Brain Size, Sex, and the Aging Brain

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Abstract: This study was conducted to examine the statistical influence of brain size on cortical, subcortical, and cerebellar compartmental volumes. This brain size influence was especially studied to delineate interactions with Sex and Age. Here, we studied 856 healthy subjects of which 533 are classified as young and 323 as old. Using an automated segmentation procedure cortical (gray and white matter [GM and WM] including the corpus callosum), cerebellar (GM and WM), and subcortical (thalamus, putamen, pallidum, caudatus, hippocampus, amygdala, and accumbens) volumes were measured and subjected to statistical analyses. These analyses revealed that brain size and age exert substantial statistical influences on nearly all compartmental volumes. Analyzing the raw compartmental volumes replicated the frequently reported Sex differences in compartmental volumes with men showing larger volumes. However, when statistically controlling for brain size Sex differences and Sex × Age interactions practically disappear. Thus, brain size is more important than Sex in explaining interindividual differences in compartmental volumes. The influence of brain size is discussed in the context of an allometric scaling of the compartmental volumes. Hum Brain Mapp 36:150–169, 2015.

Key words: brain size; sex differences; morphometry; magnetic resonance imaging; neuroanatomy

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INTRODUCTION

With the advent of modern brain imaging techniques lots of studies have been conducted to examine anatomical differences between male and female brains. A well-

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established finding of these studies is that men demonstrate an on average larger total brain volume than women with an approximate 8-15% larger brain in men than in women [Ruigrok et al., 2014]. Some of these studies have also examined Sex differences of subcortical volumes including the thalamus, amygdala, hippocampus, putamen, pallidum, and accumbens, but identified hardly any sex differences especially when brain size is included as covariate variable in the analysis [Fjell et al., 2009b; Rijpkema et al., 2012; Tang et al., 2013]. One study revealed larger gray matter (GM) volumes for the caudate in men compared with women [Filipek et al., 1994] while a further study revealed larger GM volumes in women (i.e., not in men) when controlling for brain size [Luders et al., 2009]. Although there are conflicting results with respect to Sex differences for subcortical areas, there are lots of articles reporting specific anatomical and functional features for

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the basal ganglia in gender-specific neuropsychiatric disorders supporting their role in sex-specific functions [Bourque et al., 2009; Cahill et al., 1995; Gershon, 2002; Postuma and Dagher, 2006; Qiu et al., 2009; Volkow et al., 2012]. Gender specific correlations between hippocampal volume and psychometric intelligence have also been reported [Colom et al., 2013].

A further line of evidence proposes that female and male brains would demonstrate different patterns of intrahemispheric and interhemispheric connectivity. Early research in this area suggests stronger and more effective interhemispheric connections in women as indicated, for example, by larger cross-sectional corpus callosum (CC) areas (representative for the number of transcallosal fibers [DeLacoste-Utamsing and Holloway, 1982]). However, the majority of follow-up studies failed to replicate this finding [Bishop and Wahlsten, 1997] especially when morphological sex differences of the CC are related to total brain size [Jancke et al., 1997; Lüders et al., 2002]. However, this issue has regained new interest because a recent study reported greater within-hemispheric connectivity in men and greater between-hemispheric connectivity in women on the basis of diffusion tensor imaging (DTI) and graph analytical approaches [Ingalhalikar et al., 2014]. Interestingly, the analyses reported in this study have been conducted without controlling for brain size influences. Thus, it is possible that the reported results represent differences in connectivity architecture between smaller and larger brains [Luders et al., 2014].

A further issue, which has received attention in the literature is the question whether a substantial Age × Sex interaction exists. Some studies have found Age × Sex interactions [Chung et al., 2006; Coffey et al., 1998; Cowell et al., 1994; Good et al., 2001b; Gur et al., 1999, 2002b; Murphy et al., 1996; Nunnemann et al., 2009; Pruessner et al., 2001; Raz et al., 1997, 2004; Resnick et al., 2000; Riello et al., 2005; Sowell et al., 2007; Taki et al., 2011a; Xu et al., 2000] while others have found no Age × Sex interactions [Fjell et al., 2009b; Greenberg et al., 2008; Lemaître et al., 2005; Salat et al., 2004]. The pattern of Age \times Sex interactions reported in the aforementioned studies is not homogenous. For example, two studies concluded that men exhibited larger age-related brain atrophy and cerebrospinal fluid (CSF) increase than women over the entire life span [Coffey et al., 1998; Gur et al., 1999] with the strongest age-effects found in frontal and temporal lobes [Gur et al., 2002a; Murphy et al., 1996; Raz et al., 1997, 2004]. One study identified Age × Sex interactions in the parietal lobe and the hippocampus [Murphy et al., 1996] while a further study identified a stronger loss of GM in the posterior putamen in men with increasing age [Nunnemann et al., 2009]. In a more recent study, Taki et al. [2011b] reported small (in terms of effect size measures) Age × Sex interactions for several brain regions mostly including the basal part of the temporal and occipital lobes and for total GM volume when total brain volume was used as covariate. Although these Age × Sex interactions are statistically significant they are based on very small effect size measures ($\mathrm{Eta}^2 < 0.06$). These small effect sizes became significant because this study is considerably overpowered since they have analyzed 1,460 subjects, which makes it easy to identify even the smallest effect. Thus, it would be necessary to rely more strongly on effect size measures in such overpowered studies to discuss only those effects, which are practically important and substantial.

Most of the studies, which have examined brain volume differences between the sexes did not explicitly control for brain size. In our view, it would be of greatest importance to control for brain size when comparing brain volume measures between men and women. The same pertains for Age × Sex interactions. Here, it is important to mention that stereotactically normalizing individual brains in the context of studies using the voxel-based morphometry technique is not sufficient to control for brain size. Although this normalization transforms all brains into a common space, the principle differences of large and small brains still remain. For example, it has been shown that larger brains demonstrate smaller relative GM volumes but larger white matter (WM) volumes than smaller brains [Lüders et al., 2002]. In addition, the CC is relatively smaller in larger brains than in smaller brains independent of sex [Leonard et al., 2008]. These brain-size-related specific features will remain even when the brains are stereotactically normalized. Thus, comparing male and female brains, which have been stereotactically normalized will still contain the brain-size-related specific anatomical features. To study "true" sex influences on brain volume measures, it is necessary to control for brain size influences, or if one is interested in studying brain size influences, one has to control for sex influences.

Thus, this study was designed to re-evaluate Sex differences and Age × Sex interactions for cortical GM and WM, CSF, CC, cerebellar, and subcortical volumes by statistically controlling for total brain size. In addition, we analyzed brain size influences on the compartmental volumes (cortical and cerebellar GM and WM, as well as GM of subcortical volumes) independent of Sex. Following our previous findings [Jancke et al., 1997; Lüders et al., 2002] and findings of other laboratories [Lemaître et al., 2005; Leonard et al., 2008] our hypothesis is that brain size is the major source explaining interindividual volume differences independent of Sex. We included 856 conventional T1-weighted magnetic resonance images (MRI) from healthy individuals stored in our lab-internal database (age range 16-93 years). Most of the subjects have been scanned in the context of previously conducted studies of our group. One part of this sample is taken from an ongoing longitudinal project during which we scan brains of more than 220 subjects older than 65 years repeatedly for several years to come (the Longitudinal Healthy Aging Brain database project, LHAB, conducted at the International Normal Aging and Plasticity Imaging Center [INAPIC], University of Zurich) [Zöllig et al., 2011]. The

advantage of this dataset is that nearly all subjects have been measured in a single laboratory. With these data we want to answer the following explicit questions:

- 1. What is the influence of brain size (independent of sex and age) on the volumes of cortical and cerebellar GM and WM? Based on the previous studies we hypothesize that total brain size will exert its largest influence on GM and WM cortical volumes [Jancke et al., 1997; Lemaître et al., 2005; Lüders et al., 2002].
- 2. What is the influence of brain size (independent of Sex and Age) on subcortical subvolumes (thalamus, caudate, amygdala, hippocampus, putamen, pallidum, and accumbens)? Interestingly, this question has been examined to the best of our knowledge only in two recent articles using much smaller samples than in our study [Li et al., 2014; Tang et al., 2013]. Thus, our study serves as a cross-validation of these previous studies, but moreover, it goes beyond earlier studies by examining whether the influence of brain size is different for cortical and subcortical volume measures. Our hypothesis is that the subcortical volumes are influenced by brain size to a much smaller degree than the cortical and cerebellar volumes.
- 3. Most important for the scope of this article is to examine whether there is a "true" and substantial influence of Sex on the brain volume measures, which is independent of total brain size. Therefore, we will use total brain size as covariate when performing between-sex comparisons.
- 4. Furthermore, we are interested to test for Sex and Age effects when controlling for brain size as covariate. Similarly as in the studies by Fjell et al. and Tang et al. [Fjell et al., 2009b; Tang et al., 2013] we hypothesize that the Sex and Age interactions will disappear or at least be substantially reduced when controlling for total brain size. Thus, our study is a replication of these two studies. Different to the aforementioned studies we rely on morphological data from one single laboratory using 3 T magnets with similar scanning sequences while the aforementioned studies combined morphological data from different research groups (2-8 different groups) mostly using 1.5 T magnets. In addition, we would like to emphasize that it is important to re-examine Sex \times Age interactions as well as Sex influences on compartmental volumes using different samples, since the inconsistency of findings with respect to these influences is striking and might be [as suggested by Fjell et al., 2009b] "caused by differences in sample characteristics, scan quality, segmentation approach, or statistical procedures." Therefore, an independent replication relying on data obtained with good scan quality, a reliable segmentation approach, and sophisticated statistical analyses is worthwhile to
- 5. Finally, we are also interested to examine the influence of Age (independent from Sex) on cortical and

subcortical volume measures as well as on cerebellar GM and WM volumes, and on the CC and to compare our findings with findings of previous studies. We are aware of the fact that many studies have examined the influence of age on brain volume measures using cross-sectional [Allen et al., 2005; Courchesne et al., 2000; Fjell et al., 2009a; Fjell and Walhovd, 2010; Good et al., 2001a; Grieve et al., 2005; Jernigan et al., 2001; Lemaître et al., 2005; Pfefferbaum et al., 1994; Raz et al., 1997; Resnick et al., 2003; Salat et al., 2004; Smith et al., 2007; Sullivan et al., 2004; Taki et al., 2004, 2011a; Walhovd et al., 2005; Westlye et al., 2010; Ziegler et al., 2012] and longitudinal designs [Driscoll et al., 2009; Du et al., 2006; Fotenos et al., 2005; Raz et al., 2005; Resnick et al., 2000; Scahill et al., 2003; Taki et al., 2011a; Thambisetty et al., 2010]. However, we are motivated to study and reasses the influence of Age in our sample of Swiss subjects scanned in our laboratory.

METHODS

Subjects

We analyzed T1-weighted brain data from our database containing brain data that have been collected within the last 9 years. This database contains brain data obtained from subjects who have been examined in the context of previously published articles of our research group (see Table I for the references). All subjects are mostly righthanders (95%) as measured with the Annett Handedness Questionnaire (AHQ) [Annett, 1970]. All subjects of these groups have been scanned during 2004-2013. As a standard procedure in our laboratory, all subjects have been screened for neurological and psychiatric disorders according to the requirements of the local ethics committee. A subgroup of subjects comprises older subjects (>65 years) who are taking part at the Longitudinal Healthy Aging Brain (LHAB) database project (INAPIC) [Zöllig et al., 2011]. In this project, more than 220 subjects are scanned on a longitudinal basis for several years to come. Here, we will use the T1-weighted brain data obtained during the first examination wave, which took place from 2011–2012. The longitudinal LHAB project was launched to recruit anatomical, physiological, and psychological data of healthy older subjects (>65 years) on a longitudinal basis. All participants of the LHAB project met the following inclusion criteria: equal to or older than 65 years old, right handed, native German speaker, mini-mental status examination scoring > 26 [Folstein et al., 1983] and passing the MRI safety standards (e.g., no metallic implants). Subjects who reported a history of neurological diseases (e.g., Parkinson disease, Alzheimer's disease), mental disorders (e.g., depression), diseases of the haematopoietic system (e.g., anemia, leukemia), traumatic brain injuries in the last

TABLE I. List of studies from which the anatomical data were obtained

Sample	Scanner	Scanner	N	Se	ex	Ag	ge	1	MRI		Spatial
index	manufacturer	platform		w	m	mean	SD	sequence	TR/TE/Flip angle	resolution (mm)	Key publications
1	3 T Philips	Intera	20	20	0	23.4	4.6	TFE	20/2.3/20	0.86/0.86/0.75	[Hanggi et al., 2010]
2	3 T Philips	Intera	38	0	38	27.3	5.8	TFE	20/2.3/20	0.86/0.86/0.75	[Jancke et al., 2009]
3	3 T Philips	Intera	23	23	0	24.6	3.0	TFE	20/2.3/20	0.86/0.86/0.75	Unpublished
4	3 T GE	Signa Excite II	42	22	20	24.9	3.6	FSPGR	9.2/2.1/20	0.94/0.94/1.0	[Imfeld et al., 2009]
5	3 T Philips	Achieva	40	0	40	28.0	6.2	FFE	8.1/3.7/8	0.94/0.94/1.0	Unpublished
6	3 T Philips	Achieva	24	16	8	33.1	6.8	TFE	20/2.3/20	0.86/0.86/0.75	[Elmer et al., 2011]
7	3 T GE	Signa Excite II	18	7	11	32.0	9.3	FSPGR	9.2/2.1/20	0.94/0.94/1.0	[Bruhl et al., 2013]
8	3 T Philips	Ingenia	35	18	17	24.9	4.4	FFE	8.1/3.7/8	0.94/0.94/1.0	[Baur et al., 2013]
9	3 T Philips	Ingenia	13	7	6	28.2	3.1	TFE	8.3/3.7/8	0.94/0.94/1.0	unpublished
10	3 T Philips	Achieva	26	13	13	25.3	4.7	FFE	8.1/3.7/8	0.94/0.94/1.0	[Elmer et al., 2013]
11	3 T Philips	Achieva	40	26	14	73.3	7.7	FFE	8.1/3.7/8	0.94/0.94/1.0	[Drobetz et al., 2014]
12	3 T Philips	Intera	96	52	44	27.2	7.6	TFE	20/2.3/20	0.86/0.86/0.75	[Meyer et al., in press]
13	3 T Philips	Achieva	95	48	47	24.1	3.5	TFE	8.1/3.7/8	0.94/0.94/1.0	unpublished
14	3 T Philips	Ingenia	51	51	0	39.7	10.6	TFE	10.4/5.7/8	1.0/1.0/1.0	[Schlumpf et al., 2013]
15	3 T Philips	Achieva	75	35	40	45.0	23.5	TFE	8.1/3.7/8	0.94/0.94/1.0	[Langer et al., 2012]
16	3 T Philips	Ingenia	220	114	106	70.6	5.0	TFE	8.2/3.8/8	0.94/0.94/1.0	[Zöllig et al., 2011]

All studies have been conducted in our Zurich laboratory over the last 9 years. Most of these studies have been published so far. Studies 3, 5, 13, and 16 are unpublished. All studies have been conducted on 3T MR scanner systems, with the Philips system being the most frequently used system. However, due to regular scanner updates the platform has changed over the years (Intera, Achieva, Ingenia). The older subjects have been scanned almost entirely on the 3T Philips Ingenia. Two studies have been conducted using a GE system (Signa Excite II). Used sequences, scan parameters, and spatial resolutions are also given. Abbreviations: FFE: Fast field echo, GE: General Electrics, MRI: magnetic resonance imaging, SD: standard deviation, T: Tesla, TE: Echo time in ms, TFE: turbo field echo, TR: Repetition time in ms). The flip angle is given in degrees. m: men; w: women.

