im Rahmen der vorliegenden Dissertation nicht möglich, den Nachweis zu führen, dass die beobachteten Korrelationen in körperlichen, zerebralen und kognitiven Maßen durch das Ausdauertraining hervorgerufen wurden. Zukünftige Forschung sollte neben einer Kontrollgruppe zusätzliche Messmodalitäten (z.B. Positronen-Emissions-Tomographie) einbeziehen, um durch eine Kombination von Informationen herauszufinden, inwiefern die Effekte von körperlicher Aktivität auf präfrontale Regionen und den Hippocampus gemeinsame oder unterschiedliche Ursachen haben und inwieweit diese wiederum interagieren.

LIST OF PAPERS

This doctoral dissertation is based on the following original papers:

Paper I

Kleemeyer, M. M., Kühn, S., Prindle, J., Bodammer, N. C., Brechtel, L., Garthe, A., Kempermann, G., Schaefer, S., & Lindenberger, U. (2016). Changes in fitness are associated with changes in hippocampal microstructure and hippocampal volume among older adults. *NeuroImage, 131,* 155–161.
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Paper II

Kleemeyer, M. M., Bender, A. R., Schaefer, S., Bodammer, N. C., Brechtel, L., & Lindenberger, U. (2017). *Correlated changes among fitness, prefrontal white-matter microstructure, and fluid cognition in old age: Results from an exercise intervention study.* Manuscript submitted for publication.

Paper III

Kleemeyer, M. M., Polk, T. A., Schaefer, S., Bodammer, N. C., Brechtel, L., & Lindenberger, U. (2017). Exercise-induced fitness changes correlate with changes in neural specificity in older adults. *Frontiers in Human Neuroscience, 11:* 123.
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LIST OF ABBREVIATIONS

AD	Axial diffusivity
ASL	Arterial spin labeling
BOLD	Blood-oxygen-level dependent
CBF	Cerebral blood flow
$CMRO_2$	Cerebral metabolic rate of oxygen
CRF	Cardiorespiratory fitness
CVR	Cerebrovascular reactivity
DA	Dopamine
DMN	Default mode network
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
HI	High-intensity
HIT	High-intensity interval exercise training
LI	Low-intensity
MCAv	Flow velocity in the middle cerebral artery
MD	Mean diffusivity
(f)MRI	(Functional) magnetic resonance imaging
(f)NIRS	(Functional) near-infrared spectroscopy
PET	Positron emission tomography
(dl)PFC	(Dorsolateral) prefrontal cortex
RD	Radial diffusivity
SPECT	Single-photon emission computed tomography
WM(M)	White matter (microstructure)

INTRODUCTION

Aging is accompanied by declining cognitive performance and profound changes in the brain (e.g., Grady, 2012; Lindenberger, 2014). Even healthy individuals aging without age-related pathologies such as Alzheimer's disease reveal structural changes (reductions in gray and white matter volumes and microstructure), as well as neurochemical changes (reductions in neurotransmitter concentration and density). Behavioral decrements have been demonstrated for various cognitive domains, including executive control functions, working memory, and perceptual speed (e.g,. Salthouse, 2010). Given that the average age in western countries has risen notably within the last decades, it becomes increasingly important for the individual, but also for society as a whole, to stay cognitively healthy well into old age. Thus, investigating possibilities to preserve cognitive capacities in aging populations attracts wide attention. A growing number of studies suggests that physical activity might be a promising approach to preserve cognitive capacities in old age (Bherer, Erickson, & Liu-Ambrose, 2013; Stillman, Cohen, Lehman, & Erickson, 2016) although little is known about the underlying mechanisms. With the present dissertation, I attempt to contribute to a better understanding of potential underlying mechanisms by extending the evidence of exercise as a means to preserve age-related decline in cerebral measures and cognitive performance. Before describing the empirical work at the core of this thesis (cf. Chapter 3.), I will provide the relevant empirical and theoretical background in Chapter 1. More specifically, I will present evidence for aging-related decrements in brain structure and function as assessed using magnetic resonance imaging (MRI) methods, and how these may relate to cognitive performance (Section 1.1). In Section 1.2, I will review the literature suggesting that exercise may be beneficial for preserving, if not reversing, those age-related changes. Finally, Chapter 4. discusses the results of the empirical work as well as its limitations and future directions.

Chapter 1.

THEORETICAL BACKGROUND

1.1 Aging

Human aging has been associated with decrements in cognitive as well as cerebral measures. Given that regular physical exercise is assumed to counteract some of the senescent changes, I will first review the corresponding literature on aging per se. The aging literature can be divided into two main design types: Cross-sectional designs investigate age differences by comparing younger and older adults on the measure of interest; however, they can only approximate true longitudinal growth and decline (Molenaar, Huizenga, & Nesselroade, 2003). In contrast, longitudinal designs measure the same individuals at different time points, thereby revealing estimates of true age-related changes, as well as individual differences in the measure of interest (Lindenberger, von Oertzen, Ghisletta, & Hertzog, 2011). For reasons of clarity and conciseness, I will restrict the literature review to studies using longitudinal designs. A more detailed picture is provided in Figure 1, which shows variables that are affected by aging including cross-sectional evidence (red dots and lines). Given that the present dissertation focuses on healthy aging, this section will be further confined to studies measuring non-pathological samples.

1.1.1 Cognitive abilities

Aging is associated with declines in cognitive performance across various tasks, including executive control, episodic memory, and perceptual speed. In general, fluid abilities (involving on-line processing/ mental manipulations or transformations)

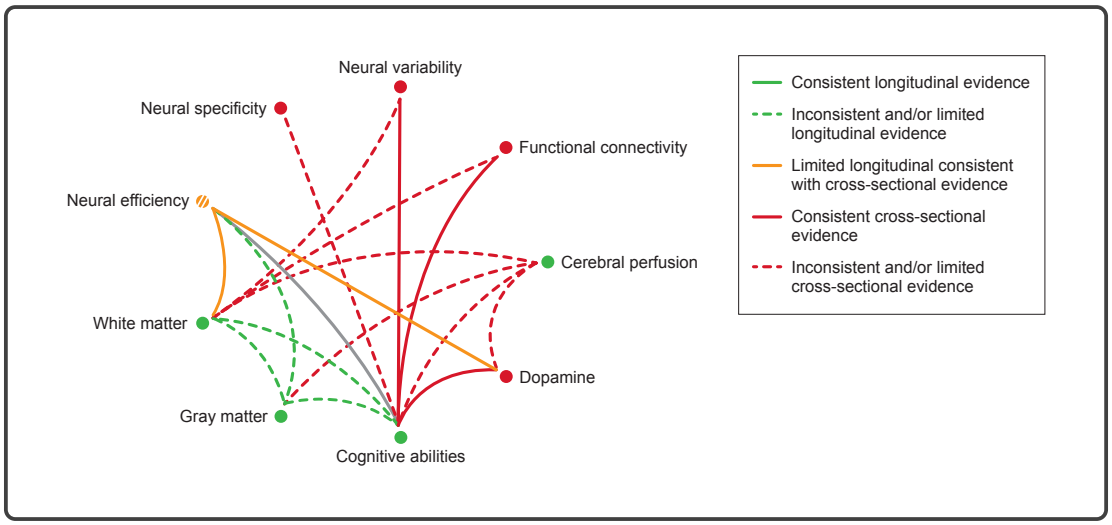

Figure 1: Schema of variables affected by aging and their interrelations. Circle colors represent whether evidence for age-related alterations in that variable comes from longitudinal (green), cross-sectional (red), or mainly cross-sectional with limited longitudinal (orange) work. Circle type indicates whether evidence is mostly consistent (plain) or inconsistent (striped). Lines between variables depict scientific evidence for a relation. Line color codes longitudinal (green), cross-sectional (red), or mainly cross-sectional with limited longitudinal (orange) evidence. Line type indicates consistent (continuous) vs. inconsistent or limited (dashed) evidence. The gray line represents an association that necessarily exists, because neural efficiency can only be examined in relation to cognitive performance.

were shown to be more affected than broad crystallized abilities (involving retrieval of accrued knowledge) (S.-C. Li et al., 2004; Salthouse, 2010; Singer, Verhaeghen, Ghisletta, Lindenberger, & Baltes, 2003). In fact, fluid abilities from distinct cognitive domains have closely corresponding age gradients (Ghisletta, Rabbitt, Lunn, & Lindenberger, 2012; Lindenberger & Ghisletta, 2009; Tucker-Drob, 2011). The most recent study suggests that the major part (60 %) of the variance in age-related cognitive decline is shared across tasks and hence, can be attributed to some general process that affects cognitive performance as a whole (Ghisletta et al., 2012). At the same time, Tucker-Drob (2011) observed domain-specific (33 %), and task-specific (28 %) variance in cognitive change, indicating that some domains may be more affected than others.

Concisely, behavioral studies demonstrated age-related deterioration in various cognitive domains, albeit tentatively more pronounced in those abilities requiring higher levels of cognitive control. A great interest in the field thus focuses on understanding

the mechanisms underlying aging-related decrements. Given that the brain provides the physical substrate of behavior, there is good reason to believe that the aforementioned behavioral deterioration should relate to cerebral changes. These changes and their relation to behavior will be discussed in the next sections.

1.1.2 Cerebral structure and function

Cerebral measures reported in the remainder of this section are based on magnetic resonance imaging methods. MRI allows in-vivo investigations of brain structure and integrity in both gray and white matter, brain activation as well as brain perfusion (among others).

Gray matter volume. Brain volumetric studies suggest an overall shrinkage of the brain parenchyma as well as thinning of the cortex with increasing age (for a detailed review, see Raz & Kennedy, 2009). Most changes have been observed over several years, but there also exist reports on decline over periods as short as 0.5–2 years (Fjell, Walhovd, et al., 2009; N. Persson et al., 2014; N. Persson et al., 2016; Raz et al., 2013). Whereas the prefrontal and inferior parietal cortices, hippocampus, caudate, and cerebellum seem to be more vulnerable to decline, primary sensory cortices remained rather stable (Fjell, Westlye, et al., 2009; N. Persson et al., 2016; Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger, 2010; Raz et al., 2005; Raz, Rodrigue, Head, Kennedy, & Acker, 2004; Sowell et al., 2003). In addition to the regional differences, there are large differences between individuals. These may in part be attributable to age-related vascular risk factors. Hypertension is probably the most common vascular risk, and to date a number of studies have indicated that hypertension is associated with an increased decline of age-sensitive brain regions as the prefrontal cortex (PFC) and/or hippocampus (Raz et al., 2005; Shing et al., 2011), as well as caudate (N. Persson et al., 2014) and cerebellum (Raz et

al., 2013). When it comes to the relationship between these volumetric declines and cognitive performance, reviews of the literature reveal inconsistent patterns of structure–cognition associations that are task-specific and hardly replicated (cf. Raz & Kennedy, 2009). This suggests that mere structural losses cannot fully explain declining cognitive abilities in aging populations.

White matter volume and microstructure. Unsurprisingly, aging also affects the brain's white matter (WM). Results from volumetric studies looking at frontal white matter volume yielded mixed results. Whereas measurements up to two years apart did not lead to observations of decline (N. Persson et al., 2014; Raz et al., 2013), two other studies spanning 2.5–5 year intervals reported declining frontal white matter volumes (Raz et al., 2010; Raz et al., 2005). The decline in prefrontal WM may follow slower age trajectories that are only detectable over more than two years.
Aging is also related with severe deteriorations in white matter microstructure (WMM). WMM is commonly investigated using parameters derived from diffusion tensor imaging (DTI). DTI measures the diffusion properties of water molecules within tissue and yields the following indices (see Bammer, 2003; Beaulieu, 2002). Fractional anisotropy (FA) indicates the diffusion tensor‘s degree of anisotropy, whereas mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) indicate how freely a water molecule can diffuse in any direction, parallel to the main fiber direction, or perpendicular to the main fiber direction, respectively. An increasing number of longitudinal studies indicates that aging is accompanied by reductions in FA (e.g., Bender & Raz, 2015; Bender, Völkle, & Raz, 2016; Lövdén et al., 2014; Rieckmann et al., 2016; Sexton et al., 2014; Vik et al., 2015) as well as increases in MD (e.g., Lövdén et al., 2014; Rieckmann et al., 2016; Sexton et al., 2014), and RD (e.g., Bender & Raz, 2015; Bender, Völkle, & Raz, 2016; Rieckmann et al., 2016; Sexton et al., 2014). The direction of age-related changes in AD are more inconsistent, with one study showing consistent increases (Sexton et al.,

2014), one study showing increases or stability (Rieckmann et al., 2016), and three studies showing both increases and decreases depending on the tract (Barrick, Charlton, Clark, & Markus, 2010; Bender & Raz, 2015; Bender, Völkle, & Raz, 2016). With respect to regional specificity, results are mixed: Cross-sectional studies suggest that prefrontal areas are most affected (reviewed in Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009), but few longitudinal studies have provided support for this (Sexton et al., 2014). In general, altered WMM is thought to reflect a process of disconnection, which should result in cognitive dysfunction. Indeed, mostly cross-sectional (for a review, see Bennett & Madden, 2014), but also a few recent longitudinal studies provide first evidence that disruptions in structural connections are associated with age-related deficits in cognitive performance (Bender, Prindle, Brandmaier, & Raz, 2016; Charlton, Schiavone, Barrick, Morris, & Markus, 2010; Lövdén et al., 2014; Ritchie et al., 2015). Yet, as is the case for gray matter volumes, the evidence varies by study, task, and measure, and has hardly been replicated so far. There is at least one study that did not observe associations between changes in white matter integrity and cognitive performance (Burzynska et al., 2017). In addition, first studies have shown that age-related alterations in WMM (indexed by FA) are inversely related to neural activations (Burzynska et al., 2013; Burzynska et al., 2015; Hakun, Zhu, Brown, Johnson, & Gold, 2015; J. Persson et al., 2006, see Section 1.1.2 below). One study was able to show that cortical thinning is apparent in areas containing the projected tract endings of WM tracts also showing age-related FA decline (Storsve, Fjell, Yendiki, & Walhovd, 2016).

Thus, longitudinal evidence suggests that aging alters WMM. It is not yet clear whether these changes translate directly to declining cognitive abilities. Rather, they may be part of a more complex interaction involving co-occurring structural and functional brain changes.

Brain activation. In addition to volumetric changes, aging alters neural activation patterns measured by means of functional MRI (fMRI). With almost no exceptions, the evidence for age differences in blood-oxygen-level dependent (BOLD) signal comes from cross-sectional comparisons between younger and older adults. Neural efficiency refers to more efficient usage of brain networks, which is typically reflected in a reduced BOLD signal, and in greater functional connectivity with increasing task demands. Evidence suggests that neural efficiency decreases with increasing age, especially in prefrontal cortex. Studies report stronger and more distributed bilateral activations during memory and attention tasks for older as opposed to younger adults when achieving similar performance levels (for a review, see Grady, 2012). D. C. Park and Reuter-Lorenz (2009) suggested that these increased frontal activations are indicative of a compensatory mechanism, necessary for preserving cognitive performance despite structural declines. This interpretation has been challenged, however, by studies finding increased activation in spite of performance decrements. Using three levels of task difficulty, Nagel et al. (2009, 2011) found that compensatory over-activation was disadvantageous to local BOLD responsivity: Whereas young adults showed increased activations with increasing task load in relevant areas, older adults' activation did not change notably with load. Many older adults had probably already recruited more prefrontal resources at low task demands, so that activations could not be further increased at higher loads, which then resulted in performance decreases. In contrast, older adults performing similarly accurately as young adults exhibited increased BOLD activity with increasing task load. Thus, the ability to adaptively modulate brain activity in response to cognitive demands appears to influence performance level and to be restricted by a potential compensatory mechanism. This may explain some of the inconsistent results obtained when using only two levels of task difficulty. In any case, maintaining a more "youth-like" pattern of brain activations is associated with better cognitive performance (Düzel, Schütze, Yonelinas, & Heinze, 2011; Fandakova, Lindenberger, & Shing, 2015; Nagel et al., 2011).

The two existing longitudinal studies do not provide much help in this regard because they yield contrasting results: Whereas Nyberg et al. (2010) showed age-related reductions in BOLD signal over six years (inconsistent with compensation), Hakun et al. (2015) provided evidence for bilaterally increased fMRI activations in three clusters within prefrontal cortex with increasing age (supporting compensation). Interestingly, Hakun et al. (2015) found FA decreases in the anterior body of the corpus callosum (a white matter tract connecting regions of the prefrontal cortex) to be associated with BOLD activity increases in prefrontal cortex clusters. The authors concluded that the increases in prefrontal activity may reflect compensatory processes for reduced structural connectivity. However, they may also reflect a loss of inhibitory control of the contra-lateral hemisphere. Notably, these results strongly resemble cross-sectional results (Burzynska et al., 2013; J. Persson et al., 2006). At the same time, BOLD activity increases in one of the prefrontal clusters were positively correlated with response time (participants with more activation were slower to respond).

Recently, Pudas, Josefsson, Rieckmann, and Nyberg (2017) separated 130 older adults into two groups with either stable or declining episodic memory over the study period. Only the memory decline group showed an increase of prefrontal functional responses during memory encoding and retrieval over 4 years. Also, only participants from this group displayed smaller volume of their right hippocampi. In the same sample, J. Persson et al. (2012) found that longitudinal reductions in hippocampal BOLD activation as well as hippocampal volume were both positively related to memory performance.

In sum, increases as well as decreases in BOLD activations seem to be indicators of adverse aging effects. Co-occurring structural changes, task difficulty level, and sample heterogeneity may contribute important insights into the origins of the observed inconsistencies.

Perfusion. Upon reporting these age-related cognitive, volumetric, and functional declines, the obvious question to arise asks why this decline occurs. Cerebral perfusion may be a key factor in this regard. An adequate blood supply is very important for all brain areas as it meets the neurons' demand for energy. This energy (i.e., oxygen and glucose) is needed to sustain the resting membrane potential, which provides the basis for proper signal conductance (the backbone of all cerebral functioning) on the one hand and ensures the cells' survival on the other hand (Anderson, Greenwood, & McCloskey, 2010).

In a rodent study, Sonntag, Lynch, Cooney, and Hutchins (1997) showed that arteriolar density on the surface of the cortex as well as arteriolar anastomoses (connections between cortical branches of different cerebral arteries) decreased with age indicating an age-related rarefaction of cerebral microvasculature (which potentially contributes to an age-related decrease in blood flow). Similarly, Bullitt et al. (2010) noted a trend toward vessel loss with age in a human sample using MR angiography. Consistent with those findings, other studies have found decreases in cerebral perfusion with advancing age cross-sectionally (Chen, Rosas, & Salat, 2011; Melamed, Lavy, Bentin, Cooper, & Rinot, 1980; Raz, Daugherty, Sethi, Arshad, & Haacke, 2017; Scheel, Ruge, Petruch, & Schöning, 2000; van Es et al., 2010) and also from longitudinal work covering three or seven years, respectively (Beason-Held, Moghekar, Zonderman, Kraut, & Resnick, 2007; ten Dam et al., 2007).

In a large aging sample, Lu et al. (2011) measured the cerebral metabolic rate of oxygen ($CMRO_2$), a proxy for oxygen demand, cerebral blood flow (CBF), a proxy for oxygen supply, and venous oxygenation level (Y_V), a proxy for how well the supply matches the demand. They provided evidence that CBF decreased with increasing age, and this was especially true for prefrontal cortex, insula, and caudate. At the same time, $CMRO_2$ increased with increasing age, resulting in a mismatch between supply and demand as indicated by reduced Y_V. In addition, cerebrovascular reactivity (CVR), that is, the blood vessels' ability to dilate in response to reduced oxygen levels, was also negatively associated with age. Hence, aging appears to not

only impact resting measures, but also the dynamic modulation of the vasculature in response to altered demands.

Interestingly, Heo et al. (2010) were able to relate regional CBF to cognitive performance: When comparing younger and older adults, they replicated the expected age-related decreases in spatial memory performance as well as the reductions in regional CBF in hippocampus and brain stem. More importantly, blood flow through the hippocampus was negatively correlated with reaction time on a spatial memory task only in the older adults. This correlation was task-specific (absent in a choice reaction-time task) as well as region-specific (absent in the brainstem).

Concisely, decreases in CBF with increasing age have been demonstrated consistently. Preliminary evidence suggests that they may be region-specific and relate to cognitive abilities.

1.1.3 Dopamine

On a more molecular level, age-related deficits in neurotransmitter function and neuromodulation, and have been observed for dopamine (DA) in particular. With age, there are widespread reductions of postsynaptic DA markers, namely D1 and D2 receptor levels as well as losses of the DA transporter (DAT), a presynaptic marker with a strong association between those two. Furthermore, cross-sectional research including lifespan samples was strikingly consistent in supporting a link between age-related nigrostriatal DA loss (all markers) and deficits in fluid abilities (reviewed in Bäckman, Lindenberger, Li, & Nyberg, 2010; Bäckman, Nyberg, Lindenberger, Li, & Farde, 2006). Neurocomputational work suggests that attenuated neuromodulation lowers a cell's responsivity, which increases noise in the system and leads to less distinct neural representations. Such a decline in neural specificity could explain impaired cognitive performance at the behavioral level (S.-C. Li & Sikström, 2002). Some evidence in favor of such a model has been documented in a cross-sectional

fMRI study: Whereas young adults showed highly distinct, region-specific activation patterns to differing categories of visual input (e.g., faces vs. houses), activations were less distinctive in older adults, indicating that neural specificity declines with increasing age (Grady et al., 1994; D. C. Park et al., 2004). In one study, reduced neural specificity was indeed associated with lower cognitive performance in a variety of cognitive tasks in older adults (J. Park, Carp, Hebrank, Park, & Polk, 2010). Most intriguing, manipulation of DA levels translates to BOLD signal *variability*. Neural variability refers to the dynamic range of neural activations using the standard deviation of the BOLD signal (whereas neural efficiency and neural specificity rely on the *average* BOLD signal). Evidence suggests that numerous brain regions display age differences in BOLD variability with the majority of regions showing less variability in older adults (Garrett, Kovacevic, McIntosh, & Grady, 2011; Garrett et al., 2015). Reductions in variability were in turn associated with decreased cognitive performance on a variety of tasks. In contrast, boosting DA levels via intake of d-amphetamine restored deficient BOLD signal variability, which in turn partly predicted working memory performance (Garrett et al., 2015).
Hence, age-related reductions in pre- and postsynaptic DA markers appear to be a prominent source of age-related cognitive deficits.

1.2 Aging and Physical Fitness

To recapitulate the previous section, the brain undergoes profound and wide-spread structural declines with increasing age. To a certain degree, however, it appears plastic enough to adapt to structural changes with functional reorganization, leading to different activation patterns but similar behavioral performance. Also, some individuals seem to be less affected than others, indicating that there may be modifiers of brain aging. Whereas vascular risk factors reside on the dark side of modifiers, accelerating age-related changes, physical exercise has now repeatedly been shown

to alleviate a variety of age-related changes and "has the most support as being protective against the deleterious effects of age on health and cognition" (Bherer et al., 2013). On the other hand, animal research has discovered that the potential for neurogenesis in the hippocampus is preserved well into adulthood and that exercise can in fact trigger hippocampal neurogenesis (van Praag, 2008). A recent review by Düzel, van Praag, and Sendtner (2016) suggests that the effects of exercise may be characterized either as preservation, when it defers age-related decline happening mainly in prefrontal cortical areas, or as improvement, when it induces structural and neurochemical changes in the hippocampus. Exercise intensity and duration may influence whether one and/or the other occurs. I will therefore divide the following literature review on fitness-related cerebral measures into two sections along these lines.

