Diploma thesis

ARTERIAL HYPERTENSION AND ATROPHY OF THE HIPPOCAMPUS AND ITS SUBFIELDS: A COMMUNITY – BASED STUDY IN OLDER ADULTS

submitted by

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AFFIDAVIT

I hereby declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

Graz, 28th March 2016 Lisa Wilhelmer, eh

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GLOSSARY

aHT	Arterial hypertension
ß	Standardized Beta
CA	Cornu ammonis
CI	Confidence interval
DG	Dentate gyrus
DM	Diabetes mellitus
EC	Entorhinal cortex
LGT-3	Bäumler's Lern- und Gedächtnistest
MRI	Magnetic resonance imaging
SD	Standard deviation
TIV	Total intracranial volume

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ABSTRACT

BACKGROUND & AIMS: Normal ageing is associated with impairment of memory performance. Age-related volume changes of the hippocampus and the hippocampal subregions appear crucial, but little is known about the susceptibility to vascular risk factors, such as arterial hypertension, which have a high prevalence in the older population. The aims of this study were to explore whether arterial hypertension relates to volume loss of the hippocampus globally or selectively to volume loss of hippocampal subfields and whether hypertension-related global and selective hippocampal volume loss, if any, is associated with memory impairment in older adults.

METHODS: We included 261 healthy older adults (62.1% females, mean age 71 years, age range 60–87 years) without clinical signs for dementia from the Austrian Stroke Prevention Family Study. Memory performance was evaluated by the "Bäumler's Lern- und Gedächtnistest". The volumes of the hippocampus and seven hippocampal subfields were calculated from 3T MRI high-resolution T1 MPRAGE scans using Freesurfer software. Relations between total hippocampus and hippocampal subfield volumes, arterial hypertension and memory performance were assessed using multiple linear regression analyses adjusted for age, sex, brain volume and coexisting vascular risk factors.

RESULTS: We found no significant relations between the presence of arterial hypertension and loss of total hippocampus volume or loss of hippocampal subfield volumes. The hippocampal subfield volume CA2-3 correlated positively with the presence of arterial hypertension (ß=0.118, CI:0.003-0.232, p=0.044). Arterial hypertension was also not associated with decreased memory performance.

CONCLUSION: The presence of arterial hypertension is neither related to global hippocampus atrophy or atrophy of specific hippocampal subfields nor to memory decline in community-dwelling older adults.

ZUSAMMENFASSUNG

HINTERGRUND & ZIELE: Der natürliche Prozess des Alterns ist assoziiert mit einer Abnahme der Gedächtnisleistung. Volumsveränderungen des Hippocampus und seiner Subregionen wurden mit dem gesunden Altern in Verbindung gebracht, doch wenig ist über die Anfälligkeit dieser Strukturen für häufig altersassoziierte vaskuläre Risikofaktoren, wie arteriellem Hypertonus, bekannt. Diese Studie untersucht, ob arterieller Hypertonus mit Atrophie des Hippocampus bzw. der Hippocampus-Subfelder assoziiert ist und ob diese Zusammenhänge, sofern vorhanden, mit der Gedächtnisleistung älterer Personen in Verbindung stehen.

METHODEN: In die Studie wurden 261 gesunde, ältere Personen (62.1% Frauen, Durchschnittsalter 71 Jahre, Altersspanne 60–87 Jahre) ohne klinische Anzeichen einer Demenz der Austrian Stroke Prevention Family Study eingeschlossen. Die Evaluierung der Gedächtnisleistung erfolgte mittels “Bäumler’s Lern- und Gedächtnistest”. Unter Einsatz der MRT-postprocessing-Software „Freesurfer“ wurden die Volumina des Hippocampus sowie von sieben Subfeldern aus den hochaufgelösten, T1-gewichteten MPRAGE Sequenzen der 3T MRT berechnet. Zusammenhänge zwischen Volumina des Hippocampus sowie der Hippocampus-Subfelder, arteriellem Hypertonus und Gedächtnisleistung wurden mittels multipler linearer Regression analysiert und für die Variablen Alter, Geschlecht, Hirnvolumen sowie co-existente vaskuläre Risikofaktoren korrigiert.

ERGEBNISSE: Es zeigte sich kein signifikanter Zusammenhang zwischen dem Vorhandensein von arteriellem Hypertonus und Volumsabnahme des globalen Hippocampus bzw. der Hippocampus-Subfelder. Das Volumen des Hippocampus-Subfelds CA2-3 korrelierte positiv mit dem Vorhandensein von arteriellem Hypertonus (ß=0.118, CI:0.003-0.232, p=0.044). Arterieller Hypertonus zeigte keine Assoziation mit einer Verringerung der Gedächtnisleistung.

SCHLUSSFOLGERUNGEN:

Das Vorhandensein von arteriellem Hypertonus bei älteren Personen steht weder mit Atrophie des globalen Hippocampus bzw. der Hippocampus-Subfelder noch mit einer Verschlechterung der Gedächtnisleistung im Zusammenhang.