2 years, or those subjects suffering from migraine, diabetes or tinnitus were excluded from participation. With all older subjects, we conducted extensive neuropsychological testing to probe their cognitive and sensorimotor functions. Neuropsychological testing has been done for the group of older subjects scanned in the context of the LHAB project but not consistently and with the same psychometric tests for the remaining subjects. All older subjects are at least of average intelligence. For the remaining subjects, we inferred average intelligence on the basis of their current profession.

Taken together, all subjects have carefully been screened for neurological and/or psychiatric diseases. Only subjects were included in this analysis showing no sign of neurological and/or psychiatric hazards. A radiologist inspected all MR images and images revealing any of the following were excluded: major infarctions, brain tumors, hemorrhages (observed in low-intensity areas in T2-weighted images). As can be seen in Table II our entire data sample comprises 856 healthy subjects (women: 452; men: 404). We also regrouped our sample into two age categories (young and old), which will be used in the following analyses of variances (ANOVAs; see Table II). We chose to categorize our data into two groups because our database comprises too few data for the age range 35-60 years. Thus, it was impossible to design a more refined categorization across the entire age range. All subjects younger than 45 years were allocated to the "young" group

(n = 533) while those older than 45 years were allocated to the "old" group (n = 323). The age of 45 was chosen since it separates the two age distributions (young and old subjects) best. However, using other cut-off ages (e.g., 50 or 40 years) does not change the results.

In Table II, age and number of subjects are depicted broken down for sex and age group.

Image Acquisitions

MRI scans were acquired either on a 3.0 T Philips (Philips Medical Systems, Best, The Netherlands) or on a 3.0 T GE whole-body scanner (GE Medical Systems, Milwaukee, WI; see Table I for detailed information about the used MRI scanner systems). For the Philips scanner, data were acquired on three different platforms: Intera, Achieva, and Ingenia and for the GE scanner the Signa Excite II platform was used. With the exception of the Ingenia platform, which used a 15 elements head coil array, an eight elements head coil was used. Both scanners were equipped with a transmit-receive body coil and a commercial head coil array capable of sensitivity encoding. The Philips platforms used either a fast field echo or turbo field echo sequence and the GE platform used a fast spoiled gradient echo sequence. Slices were acquired either in the sagittal or axial plane. The imaging parameters of the sequences applied are summarized in Table I. To test for a systematic influence of the

TABLE II. Mean and standard deviations (SD) of age and number (N) of subjects broken down for age and sex

		Mean	SD	Minimum	Maximum	N
Young	Women	25.6	5.7	16	45	275
0	Men ^a	26.8	5.6	17	45	258
	Total	26.2	5.7	16	45	533
Old	Women	67.8	8.5	46	92	177
	Men	70.8	6.2	48	93	146
	Total	69.1	7.7	46	93	323
Total	Women	42.1	21.7	16	92	452
	Men	42.7	21.9	17	93	404
	Total	42.4	21.8	16	93	856

^aFor five subjects, we replaced the missing age by the mean age of the particular study from which the subjects were selected (24.3 years).

used platform and sequence parameters on the compartmental volumes, we performed a regression analysis with platform as regressor (dummy coded vector according to Table I) and the volumes as dependent variables. For this regression, there was no linear and/or nonlinear influence of platform on the compartmental volumes. Thus, we decided to use the data without controlling for platform and sequence parameters.

Image Analysis of Global and Local Subvolumes

Cortical and subcortical volumetric segmentation were performed with the FreeSurfer image analysis suite (version 5.3.0), which is documented and available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications [Fischl and Dale, 2000; Fischl et al., 2001, 2002, 2004].

The cortical and subcortical segmentation procedure used to measure the volume of the subcortical structures takes into account three different kinds of information to disambiguate labels: (i) the prior probability that a given tissue class occurs at a specific location in the atlas, (ii) the likelihood of the image given that tissue class, and (iii) the probability of the local spatial configuration of labels given the tissue class. This latter term represents constraints on the space of allowable segmentations and prohibits label configurations that never occur in reality (e.g., the hippocampus is never located anterior to the amygdala). Finally, the volumes of the subcortical structures as well as global brain measures were computed based on these segmentations. This technique has previously been shown to be comparable in accuracy to manual labeling [Fischl et al., 2002].

Volume Measures

For this article, we estimated compartmental volumes using the FreeSurfer segmentation tool. With this tool the volumes of many of anatomical regions of interest are measured with strong reliability and validity [Fischl et al., 2004]. Here, we used the following compartmental volumes: cortical GM, cerebral WM, cerebellar GM, cerebellar WM, CC, CSF, thalamus, caudatus, putamen, pallidum, hippocampus, amygdala, and the accumbens (Fig. 1). A special note is warranted for the CC. In FreeSurfer, the CC is not only segmented into the midsagittal slice but continues laterally (2.5 mm to the right and to the left) into both hemispheres, thus producing a multislice threedimensional slab [Francis et al., 2011]. Thus, FreeSurfer provides a three-dimensional segmentation of the CC, which is different to many studies using CC measures to study structure-function relationships. However, several recent studies have used this new three-dimensional CC measure [Francis et al., 2011] demonstrating reasonable results. In addition, a recent PhD thesis uncovered strong correlations between midsagittal CC areas and FreeSurfer CC volumes with Pearson correlations between both measures of r > 0.95 [Khan, 2012]. To examine whether the Free-Surfer CC volume correlates with the traditional midsagittal CC area also in our sample, we randomly selected 10 brains of our sample for which we additionally manually measured the midsagittal CC area. The correlation between both the FreeSurfer CC volume and the CC midsagittal CC area turned out to be high (r = 0.9). Using a volume measure for the CC offers the important advantage to adjust the CC volume to brain size since all measures are volumes.

To estimate brain size, we used two measures. One is the estimated intracranial volume (ICV), which is an automated estimate of total ICV in native space derived from the atlas-scaling factor (http://surfer.nmr.mgh.harvard. edu/fswiki). This scaling factor is used to transform the native space brain and skull to the atlas. This automated measure excellently corresponds to manually traced ICV measures and is widely used in morphometry studies [Klauschen et al., 2009; Li et al., 2014]. This volume measure represents a global head size measure since it includes the brain tissue but also the CSF, the meninges, and the interstitial volumes between the skull and the brain tissue. As a second brain size measure, we used the entire forebrain volume (FBV) calculated as the sum of cortical GM and WM, cerebellar GM and WM, and subcortical GM. Therefore, this volume measure contains all brain parts except for the brain stem. The Pearson correlation between both brain size measures for our sample is strong but not perfect (r = 0.765, $r^2 = 58\%$).