1.2.1 Exercise and the deferral of age-related decline

Cognition. While cross-sectional studies find positive associations between participants' cardiorespiratory fitness (CRF) and behavioral performance in multiple domains including perceptual speed, attention, executive control, and memory (Eggermont, Milberg, Lipsitz, Scherder, & Leveille, 2009; Flöel et al., 2010; Hillman et al., 2006; Netz, Dwolatzky, Zinker, Argov, & Agmon, 2011), longitudinal studies converge quite consistently on findings of improved or sustained aspects of executive functions and/or (spatial) working memory in aerobically trained vs. control participants (Baker et al., 2010; Kramer et al., 1999; Masley, Roetzheim, & Gualtieri, 2009; Ruscheweyh et al., 2011; Voelcker-Rehage, Godde, & Staudinger, 2011). Although some studies failed to show positive associations between aerobic fitness and cognition (Pierce, Madden, Siegel, & Blumenthal, 1993; Smiley-Oyen, Lowry, Francois, Kohut, & Ekkekakis, 2008; Thomas et al., 2016), meta-analyses suggest that exercise enhances cognitive abilities, particularly those requiring higher levels of cog-

nitive control, if the training lasted more than six months (Colcombe & Kramer, 2003; Smith et al., 2010). These results nicely add to the available evidence on cognitive aging and support the idea of exercise as a means to defer age-related decline (i.e., those abilities most affected by age also benefit most from exercise). Unfortunately, published results usually only report on single cognitive tasks, challenging the interpretation of the results (cf. Hötting & Röder, 2013). There are no analogous reports to the aging literature that examined general vs. domain- or task-specific effects. Still, the observed benefits on cognition triggered a search for underlying mechanisms. These will be discussed in the following sections.

Gray matter volume. Mirroring the behavioral results, fitness-related longitudinal data converge on rather specific volumetric increases or reduced declines in frontal gray matter (Colcombe et al., 2006; Ruscheweyh et al., 2011), whereas the volumetric enlargements for fitter participants were further distributed over frontal, parietal, and temporal areas in cross-sectional studies (Bugg & Head, 2009; Colcombe et al., 2003; Fletcher et al., 2016; Flöel et al., 2010; Gordon et al., 2008). Studies comparing the effect of age and fitness within the same sample are scarce and controversial: Among a group of older participants, regions that were most severely affected by aging also showed the greatest benefits from aerobic fitness (Colcombe et al., 2003). Conversely, comparing younger and older adults, Fletcher et al. (2016) found some regions that were most influenced by age to be entirely unaffected by fitness, although there was still some overlap. Regarding associations between volume and behavior, the majority of studies has looked specifically at the hippocampus and will be discussed in Section 1.2.2 below. Two cross-sectional studies showed trifold associations between fitness level, executive control (inhibition or switching), and PFC or caudate nucleus volume (Verstynen et al., 2012; Weinstein et al., 2012). One recent longitudinal study provided evidence for behaviorally relevant volume changes: Changes in a global cognitive score and also in executive control were asso-

ciated with change in dorsolateral prefrontal cortex (dlPFC) volume. These changes were not directly related to fitness changes, but were more pronounced in the aerobic exercise as opposed to the stretching control group (Jonasson et al., 2016).
In sum, evidence suggests that exercise can protect prefrontal areas against age-related volume losses, while there is no evidence for protective effects on other structures vulnerable to decline (e.g., caudate, cerebellum). Evidence for structural changes underlying cognitive changes (beyond the hippocampus) is scarce.

White matter volume and microstructure. Regarding WM volume, there is one intervention study that found volumetric increases in roughly the anterior third of the corpus callosum in the exercise as opposed to the stretching group (Colcombe et al., 2006). Studies on WMM are slightly more numerous. All of them converge on higher fractional anisotropy values either in relation to fitness or after an intervention, but the WM regions are rather diverse, including cingulum, corpus callosum, and uncinate (Johnson, Kim, Clasey, Bailey, & Gold, 2012; Marks et al., 2007; Tian et al., 2014). Only recently, Oberlin et al. (2016) found a trifold association including cognition: Higher fitness-related FA in a diverse network was positively associated with spatial working memory performance.
To date, only two intervention studies assessed WMM and obtained more or less opposing results: Whereas Voss, Heo, et al. (2013) could not detect a Time × Group interaction with respect to FA measures, changes in prefrontal and temporal FA were correlated with changes in fitness. No effects for AD and RD were observed, and the training-induced changes seen in FA did not map onto changes in cognitive performance. On the other hand, a very recent study only found a Time × Group interaction, with six months of dancing being the only effective intervention to increase FA in the fornix (out of 20 tracts), whereas brisk walking could not defer age-related FA decreases (Burzynska et al., 2017). The authors reported no relation to fitness changes or cognitive performance.

Taken together, there is preliminary evidence that exercise exerts beneficial effects on prefrontal WM. However, it is not clear from the aging literature whether prefrontal WM is also most affected by aging (cp. 1.1.2). Studies have failed to show that exercise-induced changes in cognition can be traced back to changes in WMM.

Brain activation. Attempts have been made to examine the effects of exercise on brain activity using fMRI. Longitudinal findings (Colcombe et al., 2004; Voelcker-Rehage et al., 2011) indicated that higher exercise-induced fitness leads to reduced frontal activations during performance of an executive control task. These findings would be consistent with the idea that exercise increases neural efficiency (or decreases the need for compensation). However, they conflict with cross-sectional studies indicating that fitter participants show greater task-related frontal activation and better cognitive performance (Prakash et al., 2011; Wong et al., 2015). Similarly, cross-sectional studies using functional near-infrared spectroscopy (fNIRS) revealed that fitter participants had superior cognitive performance and increased prefrontal oxygenation (which is comparable to BOLD activation) during the execution of an executive control task (Albinet, Mandrick, Bernard, Perrey, & Blain, 2014; Dupuy et al., 2015; Hyodo et al., 2016). One study found that oxygenation in left dlPFC was positively associated with fitness and interference reaction time (Hyodo et al., 2016).

To investigate fitness effects on the hypothesized age-related increase in bilateral activation (Cabeza, Anderson, Locantore, & McIntosh, 2002, cf. Section 1.1.2), McGregor et al. (2011) elegantly used a very simple motor task. Whereas physically active older adults showed ipsilateral motor patterns similar to younger adults, sedentary individuals revealed decreases in measures of ipsilateral inhibition. The authors conclude that physical activity may protect against aging-related decline in interhemispheric inhibition.

Hence, like aging per se, exercise- or fitness-related alterations in prefrontal ac-

tivations patterns have been documented, though cross-sectional and longitudinal studies seem to yield contrasting results. Based on the aging literature, considering the interrelation of neural efficiency and white matter microstructure may help resolve some inconsistencies. Also, no study has looked at more than two levels of task difficulty level to more closely investigate the effects of exercise on the ability to adaptively modulate brain activity in response to cognitive demands.

Perfusion. As mentioned in Section 1.1.2, cerebral perfusion might be a potent player in mediating the beneficial effects of exercise, and some studies have actually provided first evidence for this. Cross-sectional studies assessing fitness effects on cerebral perfusion have consistently found positive correlations. In an age-comparative study, Ainslie et al. (2008) confirmed negative adult age differences in CBF by measuring blood flow velocity in the middle cerebral artery (MCAv) via ultrasound. Though the decline occurred regardless of fitness level, that is, in endurance-trained as well as sedentary participants, MCAv in endurance-trained men was elevated by $\sim$17 % throughout the age range. This constituted an approximately 10-year rejuvenation in MCAv as compared to sedentary participants. Using arterial spin labeling (ASL), Zimmerman et al. (2014) reported a positive correlation between blood flow in gray matter and CRF, as well as a negative correlation between CBF and age. Johnson et al. (2016) showed that fitness was positively correlated with CBF in regions belonging to the default mode network (DMN). With respect to vasculature, Bullitt et al. (2009) assessed capillary density by means of MR angiography and found an increased number of small vessels in fitter participants.

Although cross-sectional work supports a positive relation between fitness and perfusion, intervention studies measuring regional CBF in relation to changes in fitness and cognitive performance are lacking.

Dopamine. The effects of exercise on neurotransmitter systems have rarely been investigated in humans. There are two studies pointing to beneficial effects of dopamine by looking at different genetic variants. In a cross-sectional study, Voelcker-Rehage, Jeltsch, Godde, Becker, and Staudinger (2015) analyzed the relation between fitness, cognitive performance, and the Catechol-O-methyltransferase (COMT) gene. COMT is an enzyme that degrades DA from the synaptic cleft. COMT is less active in Met allele carriers, leading to greater DA availability than in Val allele carriers. The authors observed the highest positive correlation between fitness and cognition in those participants with lower DA levels based on their genotype (val/val COMT gene homozygotes). Thus, a high fitness level might compensate for a lower performance accuracy associated with the val/val COMT genotype. Along the same lines, Stroth et al. (2010) showed that young adults carrying the val/val COMT polymorphism exhibited greater cognitive improvements after a 4-month exercise intervention compared with all other genotypes (met/met or met/val carriers). There is no direct or indirect evidence for long-term exercise effects on dopamine levels in older adults.

1.2.2 Exercise and the hippocampus

The hippocampus has attracted much attention in the exercise literature because animal work has yielded some of the most compelling evidence for exercise-induced changes in this structure. The hippocampus is a small brain region located in the medial temporal lobes, and plays a major role in spatial and contextual memory formation (Squire, Stark, & Clark, 2004). Importantly, the hippocampus is responsive to exercise in animal models, that is, wheel running enhances hippocampal neurogenesis, angiogenesis, and synaptogensis (formation of new neurons, blood vessels, synapses, respectively) in mice and rats, thereby promoting memory and learning (reviewed in van Praag, 2008). Attempting to link animal and human work, Pereira

et al. (2007) showed that in mice, exercise induces neurogenesis and the number of new neurons in the hippocampus is correlated with hippocampal blood volume. In humans, 12 weeks of exercise training led to increased fitness level in most participants. Change in fitness was positively correlated with cerebral blood volumes in the dentate gyrus of the hippocampus (measured using gadolinium as a contrast agent). Furthermore, fitness change was positively correlated with memory performance in a free recall task at posttest (not change). Importantly, the increase in cerebral blood volumes within the dentate gyrus was also positively correlated with memory performance. Extrapolating from animal data, Pereira and colleagues thus claimed to have indirect evidence that exercise triggers neurogenesis in humans, too. Ever since, the hippocampus and hippocampus-dependent memory has been intensively studied in relation to exercise and fitness. Human behavioral results still remain equivocal: while some studies did find improved memory performance upon exercise longitudinally (Erickson et al., 2011; Klusmann et al., 2010; Ruscheweyh et al., 2011) or cross-sectionally (Erickson et al., 2009; Flöel et al., 2010), other studies did not (Baker et al., 2010; Jonasson et al., 2016; Masley et al., 2009; Thomas et al., 2016). In spite of the inconclusive behavioral results, a growing body of literature suggests that fitness level (cross-sectionally, Erickson et al., 2009; Szabo et al., 2011) or fitness change (longitudinally, Erickson et al., 2011; Jonasson et al., 2016; Maass et al., 2015; Niemann, Godde, & Voelcker-Rehage, 2014; Thomas et al., 2016), is positively associated with hippocampal volumes. The volumetric increases were in turn positively related to spatial memory performance for a subset of studies (Erickson et al., 2011; Maass et al., 2015). Maass et al. even provided first evidence for a a mediating role of perfusion, with a path model suggesting the following sequence of events: changes in fitness lead to changes in perfusion, which in turn lead to changes in volume, which in turn lead to preserved memory functions. The same model without volume fit the data equally well, indicating that the volumetric changes may be a mere reflection of the perfusion changes and not contribute additional information. Although these findings are extremely intriguing, they were

only correlational and do not permit strong claims about causality (see also Tidwell, Dougherty, Chrabaszcz, Thomas, & Mendoza, 2014). More than two measurement occasions and a no-intervention control group would be needed to directly test this proposed sequence.

Taken together, there is compelling evidence for exercise-induced changes in the hippocampus and hippocampus-dependent cognitive abilities, with perfusion changes constituting a key factor. The observed perfusion increases may reflect either dilation of existing blood vessels or generation of new blood vessels. Assessing hippocampal microstructure may help resolve these ambiguities (Lövdén, Wenger, Mårtensson, Lindenberger, & Bäckman, 2013). Some evidence in favor of angiogenesis comes from a cross-sectional study, relating lower mean diffusivity in the hippocampus to higher fitness levels (Tian et al., 2014). Lower MD is indicative of more cell membranes (e.g., from blood vessels, neurons, glia cells etc.) acting as diffusion barriers. However, longitudinal evidence is missing, and the relation between microstructure and volume also remains to be clarified.

Chapter 2.

SUMMARY AND RESEARCH QUESTIONS

Aging research has repeatedly demonstrated declines in cognitive performance, brain volume, white matter microstructure, dopaminergic neuromodulation, as well as altered patterns of brain activation. Fortunately, fitness and intervention research has accumulated increasing evidence that exercise prevents or at least defers some age-related degeneration. In this context, two brain structures have attracted most attention: hippocampus and prefrontal cortex.

Animal studies were the first to show that exercise triggers hippocampal neurogenesis, angiogensis, and synaptogensis. Subsequent human studies yielded some corresponding findings, for instance, increased volumes and perfusion of hippocampal regions (see Section 1.2.2). However, hippocampal microstructure has not yet been investigated, although this would extend the understanding of underlying tissue changes.

The prefrontal cortex emerged as important structure in this context in human aging and exercise studies. The evidence suggests that the PFC is among the first regions to experience age-related decline in multiple cerebral measures, such as decreasing volume or functional activation. Conversely, beneficial effects of exercise have repeatedly been shown for PFC volume and functional activation (see Section 1.2.1). Yet, white matter microstructure in tracts connecting prefrontal areas and its relation to cognitive abilities have not been studied in relation to exercise.

Finally, the dopaminergic system and its association with exercise in older adults has not been examined so far (see Section 1.2.1). Given that declines in DA provide a highly plausible explanation for a wide range of aging-induced cognitive deficits (S. C. Li, Lindenberger, & Sikström, 2001), it appears worth exploring potential contributions of DA to the beneficial effects of exercise.

In my dissertation project, I therefore conducted an exercise intervention study among older adults. Fifty-two healthy participants aged 59–74 years were randomly assigned to one of two aerobic training regimens that differed in intensity. Participants trained three times a week over a period of six months. Before and after the training intervention, we assessed their cardiorespiratory fitness level, broad cognitive abilities, as well as cerebral measures via multi-model imaging. My goal was to further extend the evidence for exercise-induced deceleration and/or reversal of age-related decline. In particular, I addressed the following questions:

1. How are exercise-induced structural changes in the hippocampus related to changes in the underlying microstructure?

2. Are exercise-induced benefits in cognitive performance related to white matter microstructure?

3. Can regular exercise defer the age-related decline in neural specificity, an indirect marker of dopaminergic neuromodulation?

The present publication-oriented dissertation answers these questions in three different papers (Kleemeyer, Bender, et al., 2017; Kleemeyer et al., 2016; Kleemeyer, Polk, et al., 2017).

Chapter 3.

OVERVIEW OF PUBLICATIONS

The present dissertation is based on three papers. All three papers are the result of an exercise intervention study that was conducted within the Sensorimotor-Cognitive Couplings project at the Center for Lifespan Psychology, Max Planck Institute for Human Development.

3.1 Paper I

Kleemeyer, M. M., Kühn, S., Prindle, J., Bodammer, N. C., Brechtel, L., Garthe, A., Kempermann, G., Schaefer, S., & Lindenberger, U. (2016). Changes in fitness are associated with changes in hippocampal microstructure and hippocampal volume among older adults. *NeuroImage, 131,* 155–161.
doi:10.1016/j.neuroimage.2015.11.026

In this paper we investigated whether previously reported exercise-induced changes in hippocampal volumes could be related to changes in the underlying microstructure. An association between volume and microstructure would provide additional evidence that cellular tissue changes such as neurogenesis or angiogenesis contribute to the observed volumetric changes.

3.1.1 Theoretical background

So far, numerous studies indicate stable or even increased hippocampal volumes in response to aerobic exercise (Erickson et al., 2011; Jonasson et al., 2016; Maass et al.,

2015; Niemann et al., 2014; Thomas et al., 2016). However, the cellular mechanisms underlying these volumetric changes remain largely unknown. Animal studies show that rodents with access to running wheels as opposed to those held in standard cages show increased capillary density, and produce new neurons (for reviews, see Kempermann, 2012; Thomas, Dennis, Bandettini, & Johansen-Berg, 2012; Voss, Vivar, Kramer, & van Praag, 2013). Two studies with human samples reported associations between changes in fitness and changes in hippocampal perfusion that were behaviorally relevant as they even translated to memory performance (Maass et al., 2015; Pereira et al., 2007). Beyond that, Maass et al. were able to show that the perfusion changes accounted for volumetric changes in the anterior part of the hippocampus, indicating that the more often observed volumetric changes may trace back to perfusion changes. At the cellular level, these observations may reflect either dilation of existing blood vessels or angiogenesis.

3.1.2 Hypothesis

This study investigated whether hippocampal tissue changes as captured by mean diffusivity potentially mediate the association between fitness changes and changes in hippocampal volumes. MD is an index of how freely water molecules can diffuse through tissue, which can be derived from diffusion tensor imaging data. In densely packed tissue, the presence of numerous cell membranes hinders diffusion, and thus MD would be low, and vice versa. In this way, DTI can complement volumetric measures with information on tissue (or barrier) density (cf. Lövdén et al., 2013). If exercise-induced volumetric increases are accompanied by increases in the bulk of membranes (hyperplasia), potentially via angiogenesis, gliogenesis, or neurogenesis, we would expect to observe an increase in barrier density, that is, a decrease in mean diffusivity. If exercise-induced volumetric increases reflect mere enlargement of cells that were already present before the intervention (hypertrophy), we would expect

diffusivity to remain unchanged.

3.1.3 Major findings

By applying latent change score modeling to measures of cardiovascular fitness, bilateral hippocampal volume, and bilateral hippocampal MD, we found that more positive changes in fitness were associated with more positive changes in tissue density in bilateral hippocampi (i.e., more negative changes in mean diffusivity). More positive changes in tissue density were in turn associated with more positive changes in hippocampal volume. In line with the animal literature, this finding suggests that previously reported increases in hippocampal volume or perfusion may in part be driven by hyperplasia.

3.2 Paper II

Kleemeyer, M. M., Bender, A. R., Schaefer, S., Bodammer, N. C., Brechtel, L., & Lindenberger, U. (2017). *Correlated changes among fitness, prefrontal white-matter microstructure, and fluid cognition in old age: Results from an exercise intervention study.* Manuscript submitted for publication.

In this paper we set out to investigate whether previously reported beneficial effects of exercise on fluid cognitive abilities are related to white matter microstructure. An association between exercise-induced fitness changes and changes in WMM would provide correlational support for beneficial effects of exercise on the senescent brain and cognitive performance.

3.2.1 Theoretical background

While there is ample evidence for the beneficial effects of exercise on cognitive function and gray matter volumes (Bherer et al., 2013), evidence for changes in white matter volume or microstructure in relation to exercise is scarce. WMM is commonly investigated through multiple indices (fractional anisotropy, radial diffusivity, axial diffusivity, mean diffusivity) derived from DTI, which reflect the diffusion properties of water molecules within tissue. A meta-analysis demonstrated significant but small effect sizes towards a positive relationship between physical fitness and white matter health (Sexton et al., 2016). Thus, better physical fitness is associated with greater WM volumes, reduced WM lesions, or improved indices in WMM. The intervention literature on WMM only comprises two studies. Voss, Heo, et al. (2013) found that greater aerobic fitness changes achieved in a one-year walking program correlated with changes in fractional anisotropy in prefrontal and temporal regions. Burzynska et al. (2017) found that six months of dancing in older adults led to FA increases in the fornix (out of 20 analyzed tracts), whereas brisk walking did not defer age-related FA decreases. It is, however, not clear from their results whether the intervention was effective in raising fitness levels in the first place. On the other hand, older age has been related to decrements in WMM and age-related decline in WMM has been associated with declining cognitive abilities. Thus, cerebral WMM may be an important factor contributing to the beneficial effects of exercise on cognition.

3.2.2 Hypothesis

This study investigated whether regular exercise changes indices of white matter microstructure and if so, whether these changes map onto changes in cognitive performance. If exercise defers/reverses age-related decline efficiently, it should affect cerebral white matter to a similar extent as gray matter with a similar regional

specificity and behavioral relevance. We hypothesized that exercise attenuates age-related decline in prefrontal WM tracts and that better (maintained) prefrontal WMM would result in superior fluid cognition.

3.2.3 Major findings

In line with our hypothesis, latent change score modeling revealed that exercise-induced fitness changes were indeed associated with more intact white matter microstructure in forceps minor, a white matter tract connecting the frontal and medial surfaces of the frontal lobes via the genu of the corpus callosum. In turn, changes in WMM were positively associated with a composite measure of fluid cognitive performance. This finding provides initial, correlational evidence that white matter microstructure may contribute significantly to the often observed beneficial effects of exercise on cognition. It furthermore is consistent with the proposition that exercise may help to defer senescent cerebral decline.

3.3 Paper III

Kleemeyer, M. M., Polk, T. A., Schaefer, S., Bodammer, N. C., Brechtel, L., & Lindenberger, U. (2017). Exercise-induced fitness changes correlate with changes in neural specificity in older adults. *Frontiers in Human Neuroscience, 11:* 123. doi:10.3389/fnhum.2017.00123

In this paper we investigated whether regular exercise postpones the age-related decline in neural specificity. Neural specificity serves as an indirect marker of dopaminergic neuromodulation and has been linked to various fluid cognitive abilities. Exercise-induced changes in neural specificity would hence provide another

mechanism for the beneficial effects of exercise on cognitive performance.

3.3.1 Theoretical background

Regular physical activity has repeatedly been shown to preserve cognitive abilities in old age, and fluid cognitive abilities in particular (for meta-analyses, see Colcombe et al., 2003). In contrast, aging has detrimental effects on cognitive performance, and again, on fluid cognitive abilities in particular (cf. S.-C. Li et al., 2004; Salthouse, 2010). At the same time, neural specificity declines with increasing age (Grady et al., 1994; D. C. Park et al., 2004) and reduced neural specificity is associated with lower cognitive performance in a variety of cognitive tasks in older adults (J. Park et al., 2010). Age-related declines in dopaminergic neurotransmitter function and neuromodulation have been suggested as underlying mechanisms, a notion supported by neurocomputational models (S.-C. Li & Sikström, 2002). Evidence from animal research suggests that exercise induces an upregulation of dopamine (Foley & Fleshner, 2008; Sutoo & Akiyama, 2003) and a small number of human studies conform to this idea (Ruscheweyh et al., 2011; Stroth et al., 2010; Voelcker-Rehage et al., 2015). Exploring neural specificity in the context of an exercise intervention would yield preliminary evidence for exercise-induced changes on the dopaminergic system.