1. Introduction

The global number of people aged 60 years or above is expected to triple in the first half of the 21th century, increasing from 606 million in 2000 to nearly two billion in 2050 (1). The ageing of a human being is a biological, irreversible process accompanied by numerous changes, both physical and mental. Cognitive decline is prevalent in the course of normal ageing which can strongly affect the daily life of an individual. The advent of automated measurement techniques of regional brain volumes allows to visualize neuroanatomical structures in great detail and to study age-related brain changes.

In the following, an overview of age-associated cognitive and neuroanatomical changes with particular view to the hippocampus and its distinct hippocampal subfields is provided and the effect of vascular risk factors, in particular of arterial hypertension, on structural changes of the hippocampus and its hippocampal subfields is broached to guide the reader towards the question of this thesis.

1.1. Age- associated cognitive changes

The biological process of normal ageing is linked to changes in cognitive ability. In particular, episodic memory, working memory and executive function are cognitive domains altered with normal ageing. Declines in these cognitive domains are thought to be late-life changes, not arising before the sixth decade (2). However, there is also research indicating a peak of abilities including problem solving, processing and learning new information as well as reasoning about unfamiliar situations around the third decade followed by a gradual decline (3,4). Decrease of processing speed with normal ageing in combination with a decreased selective attention (difficulties in ignoring irrelevant information) and reduced application of strategies to enhance memory and learning performance may have a critical impact on any timed cognitive test performance (4,5). Further cognitive domains, which are affected in normal ageing are complex attention tasks (selective and divided attention), visual construction skills and, to a minor extend, simple attention span. Language ability, semantic memory, procedural memory and visuospatial abilities are assumed to remain stable up to the sixth or seventh decade, but may also decline with ageing (3,4).

1.2. Age- associated neuroanatomical changes

Normal ageing is associated with global-and regional brain volume changes and shows high regional variability. In a longitudinal study, Driscoll et al. investigated global (total brain volume, grey and white matter), lobar (frontal, parietal, temporal and occipital) and regional volume measures (including orbitofrontal cortex, cingulate gyrus and hippocampus) in healthy adults after the age of 64 years. Results suggested an annual volume decline in all examined regions. Highest annual volume loss was shown for frontal and temporal lobes. Total brain volume was estimated to decline significantly (approximately 7.0 cm^3 per year) (6). Walhovd et al. analysed 16 subcortical structures, cerebral cortex volume and total brain volume in people aged 18–95 years. Strongest age-related volume differences were found for cerebral cortex, pallidum, putamen and nucleus accumbens. A non-linear association with age has been found for hippocampus volume, total brain volume, cerebral white matter, caudate and ventricle volume (7). In addition, there is evidence suggesting that normal ageing is associated with a greater decrease of white matter than grey matter volume (6,8).

1.3. Structural changes of the hippocampus and the hippocampal subfields associated to ageing and cognition

The structure of the hippocampus is of great interest in the context of normal ageing, especially according to its role for cognitive ability. The hippocampus, which is known to play a crucial role in memory processes (9), appears to be very susceptible to age-related neurodegenerative and neuropathological processes (10,11). As part of the hippocampal formation, the hippocampus is a heterogeneous structure which encompasses a complex circuit of functionally and molecularly specific subregions (12). Broadly used is the definition of the hippocampal formation by comprising the cornu ammonis (CA 1-4), the dentate gyrus (DG) and the areas of presubiculum, subiculum, parasubiculum and entorhinal cortex (EC) (13).

Structural magnet resonance imaging (MRI) techniques have been originally applied to evaluate age-associated volumetric changes of the hippocampus as a whole. Longitudinal studies from Raz et al. showed that volume loss of the hippocampus was progressive and strongly age-related (11,14). In 2011, a review from Walhovd et al. summarized 15 MRI studies conducted from 1990 to 2008 (by the majority cross-sectional studies), which assessed the effect of age on hippocampus volume. Nine out of 15 studies showed hippocampus atrophy with normal ageing, of which three reported a non- linear relationship with age and one indicated accelerated

hippocampus atrophy by the effect of age (7). In more recent cross-sectional studies, Frisoni et al. showed a negative correlation of hippocampus volume with age in healthy older study participants (15). Gattringer et al. reported an inverse relation of advanced age (> 73 years of age) with absolute and normalized hippocampus volume in non-demented, healthy study participants from the Austrian Stroke Prevention study (16) and Pereira et al. found smaller hippocampus volumes associated with ageing in a study population of 55 cognitively healthy older adults (17). Despite the strong evidence for the relation between hippocampus atrophy and normal ageing, there are also studies indicating that the hippocampus may be relatively preserved in normal ageing (18–21). Amongst others, Grieve et al. investigated age-associated grey matter volume loss in 223 healthy study participants and reported that relative to the observed significant global grey matter volume loss, grey matter volume of the limbic system including the hippocampus was significantly preserved in ageing (19). Head et al. showed no significant differences in hippocampus volume in young adults compared to non-demented older adults in a sample of 100 subjects (20).