Statistical Analysis

IBM SPSS Statistics for Mac OSX, Version 22.0.0.0 was used for data analysis (Armonk, NY: IBM Corp). General linear models were used to explore the associations between Sex, Age, ICV, and FBV and the compartmental volumes. We used the raw compartmental volumes as well as proportional compartmental volumes. The

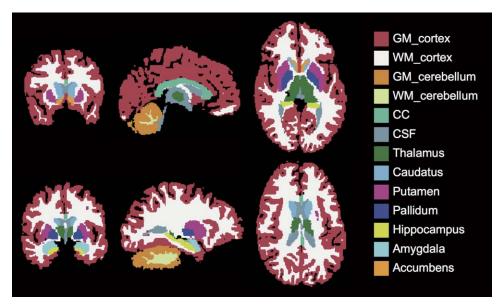


Figure 1.Coronal, sagittal, and horizontal slices demonstrating the segmentations given by FreeSurfer.

proportional compartmental volumes are the raw volumes related either to ICV or FBV (in %). With these measures, we performed several steps of statistical analyses:

- 1. To analyze the influence of brain size on compartmental volumes independent from Sex and Age, partial correlations are computed between the compartmental volumes and ICV and FBV controlled for Age and Sex. Sex effects were evaluated using a dummy-coded vector representing Sex (1 = men, -1 = women). We also calculated partial correlations between the proportional compartmental volumes and ICV as well as FBV using Sex and Age as covariates. In these analyses, interaction terms and quadratic as well as cubic brain size measures have been excluded since they are unimportant in explaining the variance of the dependent variables. These partial correlations are depicted in Figure 2.
- In a second step, we calculated two-way ANOVAs with Age (young vs. old) and Sex (women vs. men) as independent variables separately for all raw compartmental volumes.
- 3. In a third step, we computed two-way analyses of covariance (ANCOVAs; same independent variables as in the ANOVA) with ICV and FBV as covariates for the volume measures to statistically control for brain size differences. Before conducting these ANCOVAs we checked whether these data meet the assumptions to be analyzed with the ANCOVA technique. All compartmental volumes fulfilled the necessary assumptions for ANCOVA analyses comprising

independence of observation, no significant outliers, normal distribution of the dependent variable for each category of the independent variable, homogeneity of variances, linear relation of the covariate to the dependent variable at each level of the independent variable, homoscedasticity, and homogeneity of regression slopes. Homogeneity of regression slopes was tested using a hierarchical polynomial regression analysis, which was also used to examine whether the relation between the brain size measures (ICV and FBV) and the compartmental volumes can also be explained by nonlinear terms (e.g., ICV², ICV³, FBV², FBV³). Within these hierarchical polynomial regression analyses higher-order trends were tested for the predictive increments they afforded over and above lower-order trends. For instance, the significance of a quadratic function was tested by evaluating the increment in the multiple r^2 value it produced beyond that obtained when a linear function was used. Sex effects were also evaluated using hierarchical polynomial regression models that included a dummy-coded vector representing Sex (1 = men, -1 = women). Sex differences in the slopes of these regressions were analyzed by significance testing of the increment in the multiple r^2 value afforded by the addition of the interaction term including Sex (i.e. Sex \times ICV, Sex \times ICV², Sex \times ICV³). Sex differences in the intercepts of these regressions were assessed by testing of the increment in the multiple r^2 afforded by the addition of the dummy-coded gender variable to the linear and higher-order terms in the regression model.

Volumes	ICV (Age, Sex)	FBV (Age, Sex)
GM_cortex	0.64	0.87
WM_cortex	0.59	0.91
GM_cerebellum	0.37	0.52
WM_cerebellum	0.41	0.61
сс	0.20	0.43
CSF	0.11	0.19
Thalamus	0.44	0.69
Caudatus	0.41	0.48
Putamen	0.29	0.48
Pallidum	0.23	0.42
Hippocampus	0.29	0.62
Amygdala	0.32	0.48
Accumbens	0.08	0.35

Proportional volumes	ICV (Age, Sex)	FBV (Age, Sex)
% GM cortex	-0.59	-0.21
% WM cortex	-0.41	0.32
% GM cerebellum	-0.39	-0.16
% WM cerebellum	-0.26	-0.07
% CC	-0.37	-0.07
% CSF	-0.37	-0.21
% Thalamus	-0.56	-0.24
% Caudatus	-0.38	-0.20
% Putamen	-0.51	-0.26
% Pallidum	-0.42	-0.16
% Hippocampus	-0.57	-0.21
% Amygdala	-0.41	-0.15
% Accumbens	-0.41	-0.09

strong

moderate

small

Figure 2.

Partial correlations (r) between ICV and FBV and the compartmental volumes (left panel). On the right panel partial correlations between the proportional volume measures and ICV as well as FBV are shown. All partial correlations are computed with Age and Sex as covariates. The partial correlations are

color-coded to demonstrate the strength of the effects according to Cohen [1992]. Large effect sizes with a partial correlation r > 0.5 are coded in red; moderate effect sizes with a partial correlation r > 0.3 are coded in yellow, and small effect sizes with a partial correlation r > 0.1 are coded in green.

- 4. Additional two-way ANOVAs with Age (young vs. old) and Sex (women vs. men) as independent variables were calculated using the proportional compartmental volumes. These proportional measures were used to examine whether this brain size correction revealed the same results as the ANCOVAs and to be comparable with previous studies which used the same brain size correction [Fjell et al., 2009b; Lemaître et al., 2005; Li et al., 2014]. The mean raw, adjusted, and proportional compartmental volumes are presented in Figures 3 and 4. The results of the ANOVAs/ANCOVAs are summarized in Figure 5.
- 5. We also examined GM/WM ratios for Sex and Age influences to compare our results with previous studies using the same measures [Gur et al., 1999; Lemaître et al., 2005]. We calculated the GM/WM ratio on the basis of the cortical GM and WM volumes.
- 6. Finally, we closer inspected the Age effects when controlling for ICV, FBV, and Sex. For this, we used the adjusted volume measures (independent from Sex) and compared them descriptively between old and young subjects using Cohen's *d*. In addition, we calculated mean volume differences across the age span to

compare volume differences in our sample with similar volume differences reported in other studies [Fjell and Walhovd, 2010; Walhovd et al., 2011; Ziegler et al., 2012]. These adjusted means and the associated Cohen's *d* values are presented in Table IV.

Alpha levels were set at 0.05 for all analyses. In total, we computed 106 statistical tests (1 \times 13 ANOVAs, 3 \times 13 ANCOVAs, 4 × 13 partial correlations, and 2 ANOVAs for GM/WM ratios = 106 statistical tests). We used a Bonferoni-Holm correction to control for alpha inflation [Holm, 1979] resulting in a P = 0.05/106 = 0.0005 as P for which the sequential procedure of adjustment started. However, since these analyses are based on a relatively large sample (n = 856), even small (and more or less unimportant) effects will be detected as significant. We evaluated this by performing a power analysis explicitly demonstrating that even small effects will become significant when using the Bonferoni-Holm corrected P values. Thus, we rely on effect size measures allowing us and other researchers to compare our results with their own results independent of sample size. Here, we use etasquared (Eta² = η^2), which is an effect-size measure

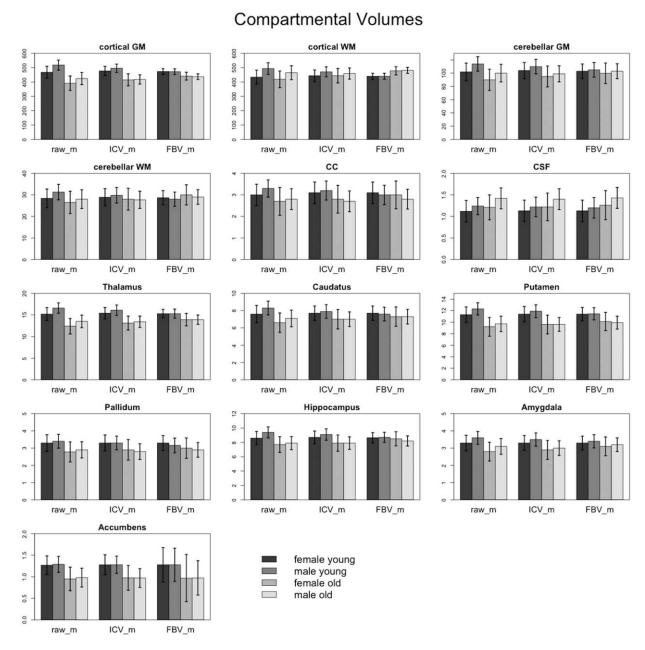


Figure 3.

Mean volumes for the raw and adjusted volumes (in ml) and standard deviations.

calculated by dividing the Type III sum of squares of the factors of interest (Age, Sex, Age \times Sex) by the total sum of squares ("Corrected Total" in SPSS) [Levine and Hullett, 2002; Olejnik and Algina, 2003; Richardson, 2011].