3.3.2 Hypothesis

This study investigated whether exercise-induced fitness changes translate to neural specificity. As suggested in earlier work (see Carp, Park, Polk, & Park, 2011), the distinctiveness of neural activation patterns in response to different stimulus categories served as an index of neural specificity. If dopamine levels (a) influence neural specificity, and (b) are affected by exercise, we would expect changes in fitness

to correlate with changes in neural specificity.

3.3.3 Major findings

Consistent with our hypothesis, changes in fitness were positively associated with changes in neural specificity. However, we did not observe associations between neural specificity and measures of cognitive performance. A possible explanation for this is a slight change of the task during data acquisition.

Chapter 4.

DISCUSSION

In this chapter, I will summarize the key findings of this dissertation and integrate them into the existing exercise literature. I will point out substantive and methodological implications as well as limitations of the present study, and provide ideas on how some of these could be addressed in future research. I will end by drawing some general conclusions.

4.1 Summary and Evaluation

4.1.1 Fitness changes are associated with changes in hippocampal microstructure

Previous research in animals has indicated that the hippocampus is responsive to exercise. Mice or rats with access to a running wheel had an increased number of new neurons, blood vessels, and synapses, thereby promoting their memory and learning (reviewed in van Praag, 2008). These findings triggered a search for exercise-induced changes in the hippocampus in human samples. To date, various intervention studies have shown that fitness changes are positively associated with hippocampal volumes (Erickson et al., 2011; Jonasson et al., 2016; Maass et al., 2015; Niemann et al., 2014; Pereira et al., 2007; Thomas et al., 2016). In some of these studies, the volumetric increases were in turn related to spatial memory performance (Erickson et al., 2011; Maass et al., 2015; Pereira et al., 2007). Moreover, Pereira et al. and Maass et al. provided first evidence for a mediating role of perfusion. Yet it remains an open question whether the observed volumetric and perfusion increases reflect dilation of existing cells or increases in the bulk of cell membranes, potentially via

angiogenesis, gliogenesis, or neurogenesis. Assessing hippocampal microstructure by measuring mean diffusivity, *Paper I* provided initial evidence for the role played by changes in tissue density: Exercise-induced changes in fitness were associated with changes in hippocampal microstructure, and changes in hippocampal microstructure accounted for changes in hippocampal volumes. Assuming that changes in MD represent changes in the barrier density of gray matter, these findings suggest that previously reported increases in hippocampal volume may, at least in part, result from hyperplastic processes, that is, a greater number of cells.

4.1.2 Changes in prefrontal white matter microstructure are associated with changes in fluid cognition

Another important and yet under-investigated question concerns exercise-induced changes in white matter microstructure and its potential contribution to cognitive benefits. Whereas the aging literature showed rather consistent declines in WMM (cf. Section 1.1.2), only three intervention studies have looked at the effects of physical activity on WM so far. Two of them converge on WM changes in prefrontal areas (Colcombe et al., 2006; Voss, Heo, et al., 2013), the third one found that dancing but not brisk walking effectively deferred age-related decline in fornix microstructure (the major efferent tract of the hippocampus; cf. Burzynska et al., 2017). Given that it is not clear from the results of this third study whether the intervention changed fitness levels in the first place, these results should be treated with caution. In any case, there is no evidence to suggest that the observed beneficial effects of exercise on cognition can be traced back to WMM. *Paper II* corroborated that exercise changes prefrontal WM. More specifically, exercise-induced fitness changes co-varied with changes in multiple indices of WMM in forceps minor, which is the main fiber bundle connecting left and right lateral and medial prefrontal cortices. Remarkably, changes in forceps minor microstructure covaried with changes in a composite

score of fluid cognitive abilities. Hence, *Paper II* provided first evidence that WMM may contribute significantly to the beneficial effects of exercise on cognition often observed.

4.1.3 Fitness changes are associated with changes in neural specificity

While age-related deficits in dopaminergic neuromodulation have been documented consistently (reviewed in Bäckman et al., 2006), there is a lack of intervention studies examining whether exercise can defer the adverse effects of aging on the dopamine system. Given that more direct measurements (e.g., PET) were not feasible within this dissertation, *Paper III* used neural specificity as a proxy for dopaminergic neuromodulation (cf. S.-C. Li & Sikström, 2002). Correlational analyses revealed that exercise-induced fitness changes were positively related to changes in neural specificity. In contrast to a previous study and inconsistently with the prediction of neurocomputational models, there was no relation to cognitive performance. Nevertheless, to the extent that neural specificity reflects dopaminergic neuromodulation, these results tentatively suggest that physical activity may protect against age-related decline in the dopamine system that has been associated with cognitive decline (Bäckman et al., 2006).

4.2 Limitations

4.2.1 Control group

We opted for a very strictly defined control group, namely participants who underwent a low-intensity training, in order to truly isolate the effects of exercise-induced fitness changes. By keeping everything identical except for the training intensity,

we hoped to exclude all potential confounds. However, we had recruited sedentary older adults to maximize possible exercise effects. This combination induced a major limitation to our study: Because these people were so unfit, some participants from the high-intensity group achieved their prescribed training heart rate at or even below the resistance of 10 W that was supposed to represent the low-intensity training. The reverse also applies: For some participants in the low-intensity group, training at 10 W already induced a heart rate that, had they been assigned to the high-intensity group, would have been prescribed to them. This means that there was some overlap in the training intensities, likely explaining the absence of group differences in fitness changes between the high-intensity and the low-intensity training groups. The fact that we observed a significant fitness increase over the training for both groups may indicate that the level of challenge provided by the low-intensity training was already effective in boosting fitness levels in sedentary older adults. In any case, the two training regimens were apparently too similar to one another to induce differential effects. Unfortunately, this deprived us of a proper control group and challenges the interpretation of some of our findings (see also Section 4.2.3 below). In the presence of a proper control group whose mean training gains in the variable of interest differed reliably from the intervention group, and that did not show a comparable change–change association, we would have been able to ascribe the observed effects to the training intervention. Given that we lack an appropriate control comparison, we cannot evaluate the intervention effects relative to a no-exercise control group, and hence we cannot provide evidence that the intervention drives the reported associations. We do not know whether we would have observed null, similar, or even larger change–change associations in the absence of any intervention, meaning that we unfortunately cannot make causal inferences about the effectiveness of the intervention in the present dissertation (cf. Tidwell et al., 2014).

4.2.2 Exercise intensity

Given that visual inspection of the training parameters after about three months of training did not show the expected increases, a high-intensity interval exercise training was included in the last 21 sessions of the initial training program. After 20 min of regular training, five intervals of two minutes each were integrated, separated by two minutes of active recovery. In these time windows, participants in the high-intensity group were pushed above their anaerobic threshold, whereas participants in the low-intensity group only increased the speed. The rather intense exercise for the high-intensity group can have detrimental effects in older age (Lucas, Cotter, Brassard, & Bailey, 2015), particularly in the presence of cerebrovascular and metabolic risk factors. These were exclusion criteria in our recruitment process and highly unlikely to be present in our sample. At the same time, some animal studies have reported negative associations between hippocampal neurogenesis and exercise duration (Naylor, Persson, Eriksson, Jonsdottir, & Thorlin, 2005) and intensity (Lou, Liu, Chang, & Chen, 2008), with the latter being quite comparable to our setup. Hence, the interval phase may have counteracted some of the positive effects of exercise training unintentionally, at least in the hippocampal measures presented in *Paper I*.

4.2.3 Preservation instead of improvement

For the majority of measures that we assessed, there was stability rather than improvement over the training, despite increased fitness levels. More specifically, in *Paper I*, average hippocampal volumes neither decreased nor increased, but remained stable. Mean diffusivity even increased over the course of the training, which is consistent with progression of age-related alterations. Neural specificity in *Paper III* also remained stable (the numerical decrease was not statistically significant). Frac-

tional anisotropy in *Paper II* decreased significantly, which is again consistent with the progression of age-related deterioration. Axial diffusivity and radial diffusivity also decreased in the majority of tracts, which in these measures is rather inconsistent with the aging literature on these measures and may be the only indication of "anti-aging" effects, together with the improvement in fluid cognitive abilities.

These findings can most likely be interpreted in terms of two opposing forces, namely aging and exercise. All of the outcome measures investigated in the present dissertation (i.e., fitness, hippocampal volume, hippocampal microstructure, neural specificity, indices of white matter microstructure, and cognitive abilities) tend to decline with age. Participants did not escape normal aging while taking part in our study, which gives reason to expect that each outcome measure would follow its estimated aging trajectory (increase or decrease) to some extent. On the other hand, training-induced fitness improvements counteracted aging-related decrements in each outcome measure, and apparently reduced or, in some cases, actually offset the effects of aging. Yet the effects of fitness improvements on cerebral measures were apparently not strong enough to reverse the adverse effects of aging. As stated above, it would have been preferable to include a no-contact control group in the study in order to document changes taking place in the absence of any intervention. Note that these findings are well in line with results from other exercise studies finding preservation rather than improvement (cf. Maass et al., 2015; Ruscheweyh et al., 2011). Reviewing the literature, Düzel et al. (2016) suggest that exercise intensity and duration may be decisive in this regard: Whereas exercise interventions with at least three sessions per week of mild-to-moderate training intensity lasting at least 12 months seem to have *preserving* effects, shorter interventions (3-6 months) with at least three sessions per week of moderate-to-high training intensity seem to have *ameliorating* effects. The intervention conducted within the present dissertation resides somewhere in the middle, with a duration of six months but mild to moderate intensity levels, and hence matches the proposition of Düzel et al. of preservation rather than improvement effects.

4.2.4 Perfusion measure

The present dissertation included arterial spin labeling to measure cerebral blood flow. Given that exercise targets the cardiovascular system, CBF appears to be a good candidate for mediating the effects of exercise (see Section 1.2.1). In fact, we used an MR sequence (Günther, Oshio, & Feinberg, 2005), which has never been acquired longitudinally before. This sequence offered an additional outcome measure, namely the cerebral arterial hemodynamic measure bolus arrival time (BAT). BAT can be seen as a measure of blood flow velocity, since it assesses the transit time of the magnetically labeled blood from the feeding arteries to its first arrival in the voxel of interest. When constructing an analysis pipeline, it became clear to us that we actually lack a calibration image, which is needed to equalize acquisition differences in image intensities between different measurement occasions. At this juncture, we may have a suitable workaround, but this awaits further validation. Perfusion changes may underlie both the gray matter changes reported in *Paper I* (as in Maass et al., 2015) and the white matter changes reported in *Paper II*. Relating perfusion changes to gray and white matter changes may thus reveal a more complete picture of the mechanisms playing a role.

4.2.5 Sample size and selectivity

As is often the case in psychological training studies, the sample size in the present study is relatively small. For most analyses, results were based on a final sample of around 50 participants. Even more importantly, the present results may reflect a bias in participant selection. Participants in the present study followed a highly sedentary lifestyle but, at the same time, were sufficiently healthy to be admitted to the study. In a cross-sectional study with similar sample selection criteria, Voelcker-Rehage et al. (2015) noticed a relatively high proportion of COMT met/met allele

carriers (related to more dopamine availability, that is, a more beneficial pattern) as compared to the general population. Our participants, in addition, felt sufficiently motivated to change their lifestyle, which may imply that they were an even more selected group. The available evidence suggests that genetic effects on cognitive abilities are magnified in old age (Lindenberger et al., 2008; Papenberg et al., 2013), resulting in lower performance for genotypes associated with suboptimal DA availability or modulation. This may indicate that our exclusion criteria led to a higher prevalence of participants carrying more "beneficial" genotypes, leaving less room for cognitive and cerebral improvement. Given the absence of genetic information in this study, this conjecture cannot be tested. It remains to be seen whether the present results replicate in larger and more representative samples.

4.3 Future Directions

4.3.1 Exercise and cardiovascular risk

The present dissertation adds to the growing body of literature suggesting that exercise can alleviate rather widespread age-related deterioration. Given this non-specificity, there may be more general factors underlying the beneficial effects of exercise on senescent brain changes. Attenuation of cardiovascular risk factors has been suggested in this context (Düzel et al., 2016), but has never been systematically investigated. There are numerous reports that pathological hypertension accelerates age-related deterioration in gray matter volume (e.g., Raz et al., 2005; Shing et al., 2011), white matter microstructure (Bender & Raz, 2015), cerebral perfusion (Bangen et al., 2014; Muller, van der Graaf, Visseren, Mali, & Geerlings, 2012), and cognition (Dahle, Jacobs, & Raz, 2009). At the same time, regular physical activity both prevents and helps treat elevated blood pressure (among other cardiovascular risk factors; see Thompson et al., 2003). A first glimpse at our data revealed that

blood pressure levels did indeed improve over the course of the training (again, equally for both groups), but in-depth analyses have not yet been conducted. It may thus be interesting to examine mediating effects of blood pressure levels on cerebral outcome measures.

4.3.2 Dopamine

As age-related decrements in dopaminergic neuromodulation have consistently been associated with the adverse effects of aging on cognitive performance, it is quite surprising that the long-term effects of exercise on dopamine have attracted so little attention. Dopamine is abundant in prefrontal areas, which is a major target for observed exercise-induced changes. Furthermore, DA constitutes a vasoactive hormone — one study (Raz et al., 2017) reported age-related reduction in cerebral blood flow only in persons carrying genetic variants associated with lower dopamine availability (COMT val homozygotes). The effects of exercise-induced changes in DA may hence unfold via different routes, influencing DA availability directly, and perfusion indirectly. Therefore, changes in DA may well contribute to all three papers' findings. First support for an association between physical activity and dopamine receptor availability comes from the Cognition, Brain, and Aging (COBRA) study. The COBRA study was set out to evaluate a wide range of brain variables in relation to aging-sensitive cognitive abilities in a longitudinal design. At three separate occasions, separated by five years each, 181 older adults will undergo testing (Nevalainen et al., 2015). Cross-sectional results from the first measurement occasion revealed that self-reported intensity of physical activities was positively associated with DA D2/D3 receptor availability in caudate, which in turn was also related to episodic memory performance (Köhncke et al., 2017). These findings suggest that exercise and DA are somewhat related. It would be of major interest to gauge more direct evidence on this issue either from longitudinal studies using objective measures of

physical fitness or from intervention work. One concern in this regard is the fact that most accurate measurements of DA are still invasive, usually obtained by means of PET or SPECT using a radioactive tracer. MR spectroscopy may provide an alternative approach, but to my knowledge, there is still no feasible way to reliably detect dopamine in the spectrum. This may change with the development of new imaging sequences and/or hardware.

4.3.3 Molecular and cellular mechanisms

Results of the present dissertation point to potential factors that may mediate the beneficial effects of exercise on cognition. Nevertheless, the precise nature of the neurobiological mechanisms underlying the observed changes remain illusive. Animal work indicates that the number of newly produced neurons is relatively small in relation to the total number of neurons in the hippocampus. This argues against volumetric changes being the human equivalent of neurogenesis in the hippocampus (Zatorre, Fields, & Johansen-Berg, 2012). Macroscopic changes, as detectable with MRI, most likely reflect a range of cellular and molecular processes including angiogenesis, dendritic spine growth, but also synaptic remodeling (Hötting & Röder, 2013; Stillman et al., 2016). These processes have in turn been linked to exercise-induced up-regulation of growth factors. Brain-derived neurotrophic factor (BDNF) has been related to neuronal growth and synaptic plasticity, vascular endothelial growth factor (VEGF) to angiogenesis, and insulin-like growth factor 1 (IGF-1) to general support of the cellular changes (Düzel et al., 2016; Thomas et al., 2012; Voss, Vivar, et al., 2013). At the same time, there is evidence that exercise induces anti-inflammatory effects, and thereby positively impacts brain aging (reviewed in Di Benedetto, Müller, Wenger, Düzel, & Pawelec, 2017). These factors all play important roles in understanding the anti-aging effects of exercise. Yet the currently available techniques provide limited opportunities to assess these factors

in living humans. Studies measuring peripheral (i.e., extra-cerebral) levels of factors such as BDNF remain inconclusive and equivocal, given that the relation between peripheral and central nervous system levels is not well established (cf. Hötting & Röder, 2013). Hence, understanding these underlying mechanisms and how they relate to the observed macroscopic brain changes in humans requires further research. Increasing exchange and comparability between animal and human investigations may be of great help in this regard.

4.3.4 Multimodal potential

One particular strength of the present dissertation is its multimodal approach. So far, the full potential of this data set has only begun to be exploited due to the time-intensive nature of MR data analyses. However, there are more data to analyze, and, more importantly, to integrate into the current results. There is, for example, another fMRI paradigm assessing spatial working memory including three levels of task difficulty that relies on prefrontal cortex activation (Nagel et al., 2009, 2011). Following Hakun et al. (2015), the observed exercise-induced changes in forceps minor microstructure could be related to BOLD activations. Hypothetically, improvements in microstructure should translate to improvements in load-dependent BOLD manipulation. Another interesting target for multimodal approaches would be the connection between prefrontal activation and hippocampal volumes (cf. Pudas et al., 2017). Noteworthily, Burdette et al. (2010) recorded data on cerebral perfusion in a pilot study by means of pulsed arterial spin labeling as well as resting state fMRI *after* a 4-month exercise intervention (there was no pre-intervention measurement). They showed increased hippocampal perfusion in the intervention as opposed to the healthy aging education control group, as well as stronger resting state functional connectivity between the hippocampus and anterior cingulate cortex. Taking hippocampal perfusion as an indicator for neurogenesis, this finding may indicate that

new neurons were directly integrated to improve prefrontal–hippocampal connections. In line with such an interpretation, Voss et al. (2010) found fitness-related increases in functional connectivity by means of resting state fMRI within the default mode and frontal executive network. These altered functional connections were shown to be behaviorally relevant, as they were related to improved performance on a composite measure of executive control. Following the accumulating multimodal approaches in the aging literature, examining the interaction between changes from different imaging modalities may extend the insights into underlying mechanisms. The present dissertation provides additional cerebral measures that may serve to test some of the hypothesized associations.

4.4 Conclusion

This dissertation extends the current literature on exercise as a means to defer the adverse effects of aging. More precisely, the results suggest (1) that exercise-induced changes in hippocampal volume may relate to changes in the underlying tissue microstructure, (2) that beneficial effects of exercise on cognition may be associated with microstructural changes in forceps minor, and (3) that regular exercise may alter neural specificity, presumably an indirect measure of dopaminergic neuromodulation. Despite their correlational character, these results warrant cautious optimism, as they point to the modifiability of brain aging in later adulthood. Future studies may clarify whether elevating the intensity and/or prolonging the duration of exercise can indeed trigger improvements, and thus provide a means to rejuvenate the senescent brain. Causal inferences drawn on the basis of the results of future studies would be strengthened considerably if no-exercise control groups were included in the research design, and if sample sizes of both control and experimental groups were sufficiently large to directly compare group differences in change–change associations.

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Paper I

Kleemeyer, M. M., Kühn, S., Prindle, J., Bodammer, N. C., Brechtel, L., Garthe, A., Kempermann, G., Schaefer, S., & Lindenberger, U. (2016). Changes in fitness are associated with changes in hippocampal microstructure and hippocampal volume among older adults. *NeuroImage, 131,* 155-161.
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Changes in fitness are associated with changes in hippocampal microstructure and hippocampal volume among older adults

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ABSTRACT

This study investigates the effects of fitness changes on hippocampal microstructure and hippocampal volume. Fifty-two healthy participants aged 59–74 years with a sedentary lifestyle were randomly assigned to either of two levels of exercise intensity. Training lasted for six months. Physical fitness, hippocampal volumes, and hippocampal microstructure were measured before and after training. Hippocampal microstructure was assessed by mean diffusivity, which inversely reflects tissue density; hence, mean diffusivity is lower for more densely packed tissue. Mean changes in fitness did not differ reliably across intensity levels of training, so data were collapsed across groups. Multivariate modeling of pretest–posttest differences using structural equation modeling (SEM) revealed that individual differences in latent change were reliable for all three constructs. More positive changes in fitness were associated with more positive changes in tissue density (i.e., more negative changes in mean diffusivity), and more positive changes in tissue density were associated with more positive changes in volume. We conclude that fitness-related changes in hippocampal volume may be brought about by changes in tissue density. The relative contributions of angiogenesis, gliogenesis, and/or neurogenesis to changes in tissue density remain to be identified.

Introduction

During the last decade, evidence has been accumulated documenting beneficial effects of exercise in preserving cognitive abilities as well as brain structure and function in older adulthood (for reviews, see, e.g., Bherer et al., 2013; Erickson et al., 2015; Hötting and Röder, 2013). The hippocampus has attracted much attention because some of the most compelling evidence for positive effects of fitness on cognition has been found for this brain region, in both animal and human studies. The hippocampus is a small brain region located in the medial temporal lobes, which plays a major role in spatial and contextual memory formation (Squire et al., 2004). It is one of the first brain regions affected by age-related atrophy and has been associated with age-related disorders such as Alzheimer's disease (Barnes et al., 2009). On the other hand, the hippocampus is one of two brain regions for which the potential for neurogenesis is preserved into late adulthood (Eriksson et al., 1998; Spalding et al., 2013). Specifically, the hippocampus is responsive to exercise in animal models (Kronenberg et al., 2003; van Praag et al., 1999).

Multiple studies point to stable or even increased hippocampal volumes after completing 3–12 months of physical exercise interventions (Erickson et al., 2011; Maass et al., 2015; Niemann et al., 2014), using either manual segmentation or automated subcortical segmentation procedures for the MR images. However, the cellular mechanisms underlying these volumetric changes remain largely unknown. Some insight can be gained from animal studies showing that animals with access to running wheels as opposed to those held in standard cages show increased capillary density, and produce new neurons (for reviews, see Kempermann, 2012; Thomas et al., 2012; Voss et al., 2013).

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Consistent with the animal literature, Pereira et al. (2007) demonstrated that after 3 months of exercise in middle-aged human adults, changes in fitness correlated with increases in dentate gyrus cerebral blood volume (CBV), and by extrapolating from animal data, claimed that these changes are related to neurogenesis. A recent study replicated this finding in older adults, reporting associations among changes in fitness, hippocampal perfusion, and volume of the hippocampal head in the context of a 3-month fitness intervention program (Maass et al., 2015). At the cellular level, these observations may reflect either dilation of existing blood vessels or angiogenesis.

Parameters derived from diffusion tensor imaging (DTI) may shed additional light on underlying changes in brain microstructure. DTI measures the diffusion of water molecules within tissue and is commonly used to determine white matter integrity (Bammer, 2003; Beaulieu, 2002). DTI can also be applied to gray matter, where it likewise provides information on how freely water molecules can diffuse through tissue. When many barriers (mostly cell membranes) are present, that is, when the tissue is densely packed, mean diffusivity (MD) would be low, and vice versa. In this way, DTI can complement volumetric measures with information on tissue (or barrier) density (cf. Lövdén et al., 2013). Thus, in the case of exercise-induced volumetric increases that are accompanied by increases in the bulk of membranes, including potentially angiogenesis, gliogenesis, or neurogenesis (hyperplasia), we would expect to observe an increase in barrier density, that is, a decrease in mean diffusivity as opposed to unchanged diffusivity, which would be more consistent with a mere hypertrophy of cells that were already present before the intervention. Tian et al. (2014) provided initial evidence for such an association between higher fitness and lower hippocampal MD, showing that the amount of self-reported exercise activities predicts lower MD in medial temporal lobe (and cingulate cortex) about 10 years later in very old adults.