More recently, high-resolution MRI techniques have been used to investigate the individual involvement of the hippocampal subregions in the process of normal ageing. The rising awareness for the concept of regional vulnerability of the hippocampus and its cytoarchitectonically distinct structures directed the focus towards the analysis of hippocampal subfield volumes rather than analysis of the hippocampus volume as a whole (12).

The following studies analysed age-related regional changes of volume and shape in the hippocampus using three- dimensional surface mapping: In a cross-sectional setting, Chételat et al. reported a relationship between increasing age and atrophy of the subiculum in healthy participants (22). Csernansky et al. analysed changes of hippocampal shape in non-demented older adults compared to young controls in a cross-sectional study and reported age-associated general flattening of the hippocampus, with inward deformations in the head as well as the tail and outward changes in the body of the hippocampus (23). In a longitudinal study, Wang et al. investigated regional hippocampal changes in subjects with mild form of Alzheimer's disease compared to cognitively healthy older adults and showed age-associated hippocampal deformation, mainly involving the hippocampal head and the subiculum (24). Moreover, ageing has been associated with atrophy in medial and lateral aspects of the hippocampal tail and body, matching CA1, and ventral aspects of the head, matching the presubiculum (15).

Various studies have been conducted applying regional morphometry of the hippocampus to evaluate structural changes of the hippocampal subfields associated to normal and pathological ageing by either manual, semi-automated or completely automated procedures (25–31).

Manual segmentation of the hippocampus and the hippocampal subfields was performed in the following studies: In a cross-sectional study from Shing et al. which analysed age-related hippocampal subfield volume differences and its association with memory performance in cognitively healthy, older adults, the hippocampal subfield CA1-2 was significantly smaller in older subjects compared to young controls. Due to the high prevalence of arterial hypertension in the older subjects, atrophy of CA1-2 was suggested to reflect special susceptibility to arterial hypertension of this area instead of reflecting the normal process of ageing (25). In 2007, Mueller et al. reported in a cross-sectional study a significant decrease of the volumes of the hippocampal subfield CA1 in healthy, older subjects with a most pronounced affection in subjects > 70 years of age (26). In two subsequent studies the negative correlation of CA1 volume and ageing was reproduced (27,28). Moreover, associations between ageing and CA3-DG volume decline were reported (27).

In an cross-sectional study, Apostolova et al. used a semi-automated hippocampal segmentation procedure and reported an effect of ageing on all examined hippocampal subfield volumes including CA1, CA3, subiculum and DG in cognitively normal, older adults (29).

Using a fully automated hippocampal segmentation procedure, Hanseeuw et al. reported significant age-related decline of total hippocampus volume and no significant results among all investigated hippocampal subfield volumes but an age-related trend for atrophy of CA4-DG (30). Similar results were obtained by Lim et al. indicating a significant correlation of age with CA4-DG volume decline in healthy, older controls (31).

In summary, data from structural neuroimaging studies investigating the effect of normal ageing on hippocampal subfield volumes remain inconsistent and indicate the necessity of further standardized exploration, given that discrepancies in defining the boundaries of hippocampal subfields, different segmentation and measuring methods along with different study designs challenge the comparability of existing results (15,22–31).

Several recent studies have assessed the role of distinct hippocampal subfields in relation to subjects' neuropsychological performance. Some small studies have also investigated the effects of age-related volume loss of distinct hippocampal subfields on cognition and function. It has been suggested that the dorsal hippocampus is primarily involved in cognitive processes

including memory, while the ventral hippocampus is thought to play a greater role in complex behaviour including emotion, stress and affect (12,32).

In a multi-center, longitudinal study from Mungas et al. with an average observation time of 4.8 ± 2.0 years, hippocampus volume loss was the main predictor for memory decline, while impairment of executive function was determined by change of multiple brain components including volumes of cortical grey matter, hippocampus and lacunes in a cohort of 103 older, cognitively healthy, mildly impaired and demented adults. Also, the results remained the same after exclusion of the demented subjects (33). Konishi et al. reported a positive relation of the hippocampus volume and the use of spatial memory strategies, a key function of episodic memory (34,35). Mueller et al. investigated the relationship between different memory domains and hippocampal subfield volumes. They found associations of early retrieval with CA3 & DG volume, and late retrieval with CA1 volume (36). Shing et al. described a relation between recognition and CA3-4 & DG volume (25). Reduced volumes of CA1 and CA3/DG (37), and of subiculum and CA2-3 (30) were reported in participants with documented memory impairment, as compared to age- matched controls. Verbal memory and visuospatial memory correlated with DG volume, while visuospatial and visual-object memory correlated with volume of posterior CA in a study on young, healthy participants (< 50 years) (38).