Since P values are more or less uninformative when calculated on the basis of such a large sample as we have used here, we will more strongly focus on effect size

measures. According to the suggestions given by Cohen [1992] an ${\rm Eta}^2 > 1\%$ is considered as small, >6% as moderate, and >14% as large. For the correlation analyses, it is also necessary to work with effect size categorizations. Here, we use also a categorization suggested by Cohen [1992]. A correlation coefficient r>0.1 is considered as small, an r>0.3 as moderate, and an r>0.5 as large.

Proportional Compartmental Volumes

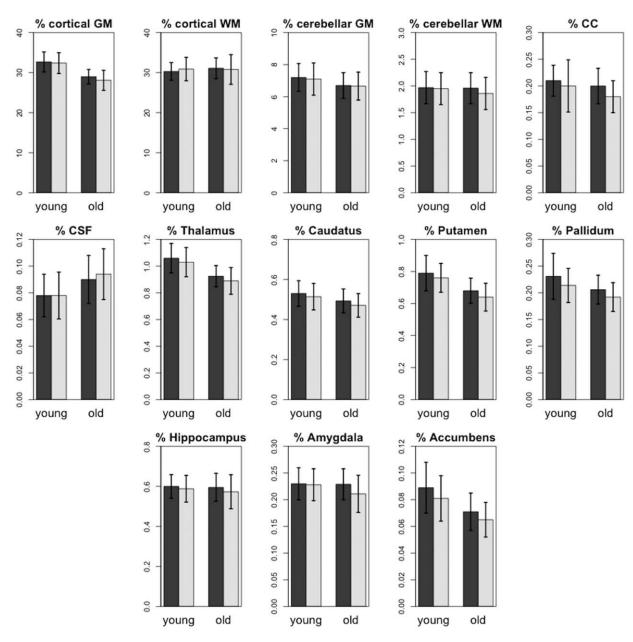


Figure 4.

Mean proportional volumes (volumes related to ICV in %) and standard deviations broken down for Age and Sex. Proportional volumes for women are shown in black and the proportional volumes for men are shown in gray bars.

RESULTS

Effects of Brain Size

First, we computed partial correlations between compartmental volumes and ICV and FBV controlled for Sex

and Age. These partial correlation analyses revealed strong correlations between the brain size measures (ICV and FBV) and the compartmental volumes even if Sex and Age were used as covariates (Fig. 2). The larger the brain size volumes the larger are most subvolumes (except for the accumbens). However, the correlations vary considerably

	Rav	Raw volumes			ICV adjusted			FBV adjusted			Proportional		
	A	s	AxS	А	s	AxS	А	S	AxS	А	s	AxS	
GM_cortex	42.7	9.9	0.5	26.9	0.5	0.4	4.4	0.0	0.0	38.4	1.1	0.2	
WM_cortex	3.5	20.3	0.3	0.2	2.4	0.3	7.7	0.0	0.0	0.4	0.1	0.5	
GM_cerebellum	16.4	11.2	0.1	9.3	2.0	0.1	0.6	0.4	0.0	5.6	0.1	0.0	
WM_cerebellum	6.7	5.5	0.5	2.2	0.1	0.4	1.2	0.6	0.0	0.7	1.4	0.4	
сс	13.9	1.3	0.7	9.5	0.0	0.6	0.7	1.0	0.2	4.4	2.8	0.5	
CSF	5.8	8.9	0.7	6.4	5.0	0.7	8.3	3.3	0.9	12.5	0.3	0.3	
Thalamus	42.6	7.9	0.2	29.9	0.9	0.2	6.9	0.0	0.0	29.2	1.0	0.0	
Caudatus	19.6	5.4	0.1	11.0	0.1	0.1	1.5	0.0	0.0	8.7	2.1	0.0	
Putamen	40.7	3.9	0.5	30.7	0.4	0.5	9.9	0.0	0.1	25.2	2.1	0.1	
Pallidum	21.6	1.3	0.0	15.2	0.0	0.0	2.6	1.0	0.1	9.2	4.2	0.0	
Hippocampus	24.8	5.4	1.4	17.1	0.7	1.4	1.4	0.3	0.5	7.9	3.4	1.1	
Amygdala	20.5	7.6	0.3	13.0	1.1	0.3	1.8	0.1	0.0	9.8	0.3	0.1	
Accumbens	32.2	0.2	0.0	27.3	0.0	0.0	8.2	1.4	0.1	19.7	2.9	0.1	
				strong moderate sr									

Figure 5.

Demonstration of ${\rm Eta}^2$ values (as % of explained variance) for the main effects Age (A) and Sex (S) as well as the interaction between Age \times Sex (A \times S) broken down for the compartmental volumes (raw, adjusted, and proportional). The effect sizes are color coded to demonstrate the strength of the effects according to Cohen [1992]. Large effect sizes with an ${\rm Eta}^2$ (in

%) >14% are coded in red; moderate effect sizes with an Eta^2 (in %) >6% are coded in yellow and small effect sizes with an Eta^2 (in %) >1% are coded in green. Degrees of freedom (df) for the main and interaction effects for the ANOVA are (1,852); the for main and interaction effects for the ANCOVAs are: (1,851).

between r = 0.08 and r = 0.64 for the partial correlations calculated with ICV and between r = 0.19 and r = 0.91 for the partial correlations calculated with FBV. With respect to ICV the strongest correlations with the subvolumes were found for cortical GM and WM volumes (r = 0.64 and r = 0.59). The volumes of cerebellar GM and WM, thalamus, caudatus, and amygdala correlated moderately strong with ICV (r = 0.32 to r = 0.44). Weak correlations were obtained for the CC, CSF, putamen, pallidum, and hippocampus (r = 0.11 to r = 0.29). The accumbens volume did not correlate with ICV at all.

The partial correlations between FBV and the subvolume measures are much stronger than the partial correlations obtained with ICV as brain size measure. The strongest partial correlations were obtained for cortical GM and WM volumes (r = 0.91 and r = 0.87, respectively). Strong correlations were also found for cerebellar GM and WM volumes (r = 0.512 and r = 0.61, respectively), the thalamus (r = 0.69), and the hippocampus (r = 0.62). A small correlation was found for the CSF, while all further volumes (CC, caudatus, putamen, pallidum, amygdala, and the accumbens) correlate moderately strong with FBV.

In a further step, we computed partial correlations (with Sex and Age as covariates) between the ICV-proportional volumes (subvolumes related to ICV) and ICV as well as partial correlations between FBV-proportional volumes (subvolumes related to FBV) and FBV. These proportional

measures were computed as % of the particular compartmental volume of ICV or FBV (Fig. 2). As shown in Figure 2, the partial correlations for the ICV-proportional volumes and ICV are all negative and at least moderately strong. This pattern of correlations indicates that with increasing ICV the relative portion of each of the compartmental volume declines. For example, larger ICVs are associated with smaller cortical GM and WM volumes. The same pertains for the subcortical structures. The partial correlations between FBV-proportional volumes and FBV are different. As can be seen from Figure 5 they are much smaller than the correlations between the ICV-proportional volumes and ICV. Most interesting is the moderately strong positive correlation (r = 0.32) between FBV and the FBVproportional volume of cortical WM. Thus, larger brains demonstrate a relatively large proportion of cerebral WM.