The present study investigates whether hippocampal tissue changes as captured by MD potentially mediate the association between fitness changes and changes in hippocampal volumes. Latent difference modeling (McArdle and Nesselroade, 1994; cf. Raz et al., 2005), a variant of structural equation modeling (SEM), was used to represent individual differences in changes before and after the intervention. This method greatly reduces the problem of unreliability in the measurement of longitudinal change by using identical sets of more than one observed variable to define equivalent latent constructs at each measurement occasion, and then computing difference scores on the basis of these latent constructs. In doing so, the method effectively separates variance at the construct level from measurement-specific variance and error, and generates difference scores that are more reliable than difference scores based on observed variables (cf. McArdle and Nesselroade, 1994). To this end, we examined the covariance of change in fitness, hippocampal MD, and hippocampal volume in the context of a fitness intervention study with healthy sedentary older adults. Elderly participants were randomly assigned to either of two levels of fitness-training intensity. Before and after the 6-month training phase, participants performed a maximal graded exercise test to assess their training-related fitness improvements, and underwent T1-weighted and diffusion-weighted MR imaging. We hypothesized: (a) a negative association between changes in fitness and changes in MD, in that an increase in fitness would lead to lower diffusivity in the hippocampus, thus reflecting higher tissue density, and (b) a negative association between changes in MD and changes in volume, in that greater decrements in diffusivity, again reflecting more positive changes in tissue density, would lead to more positive changes in volume.

Materials & methods

Participants

Seven hundred and twenty-three community-dwelling older adults were contacted via local newspaper advertisements; from this initial pool, fifty-seven individuals were enrolled in the study. Participants met the following inclusion criteria: (1) age between 59 and 75 years; (2) physical inactivity prior to study enrollment (metabolic rate < 40 based on the Freiburg Questionnaire of physical activity in German, Frey et al., 1999); (3) MMSE score ≥ 26; (4) free of neurological, psychiatric, or cardiovascular diseases; (5) right handedness; (6) no contraindication for heart-rate controlled exercise training (e.g., beta blockers); and (7) suitability for an MR environment (e.g., no magnetic implants, claustrophobia). During training, three participants dropped out due to health issues unrelated to the study, and two dropped out due to motivational issues, resulting in an effective final sample of fifty-two participants who completed the intervention (mean age = 66.0 years, SD = 4.36, age range = 59–74 years; 20 men). Participants gave informed written consent to the study procedure, which was approved by the Ethics Committee of the German Psychological Society (DGPs), and were paid for study participation. Study adherence was incentivized with a bonus system.

Design

Before (pretest) and after (posttest) the 6-month aerobic fitness intervention, as well as another six months after completing the intervention (maintenance), participants underwent a comprehensive assessment distributed over six testing sessions including a battery of questionnaires, cognitive tests, and motor tests as well as cardiovascular fitness assessment and an MR session. A mock scanner session was also included prior to the very first MR assessment to familiarize participants with the MR procedure.

Training

After completing the pretest assessment, participants were randomly assigned to either of two training regimens, high intensity (HI) or low intensity (LI). Groups were counterbalanced for age, sex, years of education, digit-symbol, and MMSE scores.

Participants in both groups came to the lab to exercise on stationary bikes. During the first three weeks, participants exercised twice a week, with each session lasting 25 min in the first week, 40 min in the second week and 55 min in the third week. After the third week, participants trained three times a week for 55 min each session. For the HI group, training intensity was calibrated to result in a heart rate at 80 % of the individual's ventilatory anaerobic threshold (Wasserman et al., 1990), as determined from a maximal graded exercise test at pretest. The LI group exercised at a constant resistance of 10 W irrespective of heart rates. During the last 21 sessions, 5 intervals of 2 min each were integrated after 20 min of training in order to further increase variance in fitness gains. Whereas the LI group only increased the cadence in these time windows from 60–70 to 80–90 cycles/min, the HI group also increased the intensity to a resistance corresponding to 110 % of the individual's ventilatory anaerobic threshold. To adapt intensity levels, heart rate was centrally and automatically monitored using the training software custo cardio concept (custo med GmbH, Ottobrunn, Germany). A staff member additionally controlled compliance for each participant and each training session. Participants exercised in groups of up to six persons at a time. Groups were not separated by intensity level, and participants were informed about differences between the two training regimens only after the termination of the study.

Cardiovascular fitness assessment

Aerobic fitness was assessed using a maximal graded exercise test on a cycle ergometer. The test started at 10 W, increased to 25 W after 2 min followed by 25 W increments every 2 min until total exhaustion or signs of cardiac or respiratory distress. A sports physician continuously monitored the cardiogram, oxygen uptake, heart rate, and blood pressure. As outcomes of the fitness assessment, four parameters

were assessed: the maximum oxygen consumption at exhaustion (VO_2max), the oxygen consumption at the ventilatory anaerobic threshold (VO_2AT), the maximum performance capacity (Wmax), and the performance capacity at the ventilatory anaerobic threshold (WAT). VO_2max for each subject and each time point was only used if at least one of the following criteria was met: (1) leveling-off of the oxygen uptake, (2) respiratory ratio ≥ 1.11, (3) maximum heart rate ≥ 200 – age, and (4) maximum oxygen equivalent ≥ 30. Note that values of performance capacity are always in relation to body weight, as heavier people can more easily achieve higher loads.

MRI data acquisition and processing

MRI protocol

Brain images were acquired on a Siemens TIM Trio 3 T MRI scanner (Siemens, Erlangen, Germany) using a 32-channel head coil. T1-weighted images were acquired using a standard magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2500 ms, TE = 4.76 ms, TI = 1100 ms, flip angle = 7°, acquisition matrix = 256 × 256 × 176, 1 mm isotropic voxels).

Diffusion-weighted images (DWI) were acquired using a single-shot EPI sequence with the following parameters: TR = 8000 ms, TE = 93 ms, 62 slices, 1 average, b-value 1000 s/mm^2, 60 diffusion encoding directions, voxel-size 2 × 2 × 2 mm^3. In addition, 7 images without diffusion weighting were acquired, which were equally distributed between the diffusion-weighted images.

Image processing

T1-weighted images. In order to remove the image inhomogeneities from the 32-channel head coil before pre-processing, the T1-weighted images were bias corrected using the N4ITK bias field correction as implemented in Slicer4.1 (http://www.slicer.org, Fedorov et al., 2012). We made the following changes to the default settings: an increase in the number of iterations to 500, 400, 300 as well as a decreased shrink factor of 2. A binary brain mask was used to improve correction quality.

Cortical reconstruction and volumetric segmentation were performed using the FreeSurfer image analysis suite version 5.3 (http://surfer.nmr.mgh.harvard.edu/). To reduce variability of results across time points and thus increase the robustness and sensitivity of the overall longitudinal analysis, images were automatically processed with the longitudinal processing stream implemented in FreeSurfer (Reuter et al., 2012). Specifically an unbiased within-subject template was created from the two time points using robust, inverse consistent registration (Reuter et al., 2010). Several processing steps, such as skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations were then initialized with common information from the within-subject template, significantly increasing reliability and statistical power (Reuter et al., 2012). All reconstructed data were visually checked for segmentation accuracy at each time point. No manual interventions with the MRI data were needed. Volumes of bilateral hippocampi in mm^3 were read out for each subject and each time point using FreeSurfer's "asegstats2table," and then included as observed variables in the LDMs.

To cross-validate the FreeSurfer analyses, we reanalyzed the T1-weighted images using FMRIB's model-based segmentation/registration tool FIRST (Patenaude et al., 2011) with its default settings, including affine registration to an MNI space template, segmentation of subcortical structures based on shape/appearance models constructed from manually segmented images, and boundary correction. Segmentations from each participant and at each time point were visually checked for segmentation accuracy. Volumes of bilateral hippocampi in mm^3 were read out for each subject and each time point to include them into the LDMs.

Diffusion-weighted images. Diffusion-weighted images (DWI) were first quality controlled using DTIprep (Oguz et al., 2014). This detects and corrects artifacts caused by eddy-currents, head motion, vibration and pulsation, venetian blind artifacts, as well as slice-wise and gradient-wise inconsistencies of signal intensity. Out of our 104 datasets, an average of 3 gradients per dataset was excluded. DWIs were then analyzed using the FMRIB Software Library (FSL, Jenkinson et al., 2012) as well as FMRIB's Diffusion Toolbox (FDT). Since motion and eddy current correction was already performed by DTIprep, we only generated a binary brain mask for the DWIs from the first non-diffusion- weighted image using the brain extraction tool (Smith, 2002) before fitting the tensor. Dtifit (part of the FDT tool in FSL) was used to fit a diffusion tensor model to every voxel included in the brain mask, producing the final diffusion maps. Participants' diffusion data were aligned into MNI space using nonlinear registration as implemented in TBSS (Smith et al., 2006). All diffusion maps were visually checked for registration accuracy at each time point. Mean diffusivity values in mm^2/s were extracted for bilateral hippocampi using anatomical regions of interest from the Harvard–Oxford Atlas.

Cognitive assessment

A comprehensive battery of cognitive tests was assessed, covering perceptual speed, executive control, episodic memory, reasoning, and vocabulary. We failed to establish a latent difference model for a pertinent cognitive ability such as episodic memory, working memory, or spatial navigation that would show reliable individual differences in change. Hence, we refrain from addressing associations between fitness-related changes in hippocampal integrity and cognitive changes in this article because the presence of reliable variance in change is a prerequisite for testing hypotheses on the covariance between changes (Hertzog et al., 2006, 2008).

Statistical analyses

Statistical analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics, V22, Armonk, NY, USA) and R (R Foundation for Statistical Computing, V 3.1.2, Vienna, Austria). To test for effects on the observed variables, we used repeated measures ANOVAs with time point as within-subject factor and training group as between-subject factor as well as age and gender as covariates.

Structural equation modeling

Given that difference scores are particularly vulnerable to the effects of measurement error, we used latent difference models to analyze data on the latent level (cf. McArdle and Nesselroade, 1994; McArdle and Prindle, 2008; Raz et al., 2005; Schmiedek et al., 2010). This method allows separation of variance at the construct level from measurement error and indicator-specific variance by defining latent factors that represent the variance common to multiple indicators (i.e., observed variables). We established a latent factor of fitness based on the observed variables VO_2AT and WAT, a latent factor of MD based on MD in the left and the right hippocampus, and a latent factor of hippocampal volume based on left and right hippocampal volumes as measured by FIRST and FreeSurfer. Using RAM notation (cf. Boker et al., 2002; McArdle and McDonald, 1984), Fig. 1 displays how latent constructs are established in the context of the latent difference score model, using the fitness factor as an example. We used the procedures described in Raz et al. (2005), with the notable exception that residual variances, which were constrained to be equal in Raz et al. (2005), were not constrained to be equal across time in the present paper in order to achieve acceptable model fit. Note, however, that the loadings of the indicators on the latent factors were constrained to be equal across time, just as in Raz et al. (2005). The resulting models retain the assumption of strong, but not strict (as in Raz et al., 2005) invariance of measurement across time.

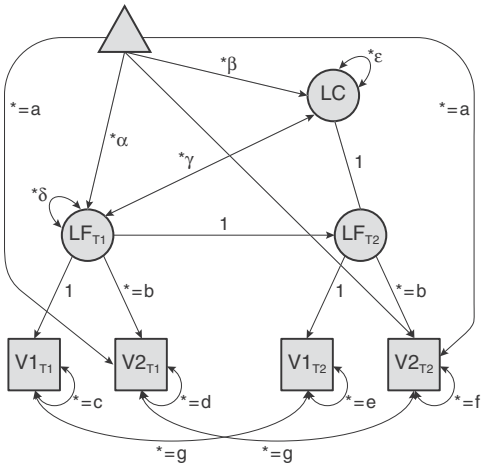

Fig. 1. Latent difference model with strong measurement invariance, adapted from Raz et al. (2005). Squares represent observed variables, circles represent latent factors, and the triangle serves to represent information regarding means and intercepts. V1–V2 = observed indicator variables (e.g., VO_2AT and WAT for fitness); LF = latent factor of construct (e.g., fitness); LC = latent change, (e.g. change in fitness from pretest to posttest); T1 = pretest (i.e., measures taken before fitness training); T2 = posttest (i.e., measures taken immediately after fitness training); α = latent mean of construct (e.g., fitness) at pretest; β = mean change in latent construct (e.g., fitness) from pretest to posttest; γ = latent covariance between individual differences at pretest and individual differences in changes; δ = latent variance at pretest; ε = latent variance in change; a = intercept of observed variable; b = loading of observed variable on latent factor; c, d, e, f = residual variances of observed variables; g = correlation between residuals; * = free parameter. Parameters with identical labels are constrained to be equal to each other to ensure identification and interpretability of the models.

To examine changes from pretest to posttest, we first computed univariate LDMs for the three constructs (fitness, MD, and volume) separately to examine whether (a) mean latent changes and (b) variances in change (i.e., individual differences in change) differed reliably from zero. Note that these two parameters vary freely, that is, individual differences in change can be reliably different from zero in the absence of reliable mean changes, and vice versa. Note also that interpreting correlations between changes would be dubious if one or both of the corresponding variances in change did not differ reliably from zero (cf. Ghisletta and Lindenberger, 2004). Age and gender were included as covariates when setting up the measurement model. All three constructs showed reliable variance in change. Hence we established a multivariate measurement model in which changes in all three constructs were allowed to correlate freely with one another (the corresponding R code can be found in Inline Supplementary R Code 1). We then imposed constraints on this measurement model, based on the guiding hypotheses of the present study. The resulting latent model specified unidirectional paths from changes in fitness to changes in hippocampal mean diffusivity, and from changes in hippocampal diffusivity to changes in hippocampal volume (see Inline Supplementary R Code 2). We used a chi-square test to examine whether this latent model, which is nested within the measurement model, was associated with a reliable decrement in fit. Given that we had clear a-priori hypotheses about the sign of the change–change associations, the statistical significance of the two path coefficients in the latent model was tested using one-tailed t-tests, with an alpha level of $p = 0.05$.

Inline Supplementary R Code 1 and 2 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2015.11.026.

To explore changes from posttest to maintenance, we used the same procedure, with one notable exception. As we had no strong hypotheses about the directionality of change–change associations during this phase of the study, we established a multivariate measurement model, in which changes in all three constructs were allowed to correlate freely with one another, but refrained from imposing a path model.

Results

Changes from pretest to posttest

Participants of the two groups did not differ at baseline with respect to age, years of education, fitness, and MMSE (more detailed sample characteristics are presented in Table 1). Also, training adherence did not differ reliably between the two groups. Table 2 provides an overview of training effects on the variables of interest for the two groups.

Univariate latent difference modeling

For fitness, model fit for strong measurement invariance (see Fig. 1) was satisfactory ($\chi^2 = 10.334$, df = 9, CFI = 0.993, RMSEA = 0.053, SRMR = 0.065). For MD, a model with strong measurement invariance provided a satisfactory fit to the data ($\chi^2 = 9.384$, df = 9, CFI = 0.999, RMSEA = 0.029, SRMR = 0.050). For volume, model fit for strong measurement invariance was acceptable ($\chi^2 = 78.657$, df = 38, CFI = 0.956, RMSEA = 0.143, SRMR = 0.056).

Inspecting changes in latent means from pretest to posttest

The intervention was effective at increasing fitness levels as confirmed by a significant mean increase in fitness (β = 0.413, SE = 0.183, p = 0.024). Contrary to expectations, the HI and LI groups did not differ in mean level of fitness change (β = 0.402, SE = 0.298, p = 0.178). Hence, the two groups were collapsed into one for all analyses reported here.

We observed a mean increase in MD at the latent level (β = 0.457, SE = 0.156, p = 0.003), as well as a mean decrease in HC volume at the latent level (β = −0.468, SE = 0.231, p = 0.043). Fig. 2 depicts the mean changes for the three constructs.

Multivariate latent difference measurement model

For the multivariate measurement model with the factors fitness, volume, and MD, strong measurement invariance provided an acceptable model fit ($\chi^2 = 195.530$, df = 131, CFI = 0.958, RMSEA = 0.097, SRMR = 0.125). As depicted in Fig. 3, changes in fitness were related to changes in mean diffusivity (ρ = −0.342, SE = 0.152, p = 0.024), and changes in MD were related to changes in volume (ρ = −0.488, SE = 0.215, p = 0.023). In contrast, the association between changes in fitness and changes in volume was not statistically reliable (ρ = 0.108, SE = 0.241, p = 0.655).

Table 1
Sample characteristics for the two training groups. Values are means (s.d.). The metabolic rate was assessed via the Freiburg Questionnaire of physical activity (Frey et al., 1999). Abbreviations: MMSE: Mini-Mental State examination, BMI: Body mass index.

Characteristic	Group		p-Value
	High intensity	Low intensity	
N	25	27	
Male	10 (40.0 %)	10 (37.0 %)	
Age	66.10 (4.16)	65.93 (4.63)	$t_{(50)} = -0.142$, p = 0.887
Education (years)	11.0 (1.57)	11.13 (1.71)	$t_{(50)} = 0.056$, p = 0.956
MMSE	29.24 (1.33)	28.85 (1.41)	$t_{(50)} = -1.020$, p = 0.313
Training adherence (max 75)	70.72 (3.82)	69.11 (7.66)	$t_{(50)} = -0.946$, p = 0.348
Hormone replacement therapy (women)	5 (33.3 %)	6 (35.3 %)	
Smoking	1 (4.0 %)	1 (3.7 %)	
Treated hypertension	4 (16.0 %)	3 (11.1 %)	
BMI	26.60 (4.33)	25.35 (3.94)	$t_{(50)} = -1.089$, p = 0.281
Digit symbol	38.13 (8.89)	39.81 (6.31)	$t_{(48)} = 0.777$, p = 0.441
Metabolic rate (self-reported)	16.92 (10.50)	20.15 (11.14)	$t_{(50)} = 1.073$, p = 0.289

Table 2
Pre- and post-intervention values (mean ± s.d.) for fitness measures, hippocampal volume, and mean diffusivity. Abbreviations: FreeSurfer HC: hippocampal volumes based on FreeSurfer, FIRST HC: hippocampal volumes based on FIRST, MD: hippocampal mean diffusivity.

	High intensity		Low intensity	
	Pre	Post	Pre	Post
Vo_2max (ml/min/kg)	21.26 (±4.76)	23.32 (±5.06)	21.69 (±5.76)	22.34 (±5.29)
Vo_2AT (ml/min/kg)	15.60 (±3.18)	16.54 (±3.49)	15.20 (±4.25)	16.05 (±3.57)
Wmax (W/kg) [*,a]	1.66 (±0.36)	1.87 (±0.41)	1.73 (±0.45)	1.81 (±0.50)
WAT (W/kg)	1.01 (±0.28)	1.14 (±0.35)	1.10 (±0.37)	1.11 (±0.34)
FreeSurfer left HC (mm^3)	3692.15 (±421.12)	3678.70 (±423.04)	3824.37 (±476.45)	3827.36 (±497.01)
FreeSurfer right HC (mm^3)	3778.10 (±399.20)	3746.16 (±404.86)	3895.97 (±468.86)	3889.15 (±465.40)
FIRST left HC (mm^3)	3658.92 (±203.53)	3612.32 (±480.09)	3674.44 (±477.30)	3648.56 (±527.43)
FIRST right HC (mm^3)	3619.24 (±479.92)	3572.00 (±457.73)	3769.44 (±447.25)	3773.63 (±455.69)
MD left HC (mm^2/s)	0.0012 (± .0001)	.0013 (± .0001)	0.0012 (± .0001)	.0012 (± .0001)
MD right HC (mm^2/s)	0.0012 (± .0002)	.0013 (± .0002)	0.0012 (± .0001)	.0012 (± .0002)

* Indicates a significant time × group interaction from the repeated measures ANOVA ($p < 0.05$).
[a] $F(1,48) = 5.207$; $p = .027$; $\eta^2 = 0.098$, follow-up ANOVAs separately for groups did not show significant changes (all $p > .11$).

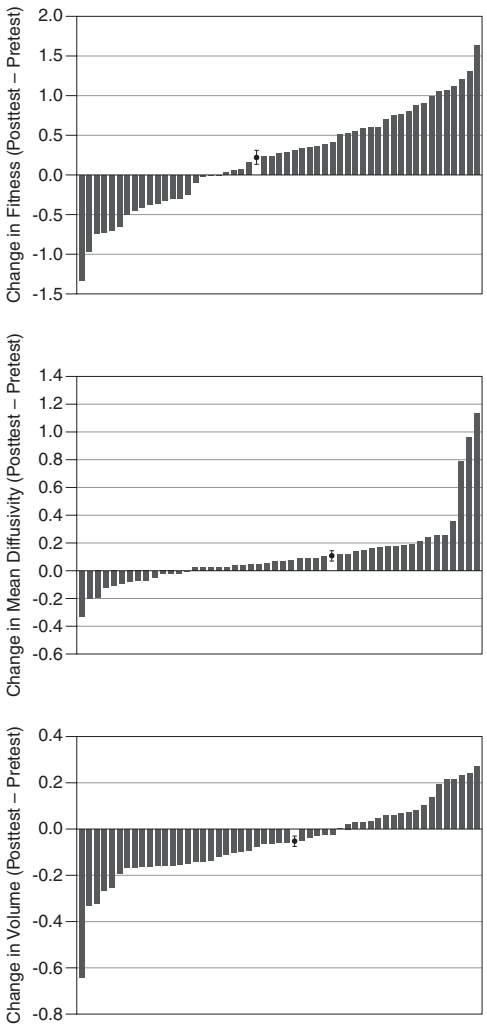

Fig. 2. Individual differences in change for fitness (top), hippocampal MD (middle), and hippocampal volume (bottom). Data for the two time points were pooled before z-transformation to preserve mean differences between time points, and then averaged across indicators. Each bar represents the posttest minus pretest difference for one participant. Difference scores are sorted in ascending order. Circles represent mean changes, with error bars corresponding to standard errors of the mean.

Multivariate latent difference path model

Next, we fit the data to a path model corresponding to the guiding hypotheses of the present study that an increase in fitness leads to lower diffusivity in the hippocampus, and that greater decrements (or lower increments) in diffusivity, reflecting more positive changes tissue density, lead to more positive (or less negative) changes in volume. This path model was nested within the multivariate latent difference measurement model. A direct comparison between the two models revealed that the path model was not associated with a reliable decrease in fit in comparison to the measurement model (−2 log likelihood ratio = 1.265; df = 1; p = 0.261). The hypothesized path model is shown in Fig. 4. The fit of the model to the data was satisfactory ($\chi^2 = 196.795$, df = 132, CFI = 0.958, RMSEA = 0.097, SRMR = 0.125). According to this model, changes in fitness account for changes in MD ($\beta = -0.330$ SE = 0.173, p = 0.028) and changes in MD lead to changes in volume ($\beta = -0.488$, SE = 0.212, p = 0.011).