1.4. Structural changes of the hippocampus and the hippocampal subfields and vascular disease

The presence of vascular risk factors might accelerate age-related hippocampal atrophy and impair hippocampal function, independent of age (11,39). The hippocampus and its subfields, in particular the hippocampal subfield CA1, show increased vulnerability to hypoxia and ischemia (39,40). Arterial hypertension (aHT) impairs cerebral perfusion including hippocampal blood flow (41,42) and might thus have detrimental effects on CA1 integrity. Age associated volume difference in the hippocampal subfield CA1 has been attributed to the presence of aHT in older study participants compared to younger controls (25). Higher blood pressure seems also to affect hippocampus volume as a whole. Beauchet et al. reported in a review that high blood pressure was associated with increased volume loss in the hippocampus (43). Volume decline of the medial temporal lobe, in particular of the hippocampus, was reported to be accelerated by the presence of aHT in otherwise healthy subjects (11,44). However, the effect of aHT on the hippocampal structure in healthy aging is not yet fully understood. Moreover, best to our knowledge, only one study investigated the effect of

hippocampal subfield volumes on memory performance in relation to the presence of aHT. Bender et al. reported an association between memory performance and volumes of the hippocampal subfields CA3-DG and CA1 in healthy adults with aHT (45).

Summing up, the effect of arterial hypertension on hippocampal subfield volumes is not yet fully understood. It is also not known, whether such changes, if present, relate to memory performance. Therefore, in this diploma thesis, the effects of arterial hypertension on hippocampus subfield volumes are assessed in a large, well-defined cohort of older adults. The effect of hypertension-related volume loss, if any, on memory performance is also assessed.

2. Objectives

The study explored whether arterial hypertension relates to volume loss of the hippocampus globally or selectively to volume loss of hippocampal subfields and whether hypertension-related global and selective hippocampal volume loss, if any, is associated with memory impairment of study participants.

3. Methods

3.1. Subjects

The study population derives from the Austrian Stroke Prevention Family Study (ASPS-Fam) and is designed as a prospective single-center, community based study. From 2006 to 2013 ASPS-Fam was established based on the Austrian Stroke Prevention Study (ASPS), which was launched in 1991 (46,47), by involving participants of ASPS and their first grade relatives in the study. Both studies were originally aimed at the investigation of the cerebral effects of vascular risk factors in community-dwelling adults of the city of Graz, Austria. Exclusion criteria were a history of stroke or dementia or abnormal neurological assessment. A total of 419 individuals were included into the study. All subjects ran through extended diagnostic and clinical assessment including medical history, blood tests, physical and neurological examination, vascular risk factor evaluation and neuropsychological testing. Except for participants with contraindications, MRI was taken from the entire cohort. Subsequently, data of 56 subjects had to be excluded due to contraindications for MRI or artefacts on MRI which did not allow analysis of hippocampal subfield volumes by applying the Freesurfer software. Thus, data of 363 individuals with age-range of 38-87 years were available for analysis in this study. Since the study focused on investigating a cohort of community-dwelling, older adults, age $\geq$ 60 years was set as an inclusion criteria which led to a study population of 261 persons. Written informed consent was received from all participating individuals and the submitted study protocol was approved by the ethics committee of the Medical University of Graz, Austria.

3.2. Neuropsychological assessment

In the course of neuropsychological assessment a thorough evaluation of memory and learning abilities, executive function (conceptional reasoning, attention, speed) and motor skills was undertaken by applying a test battery as described in Schmidt et al. 1999 (47). For all test performances the same order and the same laboratory conditions were maintained. The selected test battery is composed of neuropsychological tests which are well established and have been widely applied in the German-speaking area.

To evaluate intermediate memory recall and learning ability, Bäumler's "Lern- und Gedächtnistest" (LGT-3) (48) was applied. LGT-3 comprises six subtests. By forming the sum of weighted scores of the subtests, the total learning and memory performance score is generated. The subtests are structured in an image paradigm recognition, two tests assessing visuospatial memory (trail and design recall) and three tests screening for verbal memory (story recall, word and digit association task). For the trail recall test (trail in an abstracted city map) and the subtests image paradigm recognition (objects), design recall (core symbol and frame), story recall (facts about construction of a library) and word associations task (German–Turkish word pairs), which are composed of 20 items each, tested persons were given one minute. The digit association task (three digit telephone numbers and names of extension holders) which consists of 13 items was presented to the tested person for two minutes. Forthwith after the learning episode, while the tested persons are confronted with the six subtests consecutively, begins the recall episode, strictly adhering to the same order. Duration from presentation to recall phase for one specific subtest varies from 7 to 11 minutes.

Executive function was evaluated by applying the Wisconsin Card Sorting Test (49), the trail making test (chapter B) (50), and a part of the revised Wechsler Adult Intelligence Scale (Digit Span Test) (51). Following Milner's criteria (52) generated results for the Wisconsin Card Sorting Test were classified into the categories *completed, perseverative errors* and *total errors*.

For the assessment of motor skills The Purdue Pegboard Test (53) was used.

By composing z-scores for the specific domains of cognitive function, summary measures were computed. Within each cognitive domain, the individual test scores were converted to z-scores of which the average value was then calculated forming summary measure. For the analysis, summary measures were preferred instead of individual tests to decrease statistical artefacts (ceiling and floor effects) and other errors in measurement.