Effects of Sex and Age on compartmental volumes (Raw, Adjusted, and Proportional)

In further steps (Steps 2–4 according to the description in the statistical analysis section), we calculated two-way ANOVAs with Age (young vs. old) and Sex (women vs. men) as independent variables separately for all compartmental volumes and proportional volumes followed by the two-way ANCOVAs with ICV and FBV as covariates for

TABLE III. Mean (M) volumes and standard deviations (SD) for the raw compartmental volumes (in ml) broken down for Age and Sex

		you	ung	old					
	won	nen	me	en	won	nen	men		
	M	SD	M	SD	M	SD	M	SD	
ICV	1,435.81	128.84	1,609.51	172.44	1,352.62	131.74	1,521.17	184.72	
FBV	1,094.40	86.14	1,223.99	109.63	979.58	82.07	1,072.50	96.00	
GM_cortex	468.37	40.15	518.55	49.59	391.99	35.81	424.04	37.86	
WM_cortex	434.59	43.48	494.55	52.71	419.46	43.57	465.65	55.57	
GM_cerebellum	102.54	12.52	114.19	16.88	90.28	9.50	100.29	10.01	
WM_cerebellum	28.36	3.62	31.27	5.04	26.53	3.98	28.13	4.47	
CC	3.08	0.43	3.30	0.60	2.75	0.49	2.79	0.43	
CSF	1.12	0.21	1.24	0.26	1.21	0.24	1.42	0.27	
Thalamus	15.18	1.46	16.62	1.70	12.47	1.23	13.53	1.34	
Caudatus	7.61	0.96	8.24	1.12	6.65	0.86	7.11	0.85	
Putamen	11.30	1.37	12.28	1.44	9.21	1.05	9.68	1.18	
Pallidum	3.29	0.55	3.43	0.49	2.79	0.37	2.90	0.41	
Hippocampus	8.58	0.81	9.40	0.94	7.71	0.86	7.97	1.09	
Amygdala	3.29	0.44	3.67	0.49	2.85	0.40	3.09	0.46	
Accumbens	1.27	0.24	1.30	0.24	0.95	0.20	0.98	0.19	

the volume measures to statistically control for brain size differences. Mean raw and adjusted volume measures (adjusted for ICV and FBV) are shown in Figure 3 and Table III (this Table only shows the raw compartmental volumes). Figure 4 depicts the mean proportional volume measures. Figure 5 shows the effect size (Eta 2) values from the ANOVAs and ANCOVAs with the compartmental volumes and proportional volumes as dependent variables and Age, Sex, and the Age \times Sex interaction as independent variables. The Eta 2 values (as % of explained variance)

are categorized and color-coded according to Cohen [1992] into large (>14%), moderate (>6%), and small effects (>1%).

As one can see from these analyses there are strong Age effects for most of the raw volumes. Moderate Age effects were found for cerebellar WM and the CC. The influence of Age on cerebral WM and CSF was small (for a more detailed description of the Age effects see below).

For the raw volumes, there are moderate and small Sex effects for most of the volumes (men > women). The

TABLE IV. Mean (M) volumes and standard deviations (SD) for the compartmental volumes (in ml) broken down for Age

	young		ol	d			
	M	SD	M	SD	% change	% change/year	d
GM_cortex	494.42	48.23	403.57	40.05	18.38	0.43	2.04
WM_cortex	464.11	55.10	439.52	56.61	5.30	0.12	0.44
GM_cerebellum	108.40	15.86	94.43	10.46	12.88	0.30	1.03
WM_cerebellum	29.76	4.53	27.24	4.39	8.49	0.20	0.57
CC	3.18	0.52	2.77	0.47	13.13	0.31	0.84
CSF	1.18	0.25	1.31	0.26	-11.21	-0.26	0.52
Thalamus	15.92	1.66	12.88	1.36	19.09	0.45	1.99
Caudatus	7.91	1.07	6.84	0.89	13.51	0.31	1.08
Putamen	11.78	1.44	9.40	1.15	20.23	0.47	1.82
Pallidum	3.36	0.52	2.84	0.39	15.54	0.36	1.13
Hippocampus	8.98	0.91	7.81	1.02	13.05	0.30	1.21
Amygdala	3.48	0.49	2.95	0.45	15.18	0.35	1.12
Accumbens	1.28	0.24	0.96	0.19	24.84	0.58	1.46

Sex was used as covariate and partialed out. In addition % change % change per year, and Cohen's *d* representing the standardized difference between young and old are shown.

% Change of the volume is calculated as: (young–old) * 100/young. % Change per year is: % Change/42.9 years (42.9 years is the mean age difference between the young and old group). Cohen's d has been calculated using the formula for pooled SDs in the context of unequal sample sizes.

strongest Sex influence was found for cerebral WM (20.3%), while the Sex influence on cortical GM, cerebellar GM, CSF, thalamus, and the amygdala turned out to by moderate. Small Sex effects were found for cerebellar WM, CC, caudatus, putamen, pallidum, and the hippocampus. For the raw volume of the accumbens, there was no Sex effect present at all. The Sex \times Age interaction was actually not present for all volumes. There was only a small interaction for the hippocampus (1.4%), which is qualified by larger hippocampal volumes for younger men than women. There was no Sex difference for the older subjects.

The ANCOVAs with ICV and FBV as covariates revealed an entirely different picture (for a summary of these analyses see Fig. 5). The Sex effects, which have been found for the aforementioned ANOVAs computed for the raw volumes nearly all disappeared in the ANCO-VAs. There are only small Sex effects (men > women) for the volumes of cerebral WM (Eta $^2 = 2.4\%$), cerebellar GM $(Eta^2 = 2.0\%),$ **CSF** $(Eta^2 = 5.0\%),$ and (Eta² = 1.1%). There was only one small Sex \times Age interaction for the hippocampus ($Eta^2 = 1.4\%$), which is qualified by larger hippocampal volumes in younger men compared to younger women. For the group of older subjects, there was no Sex difference for this volume measure. This interaction also appeared for the raw hippocampal volumes (see above). The ANCOVA with FBV as covariate revealed a similar picture. Small Sex effects only remained for the CC (women > men), CSF (men > women), and the accumbens (women > men) with Eta² values ranging between 1 and 3.3%. There was no substantial Sex \times Age interaction. A similar picture emerged for the ANOVAs computed for the proportional measures. The uncovered effect sizes are roughly similar to the effect sizes found for the ANCOVA with ICV as covariate.

Comparing the Eta² values for the Sex effects (using the test suggested by Steiger [1980]) obtained by the ANOVA and ANCOVAs revealed for nearly all volume measures (except for the volume of accumbens, CC, and pallidum) significant differences. Thus, the influence of Sex on the compartmental volumes is substantially smaller or even absent after adjusting for ICV and FBV. The Age effects are strong in the ANOVA as well as in the ANCOVAs, however, for most volume measures the Age effects became slightly smaller in the ANCOVAs (e.g., cortical GM, cerebellar GM and WM, subcortical GM, CC, thalamus, putamen, pallidum, hippocampus, amygdala, and accumbens).

GM/WM Ratios in Relation to Sex and Age

We also examined GM/WM ratios for Sex and Age influences. We calculated the GM/WM on the basis of the cortical GM and WM volumes. These ratios are larger than 1 for the young subjects (women: 1.08, men: 1.05) but smaller than 1 for the older subjects (women: 0.93, men:

0.91) indicating a strong decrease in GM in old subjects. Descriptively the GM/WM ratio is slightly larger in women compared with men for both young and old subjects. A 2 \times 2 ANOVA (Sex and Age as independent variables) revealed a strong Age effect (Eta² = 37.5%) and a small Sex effect (Eta² = 1.2%). The Sex \times Age interaction was marginal (Eta² = 0.04%). Conducting an ANCOVA with FBV as covariate resulted in a massively reduced Sex effect (Eta² = 0.06%). The influence of Age remained as strong as in the ANOVA with the raw ratios (Eta² = 34.5%). The Sex \times Age interaction was still not present (Eta² = 0.001%).

Age and Compartmental Volumes

Table IV shows the adjusted volume measures independent from Sex. As one can see, older subjects demonstrate substantial lower volumes than younger subjects. The mean age difference between young and old subjects is 42.9 years. The volume differences across this age span range from 5.3% (cerebral WM) to 25% (accumbens). Using Cohen's d (which is a more valid measure to demonstrate the change across the life span since the standard deviation is used for normalization) all differences can be ranked as large (except for cortical and cerebellar WM as well as CSF which demonstrate moderate aging changes).