Changes from posttest to maintenance

Univariate latent difference modeling

For fitness, model fit for strict measurement invariance was satisfactory ($\chi^2 = 14.153$, df = 12, CFI = 0.990, RMSEA = 0.059, SRMR = 0.054). For MD, a model with strong measurement invariance provided a satisfactory fit to the data ($\chi^2 = 14.764$, df = 9, CFI = 0.986, RMSEA = 0.111, SRMR = 0.054). For volume, model fit for strong measurement

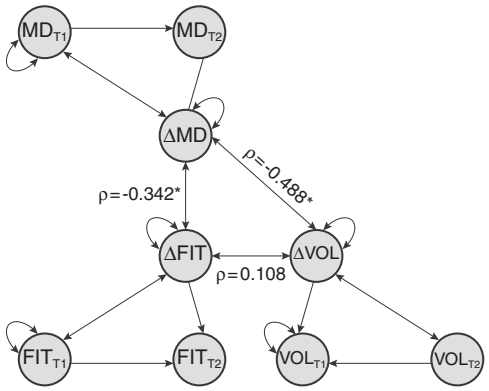

Fig. 3. Multivariate latent difference model. Changes in fitness were reliably associated with changes in MD, and changes in MD were reliably associated with changes in volume. Only latent constructs are shown. Abbreviations: FIT: latent factor fitness, VOL: latent factor volume, MD: latent factor mean diffusivity, T1: before training, T2: after training, Δ: latent change. * indicates $p < 0.05$.

Fig. 4. Path model. Only latent constructs are shown. Abbreviations: FIT: fitness, VOL: volume, MD: mean diffusivity, Δ: latent change. * indicates $p < 0.05$.

invariance was acceptable ($\chi^2 = 81.693$, df = 38, CFI = 0.951, RMSEA = 0.149, SRMR = 0.064).

Inspecting changes in latent means from posttest to maintenance

Six months after cessation of the controlled intervention mean fitness levels decreased significantly ($\beta = -0.480$, SE = 0.172, $p = 0.005$), and the two intensity groups again did not differ in mean level of fitness change ($\beta = -0.211$, SE = 0.287, $p = 0.461$). Mean latent changes in MD did not differ reliably from zero ($\beta = -0.012$, SE = 0.173, $p = 0.946$), nor did latent changes in volume ($\beta = 0.159$, SE = 0.208, $p = 0.445$).

Multivariate latent difference measurement model

We established a multivariate measurement model with the factors fitness, volume, and MD, assuming strict measurement invariance for fitness, and strong measurement invariance for MD and volume over time. This model yielded an acceptable model fit ($\chi^2 = 241.285$, df = 151, CFI = 0.943, RMSEA = 0.107, SRMR = 0.145). Changes from posttest to maintenance were not reliably associated across the three constructs (changes in fitness and changes in mean diffusivity: $\rho = -0.048$, SE = 0.163, $p = 0.768$, changes in MD and changes in volume: $\rho = -0.339$, SE = 0.208, $p = 0.103$, changes in fitness and changes in volume: $\rho = -0.000$, SE = 0.217, $p = 0.999$).

Discussion

In this study we investigated whether exercise-induced fitness improvements are associated with changes in mean diffusivity and volumes of the hippocampus. Using latent difference modeling (McArdle and Nesselroade, 1994), we were able to show that exercise-induced changes in fitness are associated with changes in hippocampal MD, and that changes in hippocampal MD are associated with changes in hippocampal volumes. The path model shown in Fig. 4 is consistent with the *assumption* of a temporal sequence in which fitness changes lead to changes in MD, which in turn lead to changes in volume. However, we note that a stringent empirical *test* of this assumption would require more than two time points.

Exercise-related changes in hippocampal volume have repeatedly been found (Erickson et al., 2011; Maass et al., 2015) but the mechanisms underlying these changes are not well understood. Given that exercise targets the cardiovascular system, improvements in brain perfusion are likely to be involved. In middle-aged adults, Pereira et al. (2007) provided initial evidence for a fitness-related increase in hippocampal perfusion. These results were recently replicated and extended in a sample of older adults by Maass et al. (2015), who reported an association between hippocampal perfusion and hippocampal volume. The association between hippocampal perfusion and hippocampal volume may reflect dilation of existing blood vessels, angiogenesis, or both. To the extent that changes in MD represent changes in the barrier density of gray matter, our findings suggest that the effects of fitness changes on volume may not be confined to dilation of preexisting vasculature but may also trigger angiogenesis. This is in line with cross-sectional findings by Bullitt et al., (2009) showing that highly active older adults exhibit a larger number of small-diameter vessels as assessed by MR angiography, and it is also consistent with relevant animal models (Bloor, 2005; Van der Borght et al., 2009). Angiogenesis may then trigger neurogenesis, synaptogenesis, or changes in the neuropil, leading to an increase in tissue volume.

In contrast to previous work (Erickson et al., 2011; Maass et al., 2015), the volumetric changes observed in this study were not directly linked to changes in fitness. The present training program included an interval phase, during which participants in the HI group were pushed above their anaerobic threshold, whereas participants in LI groups only increased the cadence. Possibly, this may have been a stressor for some participants in the HI group. Given that stress appears to suppress adult neurogenesis (Kim and Diamond, 2002; Lucassen et al., 2010), the interval phase may have counteracted some of the positive effects of fitness training. Some animal studies reported a negative association between hippocampal neurogenesis and exercise duration (Naylor et al., 2005) and intensity (Lou et al., 2008), with the latter being quite comparable to our setup. In line with this interpretation, visual inspection of the volumetric changes suggested increased volumes in the LI group as compared to stable volumes in the HI group, though the difference was not statistically reliable.

Unfortunately, the present study lacks measures of hippocampal perfusion and/or angiography and therefore cannot disentangle the contribution of these different factors to the observed volumetric changes. More research using quantifiable and mechanistically interpretable MR parameters are needed to elucidate the ways in which fitness training changes the physiology of the aging human brain, and helps to promote healthy cognitive aging.

Unlike most published reports on fitness interventions, we used LDM to analyze associations among changes. LDM establishes latent constructs of interest by using multiple indicators and represents variance in change at the latent level. It effectively separates construct variance from residual variance, and is likely to increase statistical power as well as replicability of results across studies. We encourage more frequent use of the LDM approach in intervention research. However, we note that the method is based on strong (but testable) assumptions about measurement invariance, and requires moderate to large sample sizes.

Contrary to expectations, we found no group differences in fitness changes between the HI and the LI groups. Perhaps the two training regimes were too similar for sedentary adults, in the sense that the LI training may have been more effective in boosting fitness than expected. Furthermore, genetic factors seem to modulate exercise-induced gains in fitness (Peter et al., 2014), thereby potentially blurring effects at the group level.

The sample size of the present study is still relatively small. In addition, the present results may, to an unknown degree, reflect a bias in participation selection. Participants in the present study followed a highly sedentary lifestyle but, at the same time, were sufficiently healthy to be admitted to the study, and felt sufficiently motivated to engage in an aerobic exercise intervention program. This combination of features may have led to the selection of a particularly resilient sedentary group. It remains to be seen whether the present results replicate in larger and more representative samples.

From posttest to maintenance, fitness, MD, and hippocampal volume showed reliable variance in change, but, in contrast to the intervention period, these changes were no longer associated across constructs. This may suggest that the different constructs vary in the rate of adaptation, that participants differ in the degree to which they continued exercising, or both. In the present study, the level of exercise between posttest and maintenance was only assessed via monthly self-report questionnaires; more detailed, objective measures are lacking. Hence the interpretation of these differences in change is unclear.

Finally, it should be kept in mind that the hypothesized sequence of changes postulated in this article is empirically under-identified. According to this sequence, which corresponds to the path model shown in Fig. 4, changes in fitness lead to changes in hippocampal tissue density, which in turn lead to changes in volume. Clearly, more than two time points are needed to observe these changes separately in time in order to test the veridicality of this sequence more directly. Future research should rely to a greater extent on research designs with multiple

scanning sessions distributed before, during, and after the intervention to identify the trajectories and dynamics of experience-dependent plasticity (cf. Lövdén et al., 2013).

Conclusions

In this study we found that exercise-induced fitness changes are associated with microstructural brain changes in the hippocampus as indexed by MD, and that changes in hippocampal MD are associated with changes in hippocampal volume. These results suggest that previously reported increases in hippocampal volume may in part be driven by an increase in cell membranes operating as diffusion barriers.

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Paper II

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Title

Correlated changes among fitness, prefrontal white-matter microstructure, and fluid cognition in old age: Results from an exercise intervention study

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Abstract

Beneficial effects of exercise on cerebral gray matter and cognitive performance are well documented, but little is known about exercise effects on cerebral white matter microstructure (WMM) and its potential effects on cognition. In a randomized exercise intervention, 49 adults aged 59 to 74 years participated for six months in either high- or low-intensive physical exercise. Before and after completing training, we assessed participants' cardio-vascular fitness levels and cognitive abilities, and acquired diffusion-weighted images. For analyses, data were collapsed across groups because mean changes in fitness did not differ reliably by training intensity. Using latent change score modeling, we examined individual differences in pretest-posttest changes in (i) fitness, (ii) WMM indices in predefined tracts of interest, and (iii) a composite fluid intelligence score. Changes in fitness were associated with changes in multiple indices of WMM in forceps minor, and changes in WMM were associated with changes in fluid intelligence. Results suggest that changes in WMM may contribute to the beneficial effects of physical exercise on cognition in healthy, sedentary seniors.

Keywords

Aging, Fitness, Physical exercise, White matter microstructure, Forceps Minor, Latent change score modeling

1 Introduction

In humans, *in-vivo* white matter microstructure (WMM) is commonly assayed with parameters derived from diffusion tensor imaging (DTI; but see Arshad et al., 2016). DTI renders the diffusion properties of water molecules within tissue and yields multiple indices (Bammer, 2003, Beaulieu, 2002): Fractional anisotropy (FA) indicates the coherence of diffusion along the principal axis of a tensor, whereas mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) indicate how freely a water molecule can diffuse in any direction, parallel to the principal axis, or perpendicular to the principal axis, respectively.

Advancing adult age is associated with presumably deleterious changes in WMM. The majority of cross-sectional studies (see Madden et al., 2012 for a review) as well as an increasing number of longitudinal studies report that aging is accompanied by reductions in fractional anisotropy (e.g. Bender and Raz, 2015, Bender et al., 2016b, Bennett et al., 2010, Burzynska et al., 2010, Lövden et al., 2014, Sexton et al., 2014, Vik et al., 2015) as well as increases in mean diffusivity (e.g. Bennett et al., 2010, Burzynska et al., 2010, Lövden et al., 2014, Sexton et al., 2014), and radial diffusivity (e.g. Bender and Raz, 2015, Bender et al., 2016b, Bennett et al., 2010, Burzynska et al., 2010, Sexton et al., 2014). The direction of age-related changes in axial diffusivity is more inconsistent, with one longitudinal study showing consistent increases (Sexton et al., 2014) and other studies showing both, increases and decreases depending on the tract (Barrick et al., 2010, Bender and Raz, 2015, Bender et al., 2016b, Bennett et al., 2010, Burzynska et al., 2010). These WM alterations are thought to reflect a process of disconnection, supposedly resulting in cognitive dysfunction. Indeed, mostly cross-sectional (reviewed in Bennett and Madden, 2014, but see Madden et al., 2017), but also few recent longitudinal studies provide first evidence that age-related alterations in structural connections are associated with concomitant changes in cognitive performance (Bender et al., 2016a, Charlton et al., 2010, Fjell et al., 2017, Lövden et al., 2014, Ritchie et al., 2015).

Growing evidence suggests that regular aerobic exercise can assuage age-related decrements in cognitive performance. Potential mechanisms underlying the cognitive benefits of aerobic exercise are reflected in increased gray matter volumes in frontal and hippocampal regions, possibly induced by angiogenesis, gliogenesis and/or neurogenesis, as well as more efficient engagement of brain networks (for reviews, see Bherer et al., 2013, Stillman et al., 2016, Voss et al., 2013b). However, relatively little is known about the potential contributions of exercise-induced alterations of cerebral white matter (WM) pathways, including their microstructural properties.

The extant findings relating cardio-respiratory fitness to WMM are mixed. A recent meta-analysis of the relationship between physical fitness and white matter health demonstrated a significant positive association, but the size of the effect was rather small (Sexton et al., 2016). They reported that better physical fitness was associated with greater WM volumes, reduced WM lesions, and more favorable WMM indices. To date, only three intervention studies have investigated exercise-induced WM changes. Colcombe et al. (2006) reported volumetric increases in anterior, commissural white matter, following a 6-month aerobic exercise intervention. Voss et al. (2013a) reported that DTI-derived white matter indices benefited from exercise-induced fitness changes. They reported that higher aerobic fitness gains, following a 1-year walking program, were positively associated with changes in fractional anisotropy (FA) in prefrontal and temporal WM, but not with other DTI indices (i.e., AD and RD). Moreover, the changes in FA were not associated with cognitive performance benefits. Voss et al. (2013a) restricted their analyses to four large regions of interest, which corresponded roughly to the four primary lobes of the telencephalon. The use of lobar-based analyses may have obscured tract-specific associations to behavior. Finally, a recent intervention study with older adults reported that six months of dancing in older adults led to an increase of FA in the fornix (out of 20 tracts), whereas brisk walking did not (Burzynska et al., 2017). However, it is not clear whether the dancing intervention was effective in increasing

fitness levels. Hence, there is preliminary evidence for a beneficial effect of regular exercise activities on white matter microstructure, but the association between such benefits and cognitive performance is unclear.

In summary, age-related decline in white matter microstructure has been associated with declining cognitive abilities. At the same time, exercise appears to protect against age-related declines in cognitive performance and WM health. Therefore, it is worthwhile investigating whether improvements in cerebral WMM following physical exercise contributes to the beneficial effects of exercise on cognition. In this study, we examined whether cardiovascular exercise leads to changes in aerobic fitness, and whether these changes are associated with changes in WM diffusivity parameters and cognitive performance. To this end, we randomly assigned 52 sedentary but healthy older adults to a high- versus low-intensity regime of aerobic fitness training. Before and after the 6-months training phase, participants performed a maximal graded exercise test to assess their training-related fitness improvements, underwent MR imaging, and completed an extensive cognitive test battery. We used latent change score modeling (LCSM, McArdle and Nesselroade, 1994, cf. Raz et al., 2005), to assess individual differences in changes in WMM, fitness, and cognition. The LCSM analytic framework separates variance at the construct level from measurement-specific variance and error, and provides change scores that are more reliable than difference scores based on observed variables (cf. McArdle and Nesselroade, 1994). We hypothesized that exercise attenuates age-related declines in prefrontal and limbic WM tracts. We also hypothesized that prefrontal WM changes would be associated with improved fluid cognition, whereas changes in limbic WM tracts would be associated with improved memory performance.

2 Materials and methods

2.1 Participants

A total of 52 healthy but sedentary older adults participated in the study. Eligibility criteria included: (1) physical inactivity prior to study enrollment (metabolic rate < 40 based on the Freiburg Questionnaire of physical activity in German, Frey et al., 1999); (2) absence of gross cognitive impairment (MMSE score ≥ 26); (3) absence of neurological, psychiatric, or cardiovascular diseases; (4) right handedness; (5) no contraindication for heart-rate controlled exercise training (e.g., no prescription of beta blockers); and (6) suitability for an MR environment (e.g., no magnetic implants, claustrophobia). Descriptive demographics are reported in Table 1. The Ethics Committee of the German Psychological Society (DGPs) approved the study, and all participants provided written informed consent to the study procedure prior to enrollment. Participants received monetary remuneration up to 1105 Euro for study completion, including training sessions. See Kleemeyer (2016) for a complete description of sample characteristics. Three participants had incomplete MR data and were excluded from all analyses, resulting in an effective sample of 49 individuals with a mean age of 66.2 years, and an age range of 59–74 years.

2.2 Study design

The study followed a 2 (training group: high-intensity vs. low-intensity) × 3 (time: pretest, posttest, maintenance) design, with 6 months between measurement occasions. The aerobic fitness training took place between pretest and posttest, with no further training sessions offered between posttest and maintenance. At each measurement occasion, participants completed a battery of questionnaires, cognitive tests, and motor tests, as well as the cardiovascular fitness and the MR assessments, distributed over six testing sessions.

2.3 Training

Following completion of all pretest assessments, we randomly assigned participants to one of the two training regimens. The training regimens included either high- or low-intensity aerobic exercise training: The high-intensity (HI) group trained at individually calibrated heart rates

corresponding to 80 % of the individual's ventilatory anaerobic threshold (Wasserman et al., 1990) at pretest. All participants in the low-intensity (LI) group trained at a constant resistance of 10 W. Each training session lasted 55 minutes, including five minutes for both warm up at the beginning and cool down at the end of each session. To further increase variance in fitness gains, the last 21 sessions also included high-intensity interval exercise training (HIT): After 20 minutes of regular training, five intervals of two minutes each were integrated, separated by two minutes of active recovery. During these time windows, the LI group increased the speed from 60–70 to 80–90 cycles/min, whereas the HI group also increased the intensity to a resistance corresponding to 110 % of the individual's ventilatory anaerobic threshold established at pretest. Experimenter-supervised training took place at the institute with up to six people training simultaneously, irrespective of group assignment. Heart rates were centrally monitored using the custo cardio concept (custo med GmbH, Ottobrunn, Germany) training software. We informed participants about the two different training regimens only at the end of the study.

2.4 Cardiovascular fitness assessment

We assessed participants' cardiovascular fitness using a graded maximal exercise test on a cycle ergometer. The test started at 10 W, increased to 25 W after 2 min followed by 25 W increments every 2 min until total exhaustion or signs of cardiac or respiratory distress. A sports physician continuously monitored the cardiogram, respiratory parameters (e.g. oxygen uptake), heart rate, and blood pressure. The maximum oxygen consumption at exhaustion (VO_2max) and the oxygen consumption at the ventilatory anaerobic threshold (VO_2AT) served as outcome measures.

2.5 Cognitive assessment

At each measurement occasion, participants completed three 90-minute cognitive testing sessions, administered on separate days in small groups of five participants. The cognitive

battery covered the following seven cognitive abilities: perceptual speed, inhibition, switching, updating, episodic memory, reasoning, and vocabulary. Participants completed identical versions of the tasks at all measurement occasions. The digit-symbol and vocabulary test were paper-pencil based, and the remaining tasks were computerized. Responses for computerized tasks were provided via response boxes or the computer keyboard. For all self-paced tasks, median reaction times (RT) were analyzed.

2.5.1 Perceptual speed

Perceptual speed was assessed using two tasks: the Digit-Symbol Substitution test (DSS test; Wechsler, 1981) as well as a figural comparison test (Schmiedek et al., 2010). In the DSS test, the first row on the paper provided a combination of nine digit-symbol pairs. The remaining rows only contained digits. Participants were instructed to assign the corresponding symbol to a given digit following the order on the paper. The number of correctly assigned symbols within 90 seconds served as outcome measure. The figural comparison task presented two colored, three-dimensional objects side-by-side on a computer screen for a maximum of 5000 ms, and participants were instructed to decide as quickly as possible, whether the objects were identical. Responses were provided via response boxes and the stimuli disappeared as soon as an answer was given or after 5000 ms. Five-hundred ms thereafter the next object pair appeared. Following 20 practice trials, participants completed 40 test trials. RT for correct responses served as outcome measure.

2.5.2 Inhibition

Inhibition was assessed using three tasks: the Stroop task (Stroop, 1935), the Flanker task (Eriksen and Eriksen, 1974), and a stop signal task (Li et al., 2006). During the Stroop task, color-words printed in either the same (compatible, e.g. red) or a different (incompatible, e.g. red) font were presented in the center of the screen for 1000 ms. Participants were asked to indicate as quickly as possible the font color, and the next stimulus only appeared 1000 ms

after an answer was given. Participants completed 24 practice trials and four blocks of 36 test trials each, with 50 % of the trials being incompatible, i.e. word and font color did not match. The RT for correct responses served as outcome measure. During the Flanker task, participants were presented with five arrows where the arrow in the middle could either point in the same direction as all other arrows (compatible, e.g. >>>>>) or in the opposite direction (incompatible, e.g. >><>>), and participants were instructed to indicate the direction of the middle arrow. The stimulus presentation duration was 1000 ms with 1000 ms inter-stimulus interval (ISI), independent of whether a response was recorded. After 24 practice trials, participants completed four blocks of 32 trials each, whereas 50 % were incompatible. Accuracy (ACC) defined as proportion correct served as outcome measure. During the stop signal task, a circle appeared on the screen and participants were asked to press a button as soon as they perceived the circle (go trials). In 25 % of the cases, the circle was followed by a cross and in those cases, participants were instructed to inhibit their prepared response and not press the button (stop trials). The interval between circle and cross (stop-signal delay, SSD) started at 200 ms and then increased or decreased adaptively: If the participant was able to inhibit the response, the interval was prolonged (+ 64 ms), otherwise it was shortened (- 64 ms). After 20 practice trials, participants completed 120 test trials. The stop-signal RT was estimated by subtracting the mean SSD from the mean go trial RT, and served as outcome measure.

2.5.3 Task switching

Three tasks were used to measure task switching abilities: the number-letter task (Rogers and Monsell, 1995), the global-local task (Kinchla et al., 1983) and the face-word task (Yeung et al., 2006). During the number-letter task, participants saw a number-letter pair appearing in one of four quadrants of the screen (top left, top right, bottom left, bottom right). In cases where the stimulus pair appeared at the top, participants had to attend to the number, and indicate

whether it was odd or even. If the stimulus pair appeared at the bottom, participants were instructed to attend to the letter, and indicate whether it was a vowel or a consonant. Stimuli included 2, 3, 4, 5, 6, 7, 8, 9, A, E, I, U, G, K, M, R. For the global-local task, participants were presented with Navon figures, i.e. large objects composed of small objects. Objects were always circles, triangles, squares, or crosses, e.g. a large circle composed of small triangles. If the objects appeared in blue, participants had to indicate the shape of the larger (global) object. If the objects appeared in black, participants had to indicate the shape of the smaller (local) objects. During the face-word task, participants saw 1-or 2-syllable words overlaid on male or female faces. A key appeared below the stimulus pair, indicating whether the face or the word should be attended. For words, participants were required to indicate whether the word had one or two syllables, whereas for faces they had to decide whether it was female or male. For all task-switching tasks, stimuli were presented for 2500 ms and the next stimulus only appeared 500 ms after an answer was given via response boxes. Trials were randomly presented, with 50 % of the trials requiring a task-switch. For every task, participants completed 24 practice trials followed by 128 test trials, divided into four separate blocks of 32 trials each. RT on correct switch trials served as outcome measure for all three tasks.

2.5.4 Updating

We assessed updating using three different tasks: letter updating, spatial updating and 2-back (all taken from Schmiedek et al., 2009). During letter updating, single letters (A, B, C, or D) appeared randomly on the screen one after the other for 2500 ms with a 500 ms ISI. The presentation sequence stopped after 7, 9, 11, or 13 letters and the task instructed participants to enter the last three letters displayed, via the computer keyboard. After four practice trials, participants completed two sequences composed of 7, 9, 11, or 13 letters each, resulting in a total of eight trials presented in randomized order. Accuracy served as outcome measure. During spatial updating, participants were shown two 3 × 3 grids, presented side-by-side on

the computer screen. At the beginning of every trial, a dot appeared simultaneously in each grid for 4000 ms and participants were instructed to remember the position of the dot. Next, arrows appeared synchronously above each grid for 2500 ms, indicating that the dot in the respective grid had to be moved one field in the direction of the arrow. After 500 ms another pair of arrows required another moving of the dots. At the end, the final position of the dots had to be marked in the grids via mouse click. After a practice trial, participants completed two easy trials including two updating operations per grid, as well as two difficult trials including three updating operations per grid. Accuracy served as outcome measure. During the 2-back, single digits (1-9) appeared randomly on the screen for 500 ms one after the other with a ISI of 3000 ms. Participants were asked to indicate, whether the currently presented digit was identical to the digit presented two displays earlier in the sequence or not. After one practice sequence (26 numbers), participants completed three test sequences (39 numbers). Accuracy served as outcome measure.