3.3. Vascular risk factors

A thorough evaluation of vascular risk factors was undertaken assessing the individual risk profile including aHT, diabetes mellitus (DM), cardiac disease, nicotine abuse, hyperuricaemia, hypertrigliceridaemia, hypercholesterolaemia, peripheral vascular disease and venous thrombotic disease. Diagnosis of vascular risk factors was made on the basis of medical history and results of medical examination and laboratory tests.

The condition aHT was coded existent if both of the two blood pressure readings measured in course of the medical evaluation were > 160/95 mmHg, if repeated blood pressure readings with values exceeding the defined limits were reported in medical history, or if a subject received treatment for aHT.

DM was determined present if in course of the medical evaluation a fasting blood glucose level > 140 mg/dl was measured or if a subject received treatment for DM at this point of time.

The following conditions were considered coding "cardiac disease present": cardiac abnormalities which are identified aetiologies causing cerebral embolism (54), coronary heart disease ascertained by following the Rose questionnaire (55), findings on echocardiography or electrocardiogram (ECG) indicating left ventricular hypertrophy or characteristic findings on ECG applying Minnesota coding (56).

3.4. Magnetic resonance imaging

MRI images were conducted with a 3T whole body scanner (TimTrio; Siemens Healthcare, Erlangen, Germany). A conventional protocol was generated consisting of a high resolution T1 weighted 3D sequence with magnetization preparation (MPRAGE), whole brain coverage (TR=1900ms, TE=2.19ms, inversion time=900ms, flip angle=9°, isotropic resolution of 1mm) and an axial FLAIR sequence (TR=10000ms, TE=69ms, inversion time=2500ms, number of slices=40, slice thickness=3mm, in-plane resolution=0.86x0.86mm²). Applying FSL software (Oxford, www.fmrib.ox.ac.uk) cortex volume was computed from T1 weighted MPRAGE images by fully automated structural image evaluation of atrophy. The individual head size of the subjects was considered and normalized cortex volumes were calculated.

3.5. Hippocampal subfield volumetry

For cortical surface reconstruction and volumetric segmentation of the brain, the Freesurfer image analysis pipeline (Version 5.3.0) was applied. Freesurfer is a cost-free software suite (http://surfer.nmr.mgh.harvard.edu, Martinos Center for Biomedical Imaging, Boston, Massachusetts): Technical details have been soundly specified in previous publications (57–68).

In brief, in the course of processing, the MRI images were adjusted for motion (69) and the non-brain tissue was removed (57). Further procedural steps included normalization of each study participant's native brain and the segmentation of the white and grey matter (58,59). Moreover, automated adjustment for topology, boundary tessellation of the grey and white matter tissue (60,70), normalization of intensity (71) and shaping of the surface were performed (61–63).

Freesurfer includes an automated stream for the measurement of volumetric brain structures, which was used to generate the volumes of the hippocampus. Bayesian inference and a statistical model of the medial temporal lobe were applied to segment the hippocampus to its hippocampal subfields. By this automated segmentation the seven hippocampal subfields CA1, CA2-3, CA4-DG, fimbria, presubiculum, subiculum, and hippocampal fissure were obtained for both, left and right hippocampi.

3.6. Statistical analysis

Categorical variables are indicated with frequencies and percentage, continuous variables with mean, ± standard deviation (SD) and range of measurement distribution. Normal distribution of data was tested with the Kolmogorov-Smirnov test. Group differences of the the aHT study cohort and the non-aHT study cohort was tested with chi-square test for independence for the categorical variables sex, education and vascular risk factors (including DM, cardiac disease, nicotine abuse, hyperuricaemia, hypertrigliceridaemia, hypercholesterolaemia, peripheral vascular disease and venous thrombotic disease). T-test for independent samples was applied to determine group differences of the aHT study cohort and the non-aHT study cohort for the continuous variables age, supratentorial volume and memory.

All volumetric measures obtained with Freesurfer and involved in the statistical analysis were normalized to the total intracranial volume by applying the following equation adopted from Seixas et al. (72):

$$nVol_i = \frac{Vol_i}{eTIV_i} \times 10000$$

with $nVol_i$ standing for the individual structure volume and $eTIV_i$ for the estimated total intracranial volume calculated with Freesurfer.

Multiple linear regression analysis was used to assess 1) the association between the presence of aHT and the specific hippocampal subfield volumes including total hippocampus volume and 2) the association between the presence of aHT and memory performance. Analyses were corrected for age, sex, supratentorial volume and for the vascular risk factors DM and hypertriglyceridaemia which were significantly different in the two study cohorts (aHT/non-aHT). Standardisation of values to z-scores was performed previously.

The presence of arterial hypertension was set as independent variable and the specific hippocampal subfield volumes including global hippocampus volume and memory performance (z-score), respectively, were set as dependent variables. The p-value and the 95% confidence interval (CI) were computed for each regression coefficient. Statistical significance was determined with a two-sided p-value of <0.05. IBM SPSS (Statistics for Windows, Version 21.0. Armonk, NY, USA) was used for all statistical analyses.