The strong Age effects on the raw volumes are also shown in Figure 5. For the raw compartmental volumes, the Eta² values range between 3.5% (cerebral WM) and 42.7% (cortical GM). However, when adjusting for ICV and FBV, the Age effects became a bit smaller for all compartmental volumes. Adjusting for ICV the Eta² values range between 0.2% (cerebral WM) to 30.7% (putamen). Adjusting for FBV results in an even stronger reduction of the Age influence on the compartmental values ranging from an Eta² of 0.6 (cerebellar GM) to 9.9% (putamen; Fig. 5).

Comparing the $\rm Eta^2$ values for the Age influence obtained for our sample with the $\rm Eta^2$ values obtained for the combined sample reported in Fjell and Walhovd [2010] revealed partly substantial differences. The Age effect adjusted for ICV in the Fjell et al. study is a bit stronger than the same effect in our sample. The mean $\rm Eta^2$ (average $\rm Eta^2$ for the Age effect across all compartmental volumes) in our study is 16% while the same effect is associated with an $\rm Eta^2$ of 30% in the study of Fjell and Walhovd. The strongest difference was obtained for cerebral WM for which we did not find a substantial Age effect at all ($\rm Eta^2 = 0.2\%$) while the Age effect is strong in the Fjell and Walhovd study ($\rm Eta^2 = 32\%$).

DISCUSSION

In the following, we will shortly summarize the findings before we will discuss and relate them to the current literature.

- We asked whether brain size might exert an influence on cortical, subcortical, and cerebellar volume measures. Based on previous studies of our group and other laboratories we hypothesized that brain size is the major factor explaining interindividual variability of the compartmental volume measures. In fact, we identified strong and moderate brain size influences on nearly all compartmental volumes.
- 2. The second study question was: is there a "true" and substantial influence of Sex on the compartmental volumes? As it has been proposed in previous articles, brain size might explain most of the variance of the compartmental volumes and if brain size is controlled for, Sex differences will disappear or become much smaller. Our data suggest that when brain size is controlled for, nearly all sex-specific influences disappear or at least become much smaller.
- 3. The third question relates to the often-reported Sex × Age interaction. Is there a significant and substantial Sex × Age interaction for the compartmental volumes? Our data suggest that there is practically no substantial Sex × Age interaction for all compartmental volume.
- 4. To compare our age-related findings with those of other groups, we are interested to delineate agerelated changes in compartmental volumes independent of Sex. In fact, we identified substantial smaller volumes in older subjects within our sample of healthy subjects.

Influence of Brain Size

As predicted brain size is an important variable explaining large portions of variance for most compartmental volumes. This strong influence of brain size on the compartmental volumes still persists even when Sex and Age were regressed out (Figs. 4 and 5). However, the brain size influence is not equally strong for all compartmental volumes and it also depends on the used brain size measure. The strongest brain size influences were found when using FBV as brain size measure. The correlations of FBV with the compartmental volumes ranged from 0.19 to 0.87. The strongest influences were found for cerebral WM and GM with an r = 0.91 and r = 0.87. Except for CSF, FBV was strongly or at least moderately related to all compartmental volumes. Basal ganglia structures were at least moderately related to FBV, while the hippocampus and the thalamus were strongly related to FBV. When using ICV as brain size measure the correlations are significantly smaller than when using FBV as brain size measure. The correlations ranged 0.08 to 0.64 with cortical GM and WM as the brain structures most strongly related to ICV. The reason why FBV correlates stronger with the compartmental volumes than ICV is most likely related to the fact that ICV is a volume measure comprising beside brain tissue volumes also meninges, CSF, and other non-brain-tissue-related parts. Thus, there is more "noise" which reduces the correlations. FBV on the other hand is the brain tissue without CSF, meninges, skull, and other parts and reflects the brain tissue which is involved in neurophysiological processing and which is most likely more relevant in the context of studies interested to disentangle structure–function relationships [Luders et al., 2010]. ICV might provide a more global and stable frame for the development of brain tissue, which is genetically determined while the brain tissue is subject to change due to plasticity, disease, or aging [Jancke, 2009; May, 2011; Zatorre et al., 2012].

Irrespective of the used brain size measure our results confirm earlier studies by showing that brain size is a major source of variability of compartmental volumes [Jäncke et al., 1997, 1999; Leonard et al., 2008; Lüders et al., 2002, 2014; O'Brien et al., 2011; Tang et al., 2013]. Of particular interest here is the finding that brain size is also important for explaining volume variability for some subcortical structures although not to the same extend as found for the cortical volumes. Thus, our results are partly in agreement with a recent study using much smaller and selected samples, for which the data are freely available to the scientific community [Tang et al., 2013]. The authors of this study found brain size influences on the amygdala, caudate, and hippocampus using a similar approach as used in the present study. However, they used two datasets from different laboratories. For both datasets, they found brain size influences for the right amygdala and for the right and left caudate. For the hippocampus, they only identified a brain size influence on the right hippocampus for dataset 2. Although the findings of our study and the study from Tang et al. are fairly similar there is a difference with respect to the hippocampus. Why they identified only a brain size influence on hippocampal volume in one dataset is unclear. The Tang et al. study used statistical parametric mapping software for analyzing global brain volumes and FMRIB software library's segmentation toolkit FIRST to calculate the compartmental volumes, while we have used the FreeSurfer software suite to compute all volumes. That the used software might introduce additional noise to the data is not that unlikely especially in the case of noisy MRI data [Gronenschild et al., 2012; Klauschen et al., 2009]. Whether this might be the reason for the partly discrepant findings has to be examined in future studies.

A further interesting finding in this context is that larger and smaller brains differ in terms of their proportional volumes (Fig. 2). The compartmental volumes related to ICV correlated moderately strongly and negatively with ICV. Thus, the larger the brain (in terms of ICV) the smaller is the ICV-proportion of the compartmental volumes (independent from Sex and Age). This holds for all volume measures used in this study even for the subcortical volumes as well as for the CC and CSF. In other words, larger brains demonstrate smaller ICV-proportional compartmental volumes. As aforementioned in the methods section,

ICV is more a head size than brain size measure since it comprises brain tissue, skull, meninges, CSF, and interstitial volumes. Thus, it comes as no surprise that the correlation between ICV and FBV is not perfect (r = 0.765, $r^2 = 58\%$ in our sample) as there is much additional variance in ICV, which is unrelated to brain tissue. Although the underlying reasons for this relation are far from being understood it is obvious that small brains in terms of ICV exploit the space within the skull more efficiently than larger brains. Conversely, there is overproportionally more space available for brain tissue in large brains.

Correlating the FBV-proportional measures with FBV revealed a partly different picture. Most of these correlations are negative indicating that with increasing brain tissue volume the relative proportion of the compartmental volumes decrease. However, these correlations are much smaller than the correlations between ICV-proportional measures and ICV. Most interestingly, the correlation between the FBV-proportional cerebral WM volume with FBV is positive and moderately strong. Thus, the larger the brain tissue volume the larger is the FBV-proportional cerebral WM volume. Interestingly, nearly similar correlations have been reported in two previous studies using the same measures [Leonard et al., 2008; Lüders et al., 2002].

But what do these correlations between proportional compartmental volumes and ICV or FBV indicate? In our opinion, these correlations indicate a general allometric principle according to which GM and WM compartmental volumes are related to brain size. With larger brain tissue volume there are larger distances to bridge between neuronal processing units, which requires a very efficient intrahemispheric and interhemispheric fiber tract system. To keep information transfer across larger distances in comparable time ranges as in smaller brains the fiber tract diameter has to be increased, which in turn will cause an increase in WM volume. Thus, the larger the brain tissue (FBV) the more WM volume is needed to keep information transfer speed in acceptable ranges resulting in positive correlations between FBV-proportional cerebral WM and FBV. Thus, larger brains (independent from Sex and Age) demonstrate larger WM but smaller GM volumes resulting in a changed GM/WM ratio with large brains showing smaller GM/WM ratios than smaller brains. The same relationship has been reported by Luders et al. [2002] and Leonard et al. [2008]. Thus, there is obviously a consistent relationship between GM as well as WM proportion and brain volume across different studies using different samples and different methods. As Leonard et al. [2008] already mentioned this relation suggests "that the optimal relation between neurons, glia, and axons depend more on surface area and speed of transmission."