2.5.5 Episodic Memory

The cognitive testing battery included three episodic memory tasks: image memory, plural memory and word lists (all taken from Schmiedek et al., 2010). In the image memory task, participants saw a sequence of six objects appearing at random positions in a 6 × 6 grid. Objects were presented for 4000 ms with an ISI of 1000 ms. Participants were instructed to remember both, object location and object identity. At the end of a trial, all objects appeared below the grid and participants were asked to drag the objects in the correct order onto the correct location in the grid using the computer mouse. After one practice trial, two trials with six objects each were completed, with no time constraints. Accuracy served as outcome measure. During word memory, participants saw a sequence of 18 words presented on the screen, one by one, for 4000 ms with 1000 ms ISI. Participants were asked to remember as many words as possible in the correct order. At the end, they entered the remembered words

via the computer keyboard. They completed a short practice comprising four words. Accuracy served as outcome measure. During plural memory, participants were presented with number-noun pairs (e.g. 12 plates), and were instructed to remember the pairings. The pairs were serially presented on the screen for 5000 ms each, with 1000 ms ISI. Subsequent to presentation, only the nouns appeared in random order on the screen, and participants had to enter the corresponding numbers. After one practice trial, participants completed two trials composed of four pairs and one trial composed of six pairs. Accuracy served as outcome measure.

2.5.6 Reasoning

Using a version of Raven's progressive matrices (Raven et al., 1998), participants saw a 3 × 3 matrix with patterns following certain regularities. The pattern on the lower right was missing, and participants were instructed to identify the correct pattern out of eight given alternatives. A total of 15 trials could be completed within a maximum of 15 minutes. Accuracy served as outcome measure.

2.5.7 Vocabulary

The MWT-A (Lehrl et al., 1991) served as a measure of vocabulary. Here, every row contained five words, including one non-word. Participants had to identify the non-word and cross it out. There were no time constraints. Accuracy served as outcome measure.

2.5.8 Data Analyses

Initial latent change score models for domain-specific cognitive abilities, including episodic memory, updating, and inhibition, failed to demonstrate reliable individual differences in change. To include a broad spectrum of cognitive performance into our analyses, we conducted a principal component analysis (PCA) to extract a composite score for cognition. We first tested the scores of all cognitive variables for normal distribution using the d'Agostino

method (D'Agostino and Pearson, 1973) as well as visual inspection and selected those tests whose scores were normally distributed at pretest and targeted executive or working memory functions. Applying these criteria reduced the number of 10 initial scores (Stroop RT, Flanker ACC, StopSignal RT, Number-Letter RT, Global-Local RT, Face-Word RT, Letter updating ACC, Spatial updating ACC, 2-back ACC, Raven ACC), down to the following five scores: Stop-signal RT, Face-word RT, Spatial Updating ACC, LetterUpdating ACC, Raven ACC. The pretest correlations among these remaining scores range from –0.38 to 0.45, with a median negative correlation of –0.30 and a median positive correlation of 0.40. When conducting the PCA, we found that only the first factor was associated with an eigenvalue greater than 1 (i.e., 2.264), accounting for 45.3 % of the variance. Hence only this first unrotated factor was retained for further analysis. All five variables showed moderate to high loadings on the first factor (Stop-signal RT: –.526, Face-word RT: –.611, SpatialUpdating ACC: .798, LetterUpdating ACC: .752, Raven ACC: .642). To retain mean differences between measurement occasions, the data were standardized to z-scores by subtracting the overall mean (across occasions) from the individual data and dividing the difference by the pretest standard deviation. The component coefficients estimated by the PCA were then applied to the standardized posttest data to compute a corresponding composite score at posttest, which could be included into our LCSM.

2.6 MR Image acquisition and processing

Brain images were acquired on a Siemens TIM Trio 3T MRI scanner (Siemens, Erlangen, Germany) using a 32-channel head coil. Diffusion-weighted images (DWI) were acquired using a single-shot EPI sequence with the following parameters: TR = 8000 ms, TE = 93 ms, 62 slices, 1 average, b-value 1000 s/mm^2, 60 diffusion encoding directions, voxel-size 2 × 2 × 2 mm^3. In addition, seven images without diffusion weighting (b = 0) were acquired.

First, DWIs were quality controlled using DTIprep (Oguz et al., 2014) to detect and correct artifacts caused by eddy-currents, head motion, vibration and pulsation, as well as slice-wise

and gradient-wise inconsistencies of signal intensity. Out of 147 datasets, an average of two gradients per dataset was excluded, with no significant differences between pre-, post-, and maintenance test. DWIs were then processed using the FMRIB Diffusion Toolbox (FDT) from the FMRIB Software Library (FSL, Jenkinson et al., 2012). Following motion and eddy current correction and before tensor fitting, we used the brain extraction tool (Smith, 2002) to generate a binary brain mask from the first non-diffusion weighted image. We used the DTIfit from FDT with an ordinary least squares linear regression to fit a diffusion tensor model to every voxel included in the brain mask, and produce the final diffusion parameter maps. Participants' FA maps were aligned into MNI space using nonlinear registration as implemented in TBSS (Smith et al., 2006), with two notable exceptions: We created our own group-wise template using DTI-TK, which served as the registration target. All diffusion maps were visually checked for registration accuracy at each time point. Next, we created a group mean FA image, which was used to create the FA skeleton, using a threshold of FA = 0.3. The FA skeleton represents the central streams of the white matter tracts. All participants' normalized FA data were projected onto the mean FA skeleton to further minimize registration errors, and the same projection assignments were applied to the AD, RD, and MD images. From these skeletonized images, we extracted mean FA, MD, AD, and RD values for the following predefined tracts of interest (TOI): dorsal cingulum (CingD), forceps minor (FMin), forceps major (FMaj), inferior fronto-occipital fasciculus (IFOF), superior longitudinal fasciculus (SLF), corpus callosum genu (CCg), body (CCb), and splenium (CCs), posterior limb of the external capsule (PLIC), as well as fornix (For). These TOIs were chosen as representing white matter tracts that extend into prefrontal or medial temporal lobes. FMaj and PLIC served as control regions. All masks were based on the JHU white-matter tractography atlas (Wakana et al., 2004). For fornix, we additionally used the Juelich atlas mask, as it included a greater extent of the structure on the WM skeleton. Except for the fornix masks ($r < 0.6$, for both left and right), test–retest correlations for these variables were all high ($r > 0.7$, for all). Therefore,

we excluded fornix from analyses. We also extracted FA, MD, AD, and RD values from the whole skeleton to assess global change.

2.6.1 Multiverse Analyses

In light of criticisms raised against TBSS analysis (Bach et al., 2014), we adopted a multiverse analysis approach (cf. Steegen et al., 2016) to verify the validity of our results. That is, we repeated our analyses using a set of alternative processing approaches to test the extent to which our results depend on our processing pipeline. Most importantly, we used DTI-TK to alternatively register the DWIs based on full tensor information, which supposedly improves registration performance (Zhang et al., 2006). DTI-TK also implements an unbiased longitudinal approach (Keihaninejad et al., 2013), which includes a 2-step registration procedure: First, within-subject registration creates an average, template image of all DWIs from one subject (i.e., from each occasion of measurement) and projects the native data to that intra-subject template. Next, between-subject registration creates an average of the intra-subject templates and registers the intra-subject templates to that group-wise template. By combining the transformation matrices, native space data are then warped to the group-wise template space, which is *not* a standardized space (e.g. MNI). In order to sample from a priori defined TOIs, another registration step is necessary to either register the TOIs to the group-wise template space or the group-wise template to the atlas space. Combining these different approaches resulted in the generation of seven different pipelines (see Table 2 for summary and comparison).

2.7 Structural equation modeling

Given that difference scores are particularly sensitive to the effects of measurement error, we estimated change over training and maintenance using latent change score models (cf. McArdle and Nesselroade, 1994, McArdle and Prindle, 2008, Raz et al., 2005). This method allows separating variance at the construct level from measurement error and indicator-

specific variance by defining latent factors that represent the variance common to multiple indicators (i.e., observed variables). All modeling was performed using the lavaan package in R (Rosseel, 2012). For the DTI measures, separate latent factors were created for each TOI. The mean values from left and right hemispheres served as dual indicators for each latent factor for each tract and DTI index (FA, AD, RD, MD). The latent factor of fitness was also based on two indicators, VO_2max and VO_2AT. Detailed modeling procedures have been described elsewhere (Kleemeyer et al., 2016). For each TOI and each DTI index (FA, AD, RD, MD), we first fitted univariate LCSMs separately to examine whether (a) mean latent changes and (b) variances in change (i.e., individual differences in change) from pretest to posttest differed reliably from zero. Covariates were included as regression paths on pretest latent variables when setting up the measurement model: age and gender for all three measures, and hypertensive treatment only for white matter indices. For all models, we used conventional model fit criteria to determine well fitting models for the data (CFI ≥ 0.95, RMSEA values ≤ 0.08, and SRMR values ≤ .08). In order to achieve an acceptable model fit for all models, residual variances of the observed variances were not necessarily constrained to be equal across time. These models retain the assumption of strong, but not strict (as in Raz et al., 2005) invariance of measurement across time and are indicated by an “a” in Table 3. For SLF and PLIC, we were not able to obtain an acceptable model fit for AD and MD, and the same is true for CingD and CCs for FA, and CingD and CCb for RD. The remaining models provided acceptable fit as well as reliable variance in change.

On this basis, we established multivariate measurement models, in which changes in DTI, fitness and fluid cognition were allowed to correlate freely with one another. The same procedures were applied to explore changes from posttest to maintenance.

3 Results

3.1 Univariate latent change score models

3.1.1 Fitness

Pretest to posttest

For fitness, a model imposing strict measurement invariance provided good fit (χ^2 = 12.486, df = 17, CFI = 1, SRMR = 0.060, RMSEA = 0, 90 % CI = 0.000 - 0.091). Female participants had significantly lower baseline fitness levels. Mean fitness level increased significantly from pre- to posttest (β = 0.464, SE = 0.178, *p* = 0.005), indicating the effectiveness of our treatment, with an average effect size of 0.305. However, the HI and LI groups did not differ in mean level of fitness change (β = 0.180, SE = 0.272, *p* = 0.511). Hence, the two groups were collapsed into one for all analyses investigating covariances of change. Variance in fitness change was reliable.

Post-test to maintenance

Good model fit was obtained when imposing strong measurement invariance (χ^2 = 18.279, df = 16, CFI = .988, SRMR = 0.067, RMSEA = 0.054, 90 % CI = 0.000 - 0.148). Mean fitness level did not change from posttest to maintenance (β = –0.011, SE = 0.224, *p* = 0.962), with again no difference between the two training groups (β = –0.118, SE = 0.294, *p* = 0.689), but significant variance in change.

3.1.2 Diffusion tensor imaging

Pretest to posttest

The results of the 44 (11 TOIs × 4 DTI indices) univariate models are summarized in Table 3. With the exception of SLF and PLIC AD and MD, CingD and CCb RD, and CingD and CCs FA, models imposing strong or strict measurement invariance fit the data reasonably well (CFI ≥ 0.950, RMSEA values ≤ 0.104, and SRMR values ≤ .085). For various tracts, older participants showed higher AD, RD, and MD values, as well as lower FA values at baseline (details are listed

in Table 3). In five cases, participants receiving antihypertensive treatment also showed higher AD, RD, and MD values, and lower FA. From pre- to posttest, we observed reliable decreases in AD in IFOF, CCb, and CCs, as well as in the overall skeleton. RD also decreased significantly in SLF and PLIC. Likewise, MD decreased in Cing, IFOF, CCb, as well as in the overall skeleton. FA decreased in FMin, CCg, CCb, as well as in the overall skeleton, but increased in PLIC. Furthermore, all TOIs exhibited reliable variance in change.

Posttest to maintenance

Given that various models did not meet our strict goodness of fit criteria, we also considered models that did not have an RMSEA below .08, as long as the 90 % CI included .05 and all other criteria were fulfilled. Condensed results are presented in Table 4. In contrast to changes from pre- to posttest, we observed reliable increases in the majority of tracts for AD, RD, and MD between posttest and maintenance. For FA however, we observed again significant decreases, predominantly in those tracts that did not show a decrease between pre- and posttest. Again, all TOIs showed reliable variance in change.

3.1.3 Fluid cognition

Pretest to posttest

For the composite score of fluid cognitive abilities, we observed a mean increase from pre- to posttest ($\beta = 0.357$, SE = 0.147, $p = 0.012$), as well as significant inter-individual variability.

Posttest to maintenance

Although there was no significant mean change in the fluid cognitive score during the maintenance period ($\beta = 0.102$, SE = 0.143, $p = 0.476$), variance in change was reliable.

3.2 Latent change score modeling

Pretest to posttest

All multivariate measurement models provided reasonable fit to the data based on the indices mentioned above. Only FMin changes were significantly associated with changes in fitness. For this tract, we observed significant change-change covariance parameters for multiple DTI indices: RD, MD, and FA. The association was negative for RD and MD: Participants with greater fitness gains showed more negative changes in RD and MD. For FA instead, the relation was positive, indicating that participants with greater fitness gains experienced less negative changes in FA. Since we did not observe such an association for CCg, which is a major part of FMin, we created a FMin≠CCg mask, which excluded voxels from CCg. Change-change associations with the FMin≠CCg tract tended to be more pronounced than the associations observed for FMin including the CCg, and were also present for AD. Furthermore, changes in FMin≠CCg DTI indices were associated with changes in fluid cognition. As depicted in Figure 1, changes in AD, RD, and MD were negatively related to fluid cognition, whereas the relation was positive for FA. Details regarding fit indices and model estimates are provided in Table 5.

Posttest to maintenance

We inspected whether the associations between fitness and DTI changes in FMin and/or FMin≠CCg would also hold over the maintenance phase. Since the univariate models for MD did not provide an acceptable model fit, we only inspected AD, RD, and FA. Model fits for these multivariate measurement models were marginal, and none of the changes in DTI indices co-varied with change in either fitness or fluid cognition (Supplementary Material, Table S1).

3.3 Multiverse testing

Results for the different analysis pipelines are presented in Table 6. In general, mean trends and covariances of change did not differ in sign across the pipelines. Yet, when using tensor-based registration as implemented in DTI-TK, some effects were no longer significantly different from zero.

4 Discussion

In this study, we found that fitness changes in a group of older adults engaged in fitness training were associated with changes in diffusion-tensor based indices of WMM in prefrontal WM, and that changes in indices of WMM were related to changes in fluid cognition. This finding resonates well with the literature on exercise and cognition. Repeatedly, studies have shown that beneficial effects of exercise are most pronounced in tasks requiring higher levels of cognitive control (Bherer et al., 2013, Stillman et al., 2016, Voss et al., 2013b). Furthermore, our results are consistent with initial evidence suggesting that exercise-induced changes in WMM can be primarily attributed to prefrontal regions (Colcombe et al., 2006, Voss et al., 2013a). The forceps minor is the main fiber bundle connecting the left and right lateral and medial prefrontal cortices, crossing the midline via the genu of the corpus callosum. Given that the effect in our data was even more pronounced when excluding the genu from the forceps minor, fibers reaching into distal prefrontal areas seem largely responsible for the effect. Finally, the covariance structure of changes observed in this study is consistent with the proposition that the beneficial effects of exercise on cognition are mediated, in part, by changes in WMM (see Figure 1). Clearly, given the correlational nature of the present study, the absence of a control group, and the large number of WM tracts considered, additional intervention studies need to be carried out to test the replicability of this finding.

Moreover, we observed tract-specific decreases in AD and RD over the training. We also observed changes in MD, but the latter reflect the average of AD and RD changes, and hence do not constitute additional independent evidence. It is tempting to interpret these mean changes in the sense that participating in the fitness intervention counteracted the increases in AD, RD, and MD typically observed with normal aging. The observed increases in AD and RD for the majority of tracts from posttest to maintenance appear to further support that idea. However, this interpretation is challenged by two recent longitudinal reports. Bender and

colleagues (2015, 2016b) observed decreases in AD and RD over two and seven years in healthy aging samples, respectively, in regions that partly overlap with the regions in which decreases were observed in the present study. The FA changes observed in the present study (significant decreases in FMin, CCg, and CCb over the training phase, but increases in PLIC) would also be consistent with age-related declines (cf. Bender and Raz, 2015). Yet, in the absence of a control group that did not receive physical exercise, it is impossible to tell whether the intervention successfully counteracted age-related alterations in WMM. Instead, the findings may mean that the intervention did not fully overcome aging-related declines, but that participants showing greater gains in fitness had less negative changes in WMM, similar to what has been observed for other measures from the same trial (Kleemeyer et al., 2016, Kleemeyer et al., 2017).

Several tracts (e.g., CingD, SLF) but not CCg showed significant reductions in FA during the maintenance phase. In trying to interpret these findings, it needs to be kept in mind that the maintenance phase of the present study was not well controlled in relation to the amount of exercise that participants carried on with. The fact that the mean fitness level did not significantly decline during the maintenance phase of the study suggests that at least some participants continued exercising on their own (in line with self-reports). Animal studies have observed various changes in WM with regard to aging, ranging from disruption of myelin sheaths to lower axonal fiber packing density, but how these changes translate to DTI indices is less clear (Zatorre et al., 2012). In particular, reductions in FA may not necessarily indicate decreasing axon number or myelination but may be reflective of other age-related structural changes, including increasing strength of crossing fiber populations or axonal diameter (cf. Bender et al., 2016a, Johansen-Berg, 2012).

We are aware of the criticisms that have recently been raised against TBSS, mainly concerning the mis-assignment of voxels during the skeletonization process (Bach et al., 2014). This mis-

assignment seems to be induced by suboptimal registration procedures when relying only on FA values. In contrast, registration improves when the full tensor information is taken into account. DTI-TK is software that enables image registration based on full tensor information (Zhang et al., 2006). It also provides an approach for the longitudinal processing of DTI data (Keihaninejad et al., 2013). However, to the best of our knowledge, the longitudinal DTI-TK approach has never been used in healthy aging research and is thus not well validated. Moreover, we did not intend to introduce another method, contributing to the challenges in comparison of findings between studies. We therefore opted for a multiverse testing approach (Steegen et al., 2016), and reanalyzed our data with DTI-TK and different combinations of within and between subject registrations. The general pattern of results tended to be similar when analyzed using the DTI-TK approach, though some of the observed effects did not reach statistical significance. Given that these deviations did not depend on within-subject registration, they may reflect differences in between-subject registration. Whereas DTI-TK creates a group template and registers all subjects data to that group template based on full tensor information, TBSS simultaneously aligns the group template to MNI space and applies the combined transformation (based on FA values) to the subject data. Consequently, TBSS results in skeletonized data in MNI space that the predefined TOI masks can directly be applied to. In contrast, DTI-TK results in skeletonized data in the group template space, requiring another transformation of the masks. This implies that we end up sampling from different numbers of voxels and potentially even from slightly different voxels, depending on how well the transformations have been performed. The considerably lower number of voxels in the skeleton for the two exclusive DTI-TK pipelines may lead to a lower signal-to-noise ratio, which increases the standard errors and hence results in smaller effects. It is also worth noting that TBSS voxels, even if subject to mis-assignment, originate from neighboring tracts. In our case, these would still be voxels from tracts reaching into the frontal cortices, and hence remain relevant to the research questions of the present study.

5 Conclusion

In the context of six months of aerobic fitness training in older adults, we found that changes in fitness are related to changes in the WMM of forceps minor, and changes in forceps minor WMM are related to changes in fluid cognition (see Figure 1). Greater gains in fitness were associated with smaller reductions in FA, and smaller reductions in FA were associated with greater improvements in fluid cognition. In contrast, greater gains in fitness were associated with less positive changes in AD, RD, and MD, which again were related to greater improvements in fluid cognition. Although the absence of a control group limits the basis for causal inference, the present results support the hypothesis that WMM contributes significantly to the link between exercise and cognition in old age.

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7 Conflict of Interest

The authors declare that the research was conducted in the absence of any personal, financial or other relationships with other people or organizations that could be construed as a potential conflict of interest. None of the authors or their institutions has agreements that could be seen as involving a financial interest in this work.

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10 Tables

Characteristic	Group		
	High Intensity (N = 24)	Low Intensity (N = 25)	
	M (SD)	M (SD)	p-value
Age	66.13 (4.17)	66.34 (4.62)	$t_{(47)}$ = .167, p=0.868
Education (years)	10.83 (1.66)	11.00 (1.73)	$t_{(47)}$ = .344, p=0.733
MMSE	29.33 (1.27)	28.86 (1.42)	$t_{(46)}$ = -1.175, p=0.246
BMI	26.21 (3.95)	25.26 (3.70)	$t_{(47)}$ = -.874, p=0.386
Digit Symbol	38.09 (9.09)	39.36 (6.01)	$t_{(46)}$ = 0.577, p=0.567
Metabolic rate (self-reported)	16.95 (10.72)	20.43 (11.52)	$t_{(47)}$ = 1.092, p=0.280
Training adherence (max 75)	70.54 (3.80)	69.08 (7.91)	$t_{(47)}$ = -0.819, p=0.417
	N (%)	N (%)	
Male	9 (37.5 %)	10 (40 %)	
Hormone Replacement Therapy	5 (33.3 %)	4 (26.7 %)	
Smoking	1 (4.2 %)	1 (4 %)	
Smoking earlier	9 (37.5 %)	13 (52 %)	
Hypertension medication	3 (12.5 %)	2 (8 %)	

Table 1: Sample characteristics as a function of training group. Values are means (M) and standard deviations (SD), or number of participants (N) and percentage within group (%), respectively. Abbreviations: MMSE: Mini-Mental State examination, BMI: Body mass index.

Table 2: Different analysis pipelines and their combinations.

Pipeline	Within-subject registration	Between-subject registration	BS registration target	Sampling space
TBSS	-	FA based	Fmrib58	MNI
TBSS JHU	-	FA based	JHU	MNI
TBSS-DTI-TK	-	FA based	Group-wise template	MNI
DTI-TK-TBSS1	Tensor-based	FA based	JHU	MNI
DTI-TK-TBSS2	Tensor-based	FA based	Group-wise template	MNI
DTI-TK-cross	-	Tensor-based	Group-wise template	Group space
DTI-TK-longi	Tensor-based	Tensor-based	Group-wise template	Group space

Table 3: Univariate model results depicting standardized latent mean changes over training as well as standardized regression coefficients of age and blood pressure medication on pretest data.