4. Results

4.1. Descriptive statistics

The total study cohort showed a mean age of 71 years ± 5.1 (60.8-86.7). Sex was distributed disproportionally with 62.1 percent women (n=162) and 37.9 percent men (n=99). 72.4 percent of the study participants (n=189) fulfilled the above mentioned criteria to be coded with aHT. 72.0 percent of the study participants had finished primary school or apprenticeship (n=188), while 28 percent had completed a high school diploma or a university degree (n=73). Further details about basic demographics, frequency of risk factors, psychological test results and MRI findings are displayed in Table 1.

Chi-square test for independence showed significant group differences in the aHT cohort and non-aHT cohort for DM: χ^2 (1, n=261) = 4.03, p = 0.045, phi = 0.137 and hyperuricaemia: χ^2 (1, n=261) = 9.52, p = 0.002, phi = 0.200. For the categorical variables sex, education, cardiac disease, hypercholesterolaemia, hypertriglyceridaemia, venous embolic disease, peripheral vascular disease and smoking, no significant group differences were found.

T-test yielded no significant group differences in the aHT cohort and non-aHT cohort for the continuous variables age, memory performance (LGT-3) and supratentorial volume. For more details about group differences and frequency of coexisting vascular risk factors in the aHT study cohort see also Table 2 and Table 3.

Table 1: Basic demographics, risk factors, neuropsychological test performance and MRI findings of the total study cohort[a]

BASIC DEMOGRAPHICS	
Age [years]	71.0 ± 5.1 (60.8 - 86.7)
Women [n, (%)]	162 (62.1)
Education [n, (%)]	
Primary school	69 (26.4)
Apprenticeship	119 (45.6)
High school diploma	47 (18.0)
University degree	26 (10.0)
RISK FACTORS [n, (%)]	
Arterial hypertension	189 (72.4)
Diabetes	34 (13.0)
Cardiac disease	78 (29.9)
Hypercholesterolaemia	211 (80.8)
Hypertriglyceridaemia	44 (16.9)
Hyperuricaemia	87 (33.3)
Peripheral vascular disease	1 (0.4)
Venous embolic disease	36 (13.8)
Current smoker	23 (8.8)*
History of smoking	96 (36.8)
NEUROPSYCHOLOGICAL TESTING (z- SCORES)	
Memory, z- scores	-3.51 - 3.46
MRI VARIABLES [cm³]	
Cortex volume	391 ± 37.0 (297 - 503)
Total intracranial volume	1423 ± 130 (1139 - 1829)
Supratentorial volume	996 ± 98.5 (769 - 1265)
Left hippocampus volume	4.10 ± 0.50 (1.89 – 5.58)
Right hippocampus volume	4.19 ± 0.49 (3.15 – 5.50)

[a] Continuous variables are indicated as mean ± standard deviation and range. Categorical variables are indicated as percentage and total number; * 4 missing values accounting for 1.5% of total study cohort.

Table 2: Chi-square test for independence[a] analysing group differences in aHT cohort and non-aHT cohort

GROUP DIFFERENCES OF CATEGORICAL VARIABLES			
	aHT cohort	non- aHT cohort	p*
Sex (women)	114 (60.3)	48 (66.7)	0.422
Education			
Primary school	51 (27.0)	18 (25.0)	0.683
Apprenticeship	89 (47.1)	30 (41.7)	
Highschool diploma	32 (16.9)	15 (20.8)	
University degree	17 (9.0)	9 (12.5)	
Diabetes	30 (15.9)	4 (5.6)	0.045
Cardiac disease	63 (33.3)	15 (20.8)	0.069
Hypercholesterolaemia	154 (81.5)	57 (79.2)	0.804
Hypertriglyceridaemia	37 (19.6)	7 (9.7)	0.086
Hyperuricaemia	74 (39.2)	13 (18.1)	0.002
Peripheral vascular disease	1 (0.5)	0 (0.0)	1.00
Venous embolic disease	25 (13.2)	11 (15.3)	0.819
Current smoker**	15 (8.1)	8 (11.1)	0.607
History of smoking	70 (37.0)	26 (36.1)	1.00

[a] *p value of <0.05 was considered significant; aHT = arterial hypertension; variables are indicated as percentage and total number; ** 4 missing values accounting for 1.5% of total study cohort

Table 3: T- test for independent samples[a] analysing group differences in aHT cohort and non-aHT cohort

GROUP DIFFERENCES OF CONTINUOUS VARIABLES						
	Group	Mean	SD	t	df	p
Age (years)	aHT	71.3	4.95	-1.48	259	0.141
	non- aHT	70.3	5.36			
Memory (LGT-3)	aHT	67.1	11.50	1.51	259	0.132
	non- aHT	69.6	12.80			
Supratentorial volume (norm.)	aHT	7006	311.5	-0.61	259	0.541
	non- aHT	6980	313.8			

[a] p value of <0.05 was considered significant; aHT = arterial hypertension;

4.2. Main findings

The multiple linear regression analyses for hippocampal subfield volumes including global hippocampus volumes showed a significant positive association of the presence of aHT and the volume of the hippocampal subfield CA2-3 (ß=0.118, CI:0.003-0.232, p=0.044). All other analysed hippocampal subfield volumes and the total hippocampus volume did not show significant associations with the presence of aHT.

The multiple regression analysis for memory performance did not show associations with the presence of aHT (ß=-0.049, CI:-0.157-0.059, p=0.374).

For more details about the results of the multiple linear regression analyses see also Table 4.

Table 4: Multiple linear regression[a]: analysing the association of arterial hypertension with hippocampal volumes and memory performance

	ß	p*	95% CI
HIPPOCAMPUS VOLUMES			
Global hippocampus volume	0.080	0.158	-0.031 - 0.191
CA1	0.104	0.100	-0.020 - 0.227
CA2-3	0.118	0.044	0.003 - 0.232
CA4-DG	0.095	0.107	-0.020 - 0.210
Fimbria	0.043	0.462	-0.073 - 0.160
Hippocampal fissure	0.071	0.265	-0.055 - 0.198
Presubiculum	0.020	0.714	-0.089 - 0.129
Subiculum	0.042	0.459	-0.069 - 0.152
NEUROPSYCHOLOGICAL TESTING (z- SCORES)			
Memory	-0.049	0.374	-0.157 - 0.059

[a] Adjusted for age, sex, supratentorial volume, DM and hyperuricaemia; *p value of <0.05 was considered significant; aHT = arterial hypertension, CI = confidence interval;

5. Discussion

In this study, we showed that the presence of arterial hypertension in healthy, older adults did not significantly correlate with atrophy of the hippocampus or atrophy of the distinct hippocampal subfields. Moreover, the presence of arterial hypertension in healthy, older subjects did not yield significant associations with memory performance. There was a significant and weak positive correlation between the presence of arterial hypertension and the hippocampal subfield volume CA2-3. This finding is biologically not plausible and it very likely represents a chance finding given the multiple comparisons when relating hypertension to hippocampal volumes.

In the field of structural neuroimaging, Beauchet et al. reported in a review that the presence of arterial hypertension was related to atrophy of the hippocampus in healthy adults (43). Over a duration of five years, Raz et al. observed volume decline of the hippocampus to be accelerated by the presence of arterial hypertension in otherwise healthy subjects (11). Apart from the global hippocampus in particular the hippocampal subfield CA1 was shown to be heightened vulnerable by hypoxia and ischemia by studies deriving form Zola-Morgan et al. and Wu et al. (39,40) and Shing et al. obtained smaller volumes of the hippocampal subfield CA1 in older subjects compared to younger controls which were attributed to the presence of arterial hypertension in the old participants (25).

These findings suggested a role of arterial hypertension in the context of age-related volume decline of the hippocampus and the hippocampal subfields. However, our study results do not corroborate these findings. We found no relationship between arterial hypertension and total hippocampus volume. We also found no association between arterial hypertension and hippocampal subfield volumes. Reasons for diverging results could be differences in study designs, sample sizes, study population including differences of parameter such as age, blood pressure levels, onset of arterial hypertension, vascular risk factor profile and concomitant disease, vascular and neuropsychological assessment, MRI scan resolution and algorithm of subfield volumetry.

There were also studies that did not find any relationship between arterial hypertension and hippocampus volume. Our findings are in line with a recent study from Raz et al. which reported age-related volume loss of the hippocampal subfield CA1-2 but did not show any relation with the presence of arterial hypertension in healthy adults (73) and with Gattringer et al. who found age-associated atrophy of the hippocampus but no effect of arterial hypertension on this relation in non-demented, older adults (16).

In comparison to our study, the cross-sectional study from Shing et al. had a considerably smaller sample size of 19 subjects (10 young controls and 19 older, cognitively healthy adults). Age characteristics were similar in mean age but differed in age range from our study with a mean age of 75.4 years ± 2.9 (range 70-78) and 71.0 ± 5.1 years (range 60-87), respectively. Moreover, Shing et al. performed a manual segmentation of hippocampal subfields contrary to our automated method (25).

The relation of arterial hypertension and the hippocampal subfield volume CA1 reported by Shing et al. may be attributable to coexisting vascular risk factors in the hypertensive subjects, which was also recently suggested by Raz et al. (25,73). According to the methods description, Shing et al. considered self-reported arterial hypertension and DM of the study participants but in contrast to Raz et al. and our study did not perform a thorough assessment of other vascular risk factors including cardiac disease, hypercholesterolaemia and nicotine abuse (25,73).

Another hypothesised explanation by Raz et al. was that the hippocampal subfield volume CA1 may be more vulnerable to the effect of arterial hypertension in late life. In a cross-sectional design, Raz et al. investigated the relation of ageing, arterial hypertension and hippocampal subfield volumes with manual segmentation in a healthy population of 80 subjects ranging from 22-84 years (mean age 57.8 ± 14.3), while subjects from the old cohort from Shing et al. were within the 7th decade of life (25,73). We analysed a well-defined cohort of older, cognitively healthy adults and did not find an association of arterial hypertension and decline of hippocampal subfield volumes. However, we did not differentiate the study cohort by decades of life which may have masked the distinct effects of arterial hypertension on hippocampal volumes in different stages of old age.