TOI	AD			RD			MD			FA		
	ΔM	age	BP	ΔM	age	BP	ΔM	age	BP	ΔM	age	BP
CingD	-0.214	-0.833	-0.057				**-1.083**	**4.659**	0.153			
FMaj	-0.226[a]	-0.324		0.199[a]	3.584	0.716	0.123[a]	4.237	0.587	-0.105	-3.345	-0.653
FMin	0.03	4.661	0.591	0.251	**6.168**	**1.144**	0.185	**6.249**	**1.062**	**-0.494**	**-5.097**	**-1.045**
FMin≠CCg	-0.026[a]	2.868	0.675	0.292	**4.992**	**1.2**	0.208	**4.799**	**1.147**	**-0.524**	-4.002	**-1.039**
IFOF	**-0.662**	**5.355**	0.058	-0.062	4.898	**1.039**	**-0.305**[a]	**6.105**	**0.861**	-0.15[a]	-2.599	**-1.116**
SLF				**-0.516**	4.999	**1.03**				0.018	-1.912	**-0.98**
CCg	-0.06[a]	**6.818**	0.192	0.211[a]	**8.974**	0.666	0.059	**8.595**	0.577	**-0.333**	**-8.381**	**-0.812**
CCb	**-0.952**	**6.364**	-0.492				**-0.641**	**9.171**	0.174	**-0.348**	**-6.512**	-0.765
CCs	**-0.71**	1.785	0.091	0.139	3.569	0.736	-0.152	3.54	0.622			
PLIC				**-1.12**	-0.537	0.105				**0.903**	2.542	-0.127
Skeleton	**-1.067**[a]	**5.601**	0.512	-0.249	**7.02**	**0.972**	**-0.683**	**7.238**	**0.916**	**-0.356**	**-4.935**	**-0.879**

People receiving hypertensive treatment are coded 0.5 as opposed to -0.5 for untreated participants. Positive estimates indicate higher values for participants treated for hypertension. Numbers printed in bold font indicate significant effect of p < 0.05. Underlined numbers indicate non-significant trends of p < 0.06. Note that the effects of age and hypertensive treatment are on DTI baseline measures.

Key: ΔM, latent mean change; AD, axial diffusivity; RD, radial diffusivity; MD mean diffusivity; FA, fractional anisotropy; CingD, dorsal cingulum bundle; FMaj, forceps major; FMin, forceps minor; FMin≠CCg, forceps minor excluding genu of corpus callosum; IFOF, inferior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; CCg, genu of corpus callosum; CCb, body of corpus callosum; CCs, splenium of corpus callosum; PLIC, posterior limb of the internal capsule; BP, blood pressure medication; DTI, diffusion tensor imaging.

[a] denotes tracts for which only strong measurement invariance constraints were imposed to improve model fit

Table 4: Univariate model results depicting standardized latent mean changes between posttest and maintenance testing.

TOI	ΔAD	ΔRD	ΔMD	ΔFA
CingD	-0.018	**0.822**	**0.707**	**-0.588**[a]
FMaj	**0.513**	**0.547**	**0.624**	-0.333
FMin	**0.601**	**0.436**		-0.209
FMin≠CCg				-0.098
IFOF	**0.733**			
SLF		**0.987**[a]	**1.1**	**-0.681**[a]
CCg	**0.823**	**0.69**	**0.911**	**-0.46**
CCb	**0.796**	**0.388**	**0.839**	-0.141
CCs	**1.353**		**1.144**	
PLIC	0.358			
Skeleton	**1.213**[b]	**0.999**[b]	**1.23**[b]	**-0.481**[b]

Numbers printed in bold font indicate significant effect of p < 0.05. Underlined numbers indicate non-significant trends of p < 0.06.

Key: Δ, latent mean change; AD, axial diffusivity; RD, radial diffusivity; MD mean diffusivity; FA, fractional anisotropy; CingD, dorsal cingulum bundle; FMaj, forceps major; FMin, forceps minor; FMin≠CCg, forceps minor excluding genu of corpus callosum; IFOF, inferior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; CCg, genu of corpus callosum; CCb, body of corpus callosum; CCs, splenium of corpus callosum; PLIC, posterior limb of the internal capsule; BP, blood pressure medication; DTI, diffusion tensor imaging.

[a] denotes models imposing only strong measurement invariance constraints to improve model fit

[b] denotes models where gender was excluded as a covariate to improve model fit

Table 5: Chi-square values, degrees of freedom, multivariate model fit indices, and standardized covariance estimates of latent changes in DTI measures with fitness and fluid cognition for FMin and FMin≠CCg

	FMin							FMin≠CCg						
	χ2	df	CFI	RMSEA	90 % CI	Δfitness	ΔCf	χ2	df	CFI	RMSEA	90 % CI	Δfitness	ΔCf
ΔAD	81.99	65	0.966	0.073	0.000 - 0.118	-0.235	**-0.327**	61.00	62	1	0	0.000 - 0.084	**-0.313**	**-0.363**
ΔRD	69.25	65	0.993	0.037	0.000 - 0.095	**-0.287**	**-0.307**	69.04	65	0.992	0.036	0.000 - 0.094	**-0.334**	**-0.318**
ΔMD	82.10	65	0.970	0.073	0.000 - 0.118	**-0.287**	**-0.325**	72.81	65	0.984	0.050	0.000 - 0.102	**-0.347**	**-0.325**
ΔFA	70.57	65	0.990	0.042	0.000 - 0.097	**0.290**	**0.270**	75.18	65	0.981	0.057	0.000 - 0.106	**0.318**	**0.265**

The first column contains chi-square values, the second column the degrees of freedom. Columns three to five provide fit indices. Column six and seven depict the standardized model estimate for the covariance between changes in DTI index and changes in fitness or fluid cognition, respectively. Numbers printed in bold font indicate significant effect of p < 0.05.

Key: Δ, latent mean change; AD, axial diffusivity; RD, radial diffusivity; MD mean diffusivity; FA, fractional anisotropy; FMin, forceps minor; FMin≠CCg, forceps minor excluding genu of corpus callosum; Cf, fluid cognition; DTI, diffusion tensor imaging.

Table 6: Chi-square values, degrees of freedom, multivariate model fit indices, standardized latent mean changes over training, and standardized covariance estimates of latent changes in DTI measures with fitness and fluid cognition for FMin and FMin≠CCg for the different analysis pipelines.

		FMin								FMin≠CCg							
AD		χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf	χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf
	TBSS	88.86	65	0.950	0.087	0.031 - 0.129	0.064	-0.224	**-0.348**	72.23	65	0.983	0.048	0.000 - 0.101	0.079	**-0.317**	**-0.352**
	TBSS-JHU	75.84	65	0.976	0.058	0.000 - 0.107	0.101	-0.234	**-0.389**	62.46	65	1	0	0.000 - 0.079	0.048	**-0.327**	**-0.353**
	TBSS-DTItk	81.99	65	0.966	0.073	0.000 - 0.118	0.030	-0.235	**-0.327**	61.00	62	1	0	0.000 - 0.084	-0.026	**-0.313**	**-0.363**
	DTItk-TBSS1	75.48	64	0.976	0.060	0.000 - 0.109	0.220	-0.242	**-0.318**	73.77	65	0.980	0.052	0.000 - 0.103	0.229	**-0.300**	**-0.307**
	DTItk-TBSS2	73.61	65	0.983	0.052	0.000 - 0.103	0.026	-0.234	**-0.346**	64.51	64	0.999	0.013	0.000 - 0.087	0.077	**-0.302**	**-0.327**
	DTItk-cross	75.52	64	0.978	0.061	0.000 - 0.109	0.302	-0.140	-0.204	69.26	64	0.989	0.041	0.000 - 0.097	0.165	-0.244	-0.241
	DTItk_longi	78.19	65	0.973	0.067	0.000 - 0.114	0.219	-0.036	-0.203	75.54	64	0.975	0.061	0.000 - 0.109	0.174	-0.158	-0.202
RD		χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf	χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf
	TBSS	85.67	65	0.966	0.081	0.014 - 0.124	0.194	**-0.287**	**-0.280**								
	TBSS-JHU	82.81	64	0.968	0.077	0.000 - 0.122	0.212	**-0.307**	**-0.285**	75.05	64	0.979	0.059	0.000 - 0.108	0.220	**-0.349**	**-0.307**
	TBSS-DTItk	69.25	65	0.993	0.037	0.000 - 0.095	0.251	**-0.287**	**-0.307**	69.04	65	0.992	0.036	0.000 - 0.094	0.292	**-0.334**	**-0.318**
	DTItk-TBSS1	86.90	65	0.963	0.083	0.022 - 0.126	0.276	**-0.277**	-0.234	92.98	64	0.947	0.096	0.048 - 0.137	0.294	**-0.332**	-0.236
	DTItk-TBSS2	80.86	1.244	0.974	0.071	0.000 - 0.116	0.213	-0.238	-0.246	86.87	65	0.960	0.083	0.022 - 0.126	0.289	**-0.294**	-0.232
	DTItk-cross	86.24	64	0.962	0.084	0.025 - 0.127	0.221	-0.247	**-0.247**	84.55	64	0.961	0.081	0.015 - 0.124	**0.418**	-0.248	-0.197
	DTItk_longi	86.16	64	0.962	0.084	0.025 - 0.127	0.243	-0.248	-0.243	86.84	64	0.957	0.085	0.028 - 0.128	0.298	**-0.285**	**-0.244**
MD		χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf	χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf
	TBSS	91.92	65	0.953	0.092	0.042 - 0.133	0.160	**-0.282**	**-0.305**								
	TBSS-JHU	86.718	64	0.960	0.085	0.027 - 0.128	0.024	**-0.286**	**-0.319**	73.89	64	0.980	0.056	0.000 - 0.106	0.033	**-0.342**	**-0.305**
	TBSS-DTItk	82.10	65	0.970	0.073	0.000 - 0.118	0.185	**-0.287**	**-0.325**	72.81	65	0.984	0.050	0.000 - 0.102	0.208	**-0.347**	**-0.325**
	DTItk-TBSS1	88.75	65	0.958	0.086	0.031 - 0.128	0.237	**-0.274**	**-0.276**								
	DTItk-TBSS2	86.86	65	0.963	0.083	0.022 - 0.126	0.161	-0.248	**-0.285**	85.38	65	0.961	0.080	0.012 - 0.123	0.234	**-0.303**	**-0.259**
	DTItk-cross	62.65	65	1	0	0.000 - 0.079	-0.273	0.227	0.087	57.34	65	1	0	0.000 - 0.064	-0.055	0.400	0.200
	DTItk_longi	82.82	64	0.967	0.077	0.000 - 0.122	0.203	-0.220	**-0.282**	82.84	64	0.964	0.078	0.000 - 0.122	**0.368**	-0.223	-0.215
FA		χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf	χ2	df	CFI	RMSEA	90 % CI	ΔM	Δfitness	ΔCf
	TBSS	87.67	65	0.960	0.084	0.026 - 0.127	**-0.397**	**0.315**	0.224	90.31	65	0.953	0.089	0.036 - 0.131	**-0.459**	**0.343**	0.216
	TBSS-JHU	80.17	64	0.971	0.072	0.000 - 0.117	**-0.423**	**0.322**	0.246	78.84	64	0.971	0.069	0.000 - 0.115	**-0.442**	**0.340**	**0.252**
	TBSS-DTItk	70.57	65	0.990	0.042	0.000 - 0.097	**-0.494**	**0.290**	**0.270**	75.18	65	0.981	0.057	0.000 - 0.106	**-0.524**	**0.318**	**0.265**
	DTItk-TBSS1	82.81	65	0.969	0.075	0.000 - 0.119	**-0.482**	0.250	0.190	85.99	65	0.960	0.081	0.017 - 0.124	**-0.495**	**0.287**	0.189
	DTItk-TBSS2	79.51	65	0.975	0.068	0.000 - 0.114	**-0.433**	0.221	0.208	85.66	65	0.961	0.081	0.014 - 0.124	**-0.490**	0.267	0.192
	DTItk-cross																
	DTItk_longi									87.38	64	0.953	0.086	0.030 - 0.129	**-0.561**	0.253	0.175

The first column contains chi-square values, the second column the degrees of freedom. Columns three to five provide fit indices. Column six indicates standardized latent mean changes and columns seven

and eight depict the standardized model estimate for the covariance between changes in DTI index and changes in fitness or fluid cognition, respectively. Numbers printed in bold font indicate significant effect of p < 0.05. Underlined number indicate non-significant trends of p < 0.06.
Key: Δ, latent mean change; AD, axial diffusivity; RD, radial diffusivity; MD mean diffusivity; FA, fractional anisotropy; FMin, forceps minor; FMin≠CCg, forceps minor excluding genu of corpus callosum; Cf, fluid cognition; DTI, diffusion tensor imaging.

Table S1: Chi-square values, degrees of freedom, multivariate model fit indices, and standardized covariance estimates of latent changes in DTI measures with fitness and fluid cognition for FMin and FMin≠CCg over maintenance.

	χ2	df	CFI	RMSEA	90 % CI	Δfitness	ΔCf	χ2	df	CFI	RMSEA	90 % CI	Δfitness	ΔCf
ΔAD	78.06	64	0.974	0.067	0.000 - 0.114	0.111	-0.071							
ΔRD	96.90	64	0.950	0.102	0.057 - 0.142	0.103	-0.122							
ΔFA	97.00	64	0.948	0.103	0.058 0.142	-0.081	0.141	94.54	64	0.948	0.099	0.052 - 0.139	0.005	0.135

The first column contains chi-square values, the second column the degrees of freedom. Columns three to five provide fit indices. Column six and seven depict the standardized model estimate for the covariance between changes in DTI index and changes in fitness or fluid cognition, respectively. Numbers printed in bold font indicate significant effect of p < 0.05.
Key: Δ, latent mean change; AD, axial diffusivity; RD, radial diffusivity; MD mean diffusivity; FA, fractional anisotropy; FMin, forceps minor; FMin≠CCg, forceps minor excluding genu of corpus callosum; Cf, fluid cognition; DTI, diffusion tensor imaging.

11 Figures

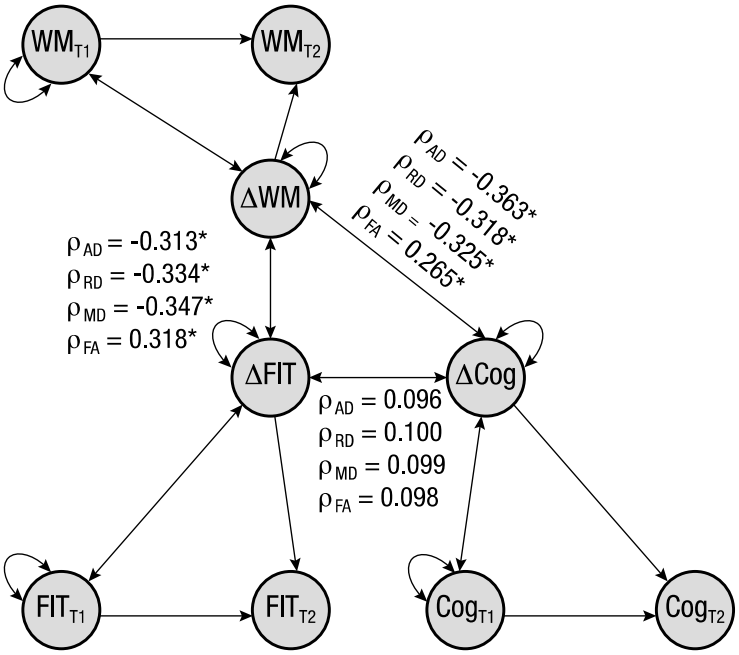

Figure 1: Multivariate latent change score model. Changes in fitness were reliably associated with changes in all four DTI indices, and changes in all DTI indices were reliably associated with changes in fluid cognition. Only latent constructs are shown. Abbreviations: FIT: latent factor fitness, composed of VO_2max and VO_2AT; WM: latent factor white matter, composed of mean values for left and right hemisphere; Cog: composite score of fluid cognition, T1: before training, T2: after training, Δ: latent change. * indicates $p < 0.05$.

Paper III

Kleemeyer, M. M., Polk, T. A., Schaefer, S., Bodammer, N. C., Brechtel, L., & Lindenberger, U. (2017). Exercise-induced fitness changes correlate with changes in neural specificity in older adults. *Frontiers in Human Neuroscience, 11:* 123. doi:10.3389/fnhum.2017.00123

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Exercise-Induced Fitness Changes Correlate with Changes in Neural Specificity in Older Adults

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Neural specificity refers to the degree to which neural representations of different stimuli can be distinguished. Evidence suggests that neural specificity, operationally defined as stimulus-related differences in functional magnetic resonance imaging (fMRI) activation patterns, declines with advancing adult age, and that individual differences in neural specificity are associated with individual differences in fluid intelligence. A growing body of literature also suggests that regular physical activity may help preserve cognitive abilities in old age. Based on this literature, we hypothesized that exercise-induced improvements in fitness would be associated with greater neural specificity among older adults. A total of 52 adults aged 59–74 years were randomly assigned to one of two aerobic-fitness training regimens, which differed in intensity. Participants in both groups trained three times a week on stationary bicycles. In the low-intensity (LI) group, the resistance was kept constant at a low level (10 Watts). In the high-intensity (HI) group, the resistance depended on participants' heart rate and therefore typically increased with increasing fitness. Before and after the 6-month training phase, participants took part in a functional MRI experiment in which they viewed pictures of faces and buildings. We used multivariate pattern analysis (MVPA) to estimate the distinctiveness of neural activation patterns in ventral visual cortex (VVC) evoked by face or building stimuli. Fitness was also assessed before and after training. In line with our hypothesis, training-induced changes in fitness were positively associated with changes in neural specificity. We conclude that physical activity may protect against age-related declines in neural specificity.

Keywords: aging, fitness, physical exercise, neural specificity, multivariate pattern analysis

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INTRODUCTION

Pictures of faces, houses, and many other stimulus categories elicit distinguishable patterns of neural response in ventral visual cortex (VVC). For example, using functional magnetic resonance imaging (fMRI), Haxby et al. (2001) found distinct neural activation patterns in response to eight stimulus categories within ventral temporal cortex. After being trained on a subset of activation patterns, machine learning classifiers can often decode the stimulus category associated with novel patterns (Haynes and Rees, 2006; Norman et al., 2006). And the more distinctive, or

specific, the patterns are, the more accurately a classifier will be able to predict the stimulus category from neural activity, so classifier accuracy is a natural way to estimate neural specificity.

Evidence suggests that neural specificity declines with increasing age (Grady et al., 1994; Park et al., 2004) and reduced neural specificity is associated with lower cognitive performance in a variety of cognitive tasks in older adults (Park et al., 2010). But why does neural specificity decline with age? Age-related declines in neurotransmitter function and neuromodulation have been suggested as underlying mechanisms. For example, the number of dopamine (DA) neurons (Bäckman et al., 2006), as well as DA receptor levels (Inoue et al., 2001) show pronounced and widespread decrements with advancing adult age. Neurocomputational models predict that attenuated neuromodulation lowers a cell's responsivity and leads to less differentiated neural responses to different stimuli (i.e., less distinct neural representations), which in turn would explain age-related deficits across a wide range of cognitive domains (Li and Sikström, 2002).

On the other hand, regular physical activity has repeatedly been shown to preserve cognitive abilities in old age (for reviews see Bherer et al., 2013; Voss et al., 2013). Evidence from animal research suggests that exercise induces an upregulation of DA (Sutoo and Akiyama, 2003; Poulton and Muir, 2005; Foley and Fleshner, 2008), possibly by stimulating DA synthesis through a calcium/calmodulin-dependent system. Consistent with this hypothesis, Ruscheweyh et al. (2011) found increased plasma concentrations of DA after 6 months of physical training in older humans. Moreover, Stroth et al. (2010) showed that young adults with a genotype associated with lower DA levels (val/val COMT gene homozygotes) exhibited greater cognitive improvements after a 4-month exercise intervention compared with other genotypes (met carriers). Since the relationship between DA and cognitive performance seems to follow an inverted U-shape, Stroth et al. (2010) hypothesized that exercise may optimize central DA availability. This especially benefits those individuals with suboptimal initial levels, as it moves them further up the curve. Similarly, a cross-sectional study with older adults showed that val/val homozygotes benefitted most from better fitness in terms of Flanker task performance. The latter result suggests that high fitness levels may compensate for being a COMT val/val homozygote (Voelcker-Rehage et al., 2015).

Taken together, the two lines of research suggest that neural specificity may provide a potential mechanism for beneficial effects of exercise in preserving cognitive functions in older adulthood. If so, improvements in exercise-induced fitness should be associated with more positive changes in neural specificity.

We are aware of four previous intervention studies that looked at exercise-related changes in BOLD activation (Colcombe et al., 2004; Voss et al., 2010; Voelcker-Rehage et al., 2011; Maffei et al., 2017). All four of them focused on changes in *neural efficiency*, that is, more efficient usage of brain networks, which is typically reflected in a reduced BOLD signal, and in greater functional connectivity with increasing cognitive load. The results of these studies suggest that an aerobic fitness training as opposed to an anaerobic fitness training or a passive control improves neural efficiency during task performance, as reflected by maintained (Maffei et al., 2017) or even reduced (Colcombe et al., 2004; Voelcker-Rehage et al., 2011) BOLD activation in task-relevant areas, as well as by strengthened functional connections within the default mode and frontal executive networks (Voss et al., 2010). In contrast to these former studies, which focused on exercise-induced changes in neural efficiency, the present study investigates exercise-induced changes in *neural specificity*, that is, the distinctiveness of specific stimulus-evoked neural activation patterns, irrespective of BOLD signal strength. Note that it is possible to observe changes in neural efficiency without changes in neural specificity, such as when two different stimuli elicit smaller, more efficient activation after the intervention that are not accompanied by reductions in activation overlap. Likewise, one may observe changes in neural specificity without changes in neural efficiency, such as when two stimuli elicit more distinct activation patterns after the intervention that are not accompanied by reductions in BOLD signal strength.

To test whether exercise-induced fitness improvements translate to neural specificity we used fMRI and multivariate pattern analysis (MVPA) within an exercise-dose-response paradigm. Elderly participants were randomly assigned to training regimens with differing levels of intensity. Before and after the 6-month training phase, participants performed a graded maximal exercise test to assess their training-related fitness improvements as well as a passive viewing task while functional brain images were acquired. As in earlier work (see Carp et al., 2011), the distinctiveness of neural activation patterns in response to different stimulus categories served as an index of neural specificity. To examine the hypothesized association between fitness and neural specificity, we correlated changes in fitness with changes in neural specificity.

MATERIALS AND METHODS

Participants

The total sample consisted of 52 community-dwelling older adults aged 59–74 (mean 65.95 $\pm$ 4.36, 20 males). All participants met the following inclusion criteria: (1) age range in years between 59 and 75; (2) physical inactivity prior to study enrollment (MET $<$ 40 based on the German version of the compendium of physical activities); (3) MMSE score $\geq$ 26; (4) free of neurological, psychiatric, and cardiovascular diseases; (5) right-handed; (6) no contraindication for heart-rate controlled exercise training (e.g., no medication with beta blockers); (7) suitability for MR assessment (e.g., no magnetic implants, no claustrophobia). This study was carried out in accordance with the recommendations of the ethics committee of the German Psychological Society (DGP). All participants gave written informed consent in accordance with the Declaration of Helsinki and participated voluntarily. They were paid for study completion; training adherence was reinforced through a bonus system. Details on the recruitment can be found in

previously published work based on the same study (Kleemeyer et al., 2016). Five participants were excluded from the MVPA analyses, four due to incomplete fMRI data, and one due to improper slice positioning at pretest. One participant was excluded from the fitness analyses due to problems in VO_2max detection. Thus, correlation analyses were based on data from 46 participants.