The longitudinal study from Raz et al. which indicated a relation between volume decline of the hippocampus and arterial hypertension included 72 healthy adults ranging from 20-77 years (mean 52.6 ± 14.1) and used latent difference modelling to evaluate changes of volumetric brain measures. While persons with a history of a severe cardiovascular condition or DM were excluded, subjects with medicated, controlled arterial hypertension or mitral valve prolapse were included in the study (11). Gattringer et al. who in a cross-sectional design did not find an effect of arterial hypertension on hippocampus volume included 287 older, cognitively healthy subjects from the Austrian Stroke Prevention Study ranging from 52-90 (mean 66.6 ± 6.6) and used a semi-automated method for the evaluation of hippocampus volume. A thorough vascular risk factor assessment was performed. In addition to the effect of arterial hypertension, the effect of other vascular risk factors including cardiac disease, DM, smoking, hyperlipidaemia,

fibrinogen and alcohol on hippocampus volume were assessed, but did not yield significant results (16). While the sample size, age characteristics and the assessment of vascular risk factors of our study are similar to and our negative results on the relation of arterial hypertension and hippocampus volume in healthy, older individuals are in line with Gattringer et al., these findings derive from cross-sectional study designs (16). But the effect of vascular risk factors on hippocampus volume may be better traced by a longitudinal observation of a healthy population as conducted by Raz et al. (11).

Interestingly, in the above discussed studies including our own, the onset of arterial hypertension, whether it concerns midlife or late life arterial hypertension has not been considered (11,16,25,73), but might be a crucial information in the context of age-related decline of hippocampal volumes in consideration of arterial hypertension.

Unexpectedly, the hypertensive group performed not significantly worse on memory tests than the normotensives. Very likely, similar study-specific parameter as for the non-existing relationship between arterial hypertension and decline of hippocampal volumes in cognitively healthy, older adults are accountable for this negative finding.

This study has limitations. A longitudinal study design would have been more appropriate to investigate the effect of arterial hypertension on hippocampal subfield volumes including total hippocampus volumes. Moreover, adjustment for family structure was not done. However, we were acting on the assumption that with the determined age cut off of 60 years, we most likely included just one generation. The condition arterial hypertension was coded existent in the course of the vascular risk factor assessment if study participants exceeded values of >160/95 mmHg at both consultation or repeatedly in past medical history or received treatment for arterial hypertension. However, this definition did not consider important factors such as first onset, duration, levels and control of blood pressure and lacked the differentiation between midlife and late life arterial hypertension. Also, we applied a summary score for memory performance, but using the different functions for memory instead may be more accurate and better enable the analysis of the relation of arterial hypertension and memory performance in older adults. In the course of automated hippocampal subfield segmentation, the hippocampal subfields CA2 and CA3 as well as CA4 and DG were each summarized to one region of interest. Separation of these subfields may reveal more precise information on the distinct affection of these hippocampal structures in the context of normal ageing and age-associated vascular risk factors.

Strengths are the large study cohort including 261 participants, its community-based design, the comprehensive, prospectively arranged radiological and clinical protocols and the precise assessment of cognitive functions and vascular risk factors. In addition, high scan resolution enabled accurate segmentation of the hippocampal subfields.

Future studies should analyse in what way a threshold effect of blood pressure levels and the distinct presence of midlife or late life arterial hypertension are associated with volume changes of the hippocampus and the hippocampal subfields and memory decline in healthy, older adults. Moreover, exploring interactive effects of arterial hypertension with other vascular risk factors including DM, cardiac disease, nicotine abuse, hyperuricaemia, hypertrigliceridaemia, hypercholesterolaemia, peripheral vascular disease and venous thrombotic disease and their relationship with volume changes of the hippocampus and the hippocampal subfields and memory decline in healthy, older adults might reveal crucial information. The separate evaluation of the left and right total hippocampus and hippocampal subfield volumes may be considered to evaluate whether age-related lateral affection of the hippocampus is prevalent. Moreover, the forming of several age groups, categorized by decade of life, might provide better insights into differences between the old and the very old adults in context of age-related volume changes of the hippocampus and the hippocampal subfields in consideration of arterial hypertension.

In general, we would like to encourage the continuous use of structural neuroimaging to investigate the relation of arterial hypertension, volumes of the hippocampus and the hippocampal subfields and memory performance, albeit other methods such as functional MRI and positron emission tomography may be of great complementary or even better value to toward this question.

6. Conclusion

In summary, our study investigated the relation of arterial hypertension, volumes of the hippocampus and the hippocampal subfields and memory performance. In a sample of healthy older adults, we neither found an effect of arterial hypertension on volumes of the hippocampus and the hippocampal subfields nor on memory performance. Notwithstanding, to help elucidating the inconclusive state of knowledge, we underscore the necessity of considering vascular risk factors, in particular arterial hypertension, in future studies of age-associated changes of the hippocampal structure and cognitive decline.

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Der Beginn aller Wissenschaften ist das Erstaunen,
dass die Dinge so sind, wie sie sind.

Aristoteles