Design

Participants completed a 6-month fitness intervention with a comprehensive test battery before (pre) and after (post) the training. Please note that we confine ourselves to describing only those methods relevant for the scope of this article, that is, training, fitness assessment, and imaging procedures. Additional information can be found in Kleemeyer et al. (2016).

Training

Participants were randomly assigned to a high-intensity (HI) or low-intensity (LI) training regimen subsequent to the pretest assessment. Groups were counterbalanced for age, sex, years of education, digit-symbol performance, and MMSE scores.

Participants in each of the two groups exercised in our lab on stationary bikes, three times a week for 55 min in each session, with a gradual increase during the first 3 weeks. Over the 6-month period, a total of 75 training sessions could be achieved. For the HI group, training intensity was calibrated to result in a heart rate at 80% of the individual's ventilatory anaerobic threshold (Wasserman et al., 1990). In contrast, the LI group exercised at a constant resistance of 10 W. For the last 21 sessions, five intervals of 2 min each were integrated after 20 min of training in order to further increase variance in fitness gains. During these 2-min time windows, the LI group only increased the cadence from 60–70 to 80–90 cycles/min, while the HI group also increased the intensity to a resistance corresponding to 110% of the individual's ventilatory anaerobic threshold. Training intensity was automatically controlled using the software custo cardio concept (custo med GmbH, Ottobrunn, Germany), with a staff member supervising compliance for each participant and each training session. Up to six participants exercised simultaneously, irrespective of intensity levels (e.g., HI and LI participants exercised together). The existence of different training regimens was conveyed only after termination of the study.

Cardiovascular Fitness Assessment

Participants performed a graded maximal exercise test on a cycle ergometer to assess their cardiovascular fitness. The test started at 10 W, increased to 25 W after 2 min followed by 25 W increments every 2 min until total exhaustion or signs of cardiac or respiratory distress. A sports physician continuously monitored the cardiogram, oxygen uptake, heart rate, and blood pressure. We computed an aggregate measure of the maximum oxygen consumption at exhaustion (VO_2max) and the oxygen consumption at the ventilatory anaerobic threshold (VO_2AT) to obtain a more robust fitness measure. Therefore, data were *z*-transformed in a way that preserves mean differences between time points, namely by subtracting the common mean from pretest and posttest data. The aggregate fitness measure VO_2 served as the outcome of the fitness assessment.

MRI Data Acquisition and Preprocessing

During functional imaging, participants passively viewed face, house, or phase-scrambled images, following the procedures of Park et al. (2010). They completed two runs, each of which consisted of four blocks per stimulus category. During every block 15 images were shown for 2 s each, resulting in 30 s per block and 6 min per run. Stimuli were presented via *E*-prime (Psychology Software Tools, Pittsburgh, PA, USA) and displayed by a projection system.

Brain images were acquired on a Siemens TIM Trio 3T MRI scanner (Siemens, Erlangen, Germany). A conventional echo-planar MR sequence was used for functional acquisitions (TR = 2000 ms, TE = 30 ms, flip angle = 80°, FOV = 216 mm) encompassing 192 volumes per run and 36 slices per volume (slice thickness 3 mm). Slices were 72 × 72 matrices acquired parallel to the Corpus Callosum. A high-resolution T1-weighted MPRAGE (TR = 2500 ms, TE = 4.76 ms, TI = 1100 ms, flip angle = 7°, acquisition matrix = 256 × 256 × 176, 1 mm isotropic voxels) was also acquired to facilitate warping masks from MNI to individual subject space. Data were preprocessed using SPM12 (Wellcome Department of Cognitive Neurology, London, UK[1]). Functional images were realigned to the mean volume. The T1-weighted image was normalized to MNI space. The inverse normalization parameters were then applied to an AAL atlas based mask of VVC (including bilateral occipital cortices, inferior temporal cortices, and fusiform gyri). As a last step, the T1-weighted image was co-registered to the mean functional image, and the same parameters were applied to the mask such that all images mapped into the subject's native space. No normalization, spatial smoothing or other transformation was applied to the functional images.

To obtain activation maps, we setup a General Linear Model (GLM) to estimate the response to each category relative to phase-scrambled control images. We defined a separate regressor for each experimental block, resulting in eight estimates of face-evoked activation and eight estimates of house-evoked activation. We also included six nuisance covariates per run in the GLM, modeling head translation and rotation.

Multivariate Pattern Analysis

Since we were interested in the responses to face and house stimuli, we restricted the analysis to voxels within VVC. We applied MVPA using correlation analysis (see Haxby et al., 2001) on individual subject data separately for pretest and posttest. More precisely, we used the 16 coefficient estimates for faces and houses vs. phase-scrambled images from the GLM and extracted the activation pattern for voxels within

[1]http://www.fil.ion.ucl.ac.uk/spm/

VVC. Next, we computed the Pearson correlation within categories (i.e., correlating the face-evoked activation patterns from odd blocks pairwise with the face-evoked activation patterns from even blocks and the same for house-evoked activation patterns) and the Pearson correlation between categories (i.e., correlating the face-evoked activation patterns from odd blocks pairwise with the house-evoked activation patterns from even blocks and vice versa). To ensure a more normal distribution, correlation coefficients were transformed into Fisher's *z*-values. Neural specificity was then defined as the difference between the mean within-category and between-category correlations.

Statistical Analyses

Statistical analyses were performed using SPSS (IBMCorp., IBM SPSS Statistics, V22, Armonk, NY, USA). To assess effects on fitness and neural specificity, we used repeated-measures analysis of variance (ANOVA) with time point as a within-subject factor and training group as a between-subject factor. To examine whether changes in fitness were associated with changes in neural specificity, we performed a two-tailed Pearson correlation analysis across participants from both groups using the absolute difference (post-training minus pre-training) in fitness and the absolute difference (post-training minus pre-training) in neural specificity. The alpha level for all analyses was set to $p = 0.05$.

RESULTS

There were no baseline differences between participants in the two training groups with respect to age, years of education, MMSE, BMI, fitness, hormone replacement therapy, and treated hypertension (see **Table 1** for means and standard deviations (SD) or proportion of participants, respectively). Also, training adherence did not differ reliably between the two groups (mean HI = 71.14, mean LI = 69.12, $t_{(44)} = -1.088$, $p = 0.282$).

The intervention was associated with increasing fitness levels ($F_{(1,49)} = 5.637$; $p = 0.022$; $\eta_p^2 = 0.103$). However, HI and LI groups did not differ in mean fitness changes ($F_{(1,49)} = 0.997$; $p = 0.323$; $\eta_p^2 = 0.020$). Consequently, data were collapsed across treatment conditions when investigating exercise effects on neural specificity.

As predicted, greater changes in fitness were associated with greater changes in neural specificity ($r_{(46)} = 0.310$, $p = 0.036$, $R^2 = 0.096$), irrespective of the training regimen's intensity (see **Figure 1**). Similar to changes in fitness, neural specificity increased from pretest to posttest in some participants, and decreased in others, especially among those whose fitness did not improve. Overall there was no significant change in neural specificity with training ($F_{(1,45)} = 1.891$; $p = 0.176$; $\eta_p^2 = 0.040$) and no reliable interaction of neural specificity changes with group ($F_{(1,45)} = 0.006$; $p = 0.936$; $\eta_p^2 = 0.000$). **Figure 2** displays mean changes in fitness and neural specificity.

To differentiate training-induced changes in neural specificity from changes in neural efficiency, we also tested whether training affected mean BOLD activation in stimulus-relevant regions, that is, the fusiform face area (FFA) for faces and parahippocampal place area (PPA) for buildings. We extracted the % BOLD signal change from four spherical ROIs (FFA left and right, PPA left and right, with 5 mm radius around center coordinates adapted from Ishai et al., 1999) for the appropriate condition (in FFA for faces, and in PPA for houses) using MarsBaR. We found a negative but non-significant relationship between change in fitness and change in BOLD activation in both the FFA ($r_{(46)} = -0.201$, $p = 0.18$, $R^2 = 0.040$) and the PPA ($r_{(46)} = -0.181$, $p = 0.23$, $R^2 = 0.033$). The average effect across both regions was also negative, but not significant ($r_{(46)} = -0.234$, $p = 0.12$, $R^2 = 0.055$). For neither region alone, nor for the average, did we observe significant training-induced changes in % BOLD signal change (all $p > 0.3$), nor were there significant interactions with training group (all $p > 0.2$). All means and SD are provided in **Table 2**.

DISCUSSION

In this study we investigated whether exercise-induced fitness improvements would be associated with enhanced neural specificity. We found a positive correlation between changes in fitness induced by a 6-month exercise intervention and changes in neural specificity, in the sense that participants whose physical fitness improved more also showed more positive changes in

TABLE 1 | Sample characteristics as a function of training group.

Characteristic	High intensity (*N* = 21)		Low intensity (*N* = 25)		*p*
	M	*SD*	*M*	*SD*	
Age (years)	66.56	4.33	65.93	4.50	0.631
Education (years)	11.05	1.63	11.36	1.63	0.520
MMSE	29.48	1.08	28.88	1.48	0.132
BMI	26.12	4.21	25.18	4.05	0.444
VO_2max (ml/min × kg)	21.39	5.14	22.06	5.79	0.683
	N	%	*N*	%	
Female	13	62	15	60	
Hormone replacement therapy	5	38	6	40	
Treated hypertension	3	14	2	8	

Values are means (M) and standard deviations (SD), or number of participants (N) and percentage within group (%), respectively. Abbreviations: MMSE, Mini-Mental State examination; BMI, Body mass index.

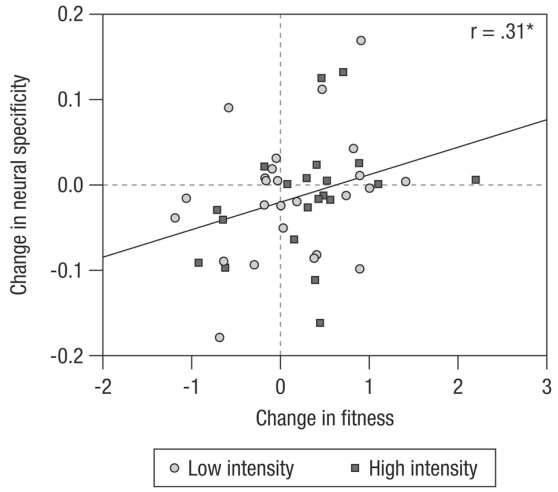

FIGURE 1 | Changes in fitness (aggregate measure of VO_2max and VO_2AT) are positively associated with changes in neural specificity. Circles represent participants from the low-intensity (LI) exercise group, and squares represent participants from the high-intensity (HI) exercise group. Change scores refer to post-training minus pre-training differences; a score of zero signifies no change. *$p < 0.05$.

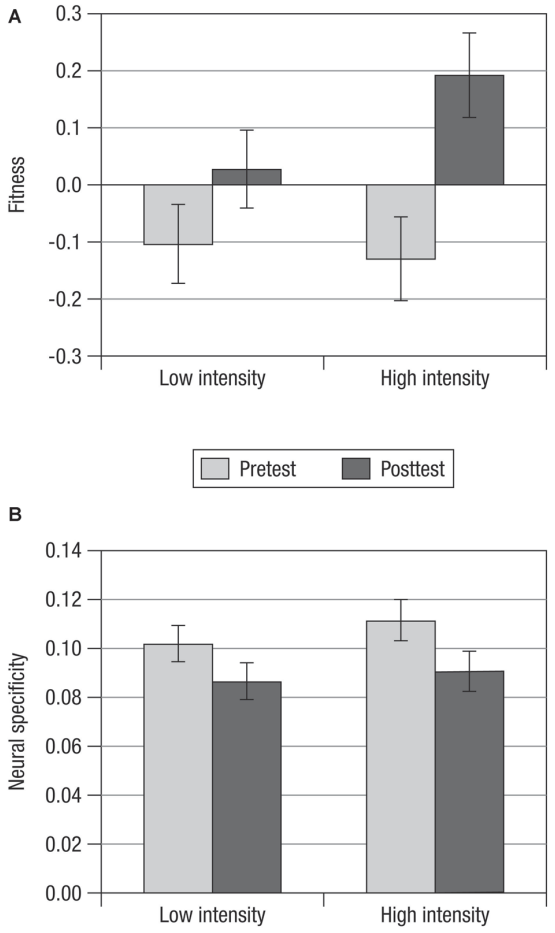

FIGURE 2 | Mean changes over the course of the training in (A) fitness (aggregate measure of VO_2max and VO_2AT) and **(B)** neural specificity. Whereas fitness increased reliably from pretest (light gray) to posttest (dark gray), changes in neural specificity were not significantly different from zero. For both measures, changes in the LI training group (left) were not reliably different from changes in the HI training group (right). Error bars depict the standard error after removing between-person variability (Cousineau and O'Brien, 2014).

neural specificity. These data suggest that regular physical activity may reduce or even reverse aging-related declines in neural specificity that have been reported in earlier studies (Park et al., 2004, 2010; Payer et al., 2006).

In terms of mechanisms, we propose that regular exercise may counteract the age-related weakening of dopaminergic neuromodulation (Bäckman et al., 2006). Neurocomputational models predict that adequate DA availability helps to keep the sigmoidal gain function within ranges that optimize signal transmission (Li et al., 2001). Optimized signaling reduces the detrimental effects of neural noise, and leads to the generation of more distinct internal representations (Li and Sikström, 2002). We note that this hypothesis could be tested more directly using Positron Emission Tomography (PET) to investigate DA binding and neural specificity before and after an exercise intervention.

Age-related losses in dopaminergic neuromodulation have more often been reported in frontal regions than in VVC. However, there is evidence for age-related losses of DA in the human temporal, parietal, and occipital cortices as well (Kaasinen and Rinne, 2002), closely resembling the dopaminergic decline repeatedly observed in the striatum. Recently, Garrett et al. (2015) found effects of d-amphetamine (boosting DA levels) on BOLD signal variability in regions beyond typical DA projections (e.g., primary visual cortices). Moreover, in a PET study with 181 healthy adults between 64 and 68 years of age, Nyberg et al. (2016) found that both caudate and hippocampal D2 DA receptor availability were positively associated with episodic memory. These findings provide further evidence for the functional significance of DA across many regions of the human brain.

It is also possible that other neurotransmitters are playing a role, instead of, or in addition to, DA. For example, in a proton magnetic resonance spectroscopy study, Maddock et al. (2016) observed increased signals for both glutamate and GABA in the visual cortex following vigorous exercise, indicating increased glutamate and GABA levels. Likewise, animal work showed an increased visual response in mouse visual cortex during running using *in vivo* calcium imaging. The suggested mechanism includes disinhibition of glutamatergic pyramidal neurons through the interaction of two different GABAergic interneurons (Fu et al., 2014). However, these results were obtained during running and there is only preliminary evidence to suggest that more exercise in the preceding week

TABLE 2 | Pre- and post-training values (mean ± SD) for fitness measures, multivariate pattern analysis, and % BOLD signal change.

	High intensity		Low intensity	
	Pre	Post	Pre	Post
VO_2max (ml/min × kg)*[1]	21.26 (± 4.76)	23.32 (± 5.06)	21.95 (± 5.70)	22.34 (± 5.29)
VO_2AT (ml/min × kg)*[2]	15.60 (± 3.18)	16.53 (± 3.49)	15.20 (± 4.25)	16.05 (± 3.57)
VO_2*[3]	−0.13 (± 0.84)	0.19 (± 0.92)	−0.10 (± 1.07)	0.03 (± 0.96)
MVPA	0.11 (± 0.07)	0.09 (± 0.06)	0.10 (± 0.07)	0.09 (± 0.07)
% Bold signal change FFA	0.18 (± 0.14)	0.15 (± 0.17)	0.20 (± 0.14)	0.18 (± 0.18)
% Bold signal change PPA	0.25 (± 0.20)	0.20 (± 0.19)	0.19 (± 0.17)	0.20 (± 0.20)

*Abbreviations: MVPA, Multivariate pattern analysis; FFA, fusiform face area; PPA, parahippocampal place area. *Indicates a significant main effect of time from the repeated measures ANOVA ($p < 0.05$). [1] $F_{(1,49)} = 4.805$; $p = 0.033$; $\eta_p^2 = 0.089$. [2] $F_{(1,50)} = 4.902$; $p = 0.031$; $\eta_p^2 = 0.089$. [3] $F_{(1,49)} = 5.637$; $p = 0.022$; $\eta_p^2 = 0.103$.*

also relates to higher resting glutamate (but not GABA) levels (Maddock et al., 2016). Furthermore, administration of GABA and a GABA agonist has been found to increase the orientation selectivity of individual visual neurons (increased neural specificity), while administration of a GABA antagonist has been found to decrease it (reduced neural specificity; Leventhal et al., 2003). Future work is needed to gauge the relative importance of different neurotransmitter systems, and their potential interactions, in mediating the effects of exercise on cognition.

Exercise has been shown to exert effects on other parameters of cerebral functioning, and these parameters may have contributed to our findings. Regarding fMRI, exercise-induced fitness changes were found to induce improved neural efficiency, that is, reductions (Colcombe et al., 2004; Voelcker-Rehage et al., 2011) or stability (Maffei et al., 2017) of BOLD activation in task-relevant areas as well as strengthened functional connections in relation to default mode and frontal executive networks (Voss et al., 2010). Though changes in neural efficiency, as investigated in these earlier studies, and changes in neural specificity, as investigated in the present study, can occur independently from each other, it seems worth exploring whether and in what way they are related empirically. Specifically, exercise training may lead to neural representations that achieve greater distinctiveness with a lower levels of neural activation.

Although our experimental task was not designed to look at neural efficiency, we were curious to see whether our training did affect mean BOLD activation in stimulus-relevant regions (FFA and PPA) and if so, in what way. Overall, we found negative, but non-significant, relationships between change in fitness and change in BOLD activation. These trends seem consistent with the hypothesis that improved fitness is associated with increased neural efficiency (i.e., less BOLD signal change). However, because we used a passive viewing task, there are no behavioral measures (e.g., reaction time, accuracy) from the scanning sessions that we could relate to the BOLD signal. We therefore acknowledge that the present study does not directly address neural efficiency, as this would require reduced BOLD activation in the absence of performance decrements.

At a more general level, exercise has been found to change brain perfusion (Pereira et al., 2007; Maass et al., 2015). Whereas Pereira et al. (2007) specifically looked at the hippocampus, Maass et al. (2015) also found fitness-related changes in perfusion to affect non-hippocampal cortical blood flow and blood volume. Changes in perfusion could potentially influence our results, as the fMRI BOLD signal measures a vascular response. Hence, we wondered whether the observed association might be due to changes in vascular response rather than changes in neural specificity. If so, one would expect to also observe an association between changes in mean activation level and changes in fitness. We therefore extracted mean activation values from VVC using MarsBaR, but found no reliable correlation with changes in fitness. Furthermore, vascular changes would not be expected to be limited to VVC, and so we tested whether associations between changes in fitness and changes in neural specificity were also present in brain regions that were not significantly activated by the task, such as medial orbitofrontal cortex, precentral gyrus, supramarginal gyrus, superior temporal gyrus, hippocampus, and parahippocampus. None of those regions, nor all of them combined, exhibited the change-change correlation observed in VVC. Each of these findings argues against a predominantly vascular interpretation of the observed effects. We note, however, that a calibrated fMRI approach would be needed to fully disentangle changes in neural activity from changes in vascular reactivity.

In contrast to Park et al. (2010), we did not observe associations between neural specificity and any of the cognitive tasks assessed in this study. One reason may be that Park et al. (2010) used a slightly different visual task in the fMRI scanner: Whereas our participants passively viewed single pictures, Park et al. (2010) presented two pictures side-by-side and asked their participants to make a same/different judgment, which may have introduced a demand characteristic that was absent in our task.

It is conceivable that exercise training as well as familiarization with the MR environment would alter participants' intention to lie quietly in the scanner. To minimize differences in head movement between pretest and posttest, all participants took part in a Mock-Scanner session before pretest. In addition, we used the FMRIB Software Library (Jenkinson et al., 2012) to check for head motion artifacts using the aggregated measures dvars as well as framewise displacement (implemented in fsl_motion_outlier). We found no evidence for significant session differences in head motion.

Also, sample size as well as sample selectivity may have influenced our results. In a study with similar sample selection criteria, Voelcker-Rehage et al. (2015) noticed a comparably high amount of COMT met/met allele carriers. Since our participants

were inactive but healthy and willing to change their lifestyle, it is rather likely that they were even more selective. Given the absence of genetic information in this study, this conjecture cannot be tested.

As discussed in Kleemeyer et al. (2016), the absence of group differences in fitness changes between the HI and the LI groups may indicate that the level of challenge provided by LI training was already effective in boosting fitness in sedentary older adults, thereby rendering the two training regimes similar to one another. The observed absence of group differences in fitness changes also provides a reasonable explanation for the absence of group differences in neural specificity changes. As for neural specificity, we did not observe a mean increase over time, but rather stability, as the numerical decrease was not statistically different from zero. We interpret this finding in terms of two opposing forces, one related to normal aging and the other related to the fitness intervention. On the one hand, neural specificity tends to decline with age. Participants did not escape normal aging while taking part in our study, and for this reason alone one would expect that neural specificity measured later in time is lower than neural specificity measured earlier in time. On the other hand, the training-induced fitness improvements apparently counteracted aging-related decrements in neural specificity, and apparently reduced or, in some cases, actually offset the effects of aging. We conclude that the effects of fitness improvements on brain functioning were not strong enough to result in a mean positive trend in neural specificity. Nevertheless, and in full agreement with our hypothesis, participants whose fitness improved more showed smaller declines, or even improvements, in neural specificity. From a design perspective, it would have been preferable to also include a no-contact control group in the study in order to document changes in fitness and neural specificity that would take place in the absence of any intervention.

To conclude, we found that exercise-related changes in fitness are positively associated with changes in neural specificity. Greater neural specificity is related to better fluid processing ability, so these results may explain some of the beneficial effects of exercise on cognition. Future longitudinal and intervention work should include higher intensity levels, longer training durations, and no-contact control groups to replicate and extend these findings.

AUTHOR CONTRIBUTIONS

MMK designed and conducted the study, analyzed the data, interpreted the results, and wrote the manuscript. TAP assisted with MVPA analysis, interpreted the results, and revised the manuscript. SS designed the study and revised the manuscript. NCB designed the neuroimaging protocol and revised the manuscript. LB performed the cardiovascular fitness assessment and revised the manuscript. UL designed the study, interpreted the results, and revised the manuscript.

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

ERKLÄRUNG

Hiermit erkläre ich an Eides statt, dass ich

- die vorliegende Arbeit selbstständig und ohne unerlaubte Hilfe verfasst habe,

- die Dissertation an keiner anderen Universität eingereicht habe und keinen Doktorgrad in dem Promotionsfach Psychologie besitze,

- die zugrunde liegende Promotionsordnung vom 3. August 2006 kenne.

Berlin, den 19. Juni 2017

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