Sex Differences

When assuming that brain size is a very important factor influencing the absolute and proportional compartmen-

tal volumes one has to question whether Sex might have an additional influence on compartmental volumes. In this context sex hormones, sex-specific or sex-unrelated genetic influences, nutritional status, as well as use-dependent influences are proposed, which might also influence brain volume measures. Among these factors Sex has often been suggested as one of the major driving forces in brain development [Geschwind and Galaburda, 1985]. A recent meta-analysis on sex differences in brain morphology confirmed that men on average have larger brain volumes than women [Ruigrok et al., 2014]. In addition, there have been regional sex differences with respect to the volumes of the amygdala, hippocampus, and insula. The authors of this meta-analysis argue that these sex-specific anatomical differences might be related somehow to "sex-biased neuropsychiatric conditions." However, the data they present in their analysis are mostly not corrected for brain size measures, thus it is not clear whether the reported sexdifferences are due to "true" sex differences or are influenced or even entirely determined by brain size differences. We, therefore, used brain size (ICV and FBV) as covariate in our ANCOVAs and also computed proportional volume measures (volumes related to ICV and FBV) and identified only some small sex-specific effects on the compartmental volumes. For example, the volumes of cerebral WM, cerebellar GM, CSF, and amygdala are marginally influenced by Sex. The variance explained by Sex as measured with the Eta² measure ranged for the aforementioned compartmental volumes between 1 and 5% when ICV was used as covariate and 1-3.3% when FBV was used as covariate, which are pretty low effect sizes compared to the explained variance by brain size and Age (see below). Similarly, the influence of Sex on the compartmental volumes is small when calculating partial correlations with Age and ICV as well as FBV as covariates (ranging between 0 and 5%). Thus, taken together, Sex represents only a weak predictor of compartmental volumes a finding which is in full agreement with the studies of Fjell et al. [2010] and Lemaitre et al. [2005], which also identified no or only minute Sex effects on volume measures when these measures are controlled for brain size differences. Thus, brain size is more important than Sex in explaining interindividual differences with respect to the volume measures.

Smaller brains (independent of Age and Sex) tended to have larger proportions of GM but smaller proportions of WM as demonstrated by the GM/WM ratios. Thus, it is unlikely that sex hormones or sex-specific genetic influences are that important in determining or modulating the compartmental volumes. Although our results are in full agreement with previous reports of our group and other laboratories [Fjell et al., 2009b; Jancke et al., 1997; Leonard et al., 2008; Lüders et al., 2002] they are at the same time in disagreement with a couple of studies, which claimed to find Sex differences [Coffey et al., 1998; Gur et al., 1999] even when the volume measures are related to brain volume [Li et al., 2014]. For example Li et al. [2014] reported

a main effect of Sex on the hippocampal volume with larger volumes in women than in men. They calculated proportional compartmental volumes by relating these volumes to FBV. The reported effect size values for this proportional measure is in the light of this study extraordinary strong (Eta² = 30% and Cohen's d = 1). However, the Li et al. study [2014] used a relatively small sample from the International Consortium for Brain Mapping, which has been made publicly available. Thus, it could be possible that this is simply a random result due to the small sample size. In addition, it could also be possible that the applied method for brain size correction might have caused the diverging results since Li et al. applied proportional measures to adjust for individual differences in brain size.

Using the raw and uncorrected GM/WM ratios we confirmed that women demonstrate slightly larger GM/WM ratios than men [Gur et al., 1999; Lemaître et al., 2005]. However, this Sex difference disappeared when the GM/WM ratio was adjusted for brain size. There was a substantial drop of this measure from young to old subjects in our sample (Eta 2 = 35%). This strong reduction in GM/WM ratio was also apparent when brain size was used as covariate.

Sex differences in brain volume are frequently related to sex differences in body height [e.g., Raz et al., 1997, 2004]. However, several studies have shown that there is no strong relation between body height and brain volume [e.g., Peters et al., 1998]. For example, Witelson et al. [2006] found that only 1-4% of the variance in brain weight could be attributed to differences in body height in postmortem analyzed brains. Other reports have found a significant correlation between height and volume in women but not men [Nopoulos et al., 2000; Peters et al., 1998] or in men but not women [Heymsfield et al., 2007; Koh et al., 2005]. In addition, body size decreases across the age with age explaining 86-87% of the variation in the rate at which body height was lost in older age [Sorkin et al., 1999]. There are in addition, substantial sex differences with respect to this age-related body size loss. Beyond the age of 45 years, women lose body size faster than men [see, for example, Fig. 4 in Sorkin et al., 1999]. Thus, using body size to normalize brain size measures would have let to an overestimation of these brain size measures for the older brains and it would also have introduced a further sex difference, which would have made it difficult to explain the anatomical data. Based on the theoretical inconsistencies raised in a our earlier article [Peters et al., 1998], the theoretical suggestions proposed by Striedter [2005], and the aforementioned age-related body size loss, we refrain from using body size as normalization variable.

Influence of Age and Sex × Age interactions

As expected, we observed strong influences of Age on nearly all raw compartmental volumes. Weak Age influences were found for cerebral WM and CSF. The Age effects decreased substantially when adjusting for brain size although they are still present. Using ICV as brain size measure the Age influence expressed as mean Eta² dropped from 22% (averaged effect size) for the raw volumes to 15% for the adjusted volumes. Using FBV the Age influences even dropped much stronger down to 4%. Nevertheless cortical GM, thalamus, putamen, and accumbens are the structures, which are most strongly influenced by age. Weak Age influences were found for cerebellar and cerebral WM, CSF, and the CC. The age effects are fairly in the same range as reported in previous studies with a % volume reduction per year ranging from 0.12% to 0.47% [Fjell and Walhovd, 2010; Walhovd et al., 2011; Ziegler et al., 2012]. However, there are also substantial differences with respect to the Age effect between our sample and the sample reported in the Fjell and Walhovd [2010] article. The Age effects reported in our study (Eta 2 = 16%) are substantially smaller than the Age effects reported in the study of Fjell and Walhovd ($Eta^2 = 30\%$). The reason for this substantial difference is difficult to explain since both studies have used the same method to delineate and compute the compartmental volumes. We only can speculate that differences in the sample characteristics might be the reason for these differences. First, the age distribution in our sample is more bimodal with two groups (young and old) of subjects while the age distribution of the sample used by Fjell and Walhovd [Fjell and Walhovd, 2010] is more equally distributed. It might also be possible that the old subjects in our sample are a bit more cognitively active than the subjects included in the Fjell and Walhovd sample since our subjects are enrolled in a longitudinal research project during which they participate over a period of 5 years at repeated psychological testing. However, which of these factors are responsible for these discrepancies has to be elucidated in future experiments.

More important in the context of this article is that we did not find any strong or even moderate Age × Sex interaction neither for the raw nor for the adjusted volumes. There was only one small Age × Sex interaction found for the hippocampus adjusted for ICV ($Eta^2 = 1.4\%$). Thus, our findings are in agreement with Lemaitre et al. [Fjell et al., 2009b; Lemaître et al., 2005; Resnick et al., 2000, 2003], but in contradiction with the common idea that male brains are more vulnerable to aging [Coffey et al., 1998]. In a sample of elderly aged from 66 to 96 years, these authors reported an increase of sulcal CSF volume in men only. In a recent study by Taki et al. [2011b] the authors reported Age × Sex interactions for cortical GM and WM as well as CSF. Although these interactions turned out to be significant, the effect sizes are relatively small and ranged from 4% to 5.4%. Due to the large sample size used in the Taki et al. study (n = 1460) even these relatively small effect sizes became statistically significant. However, discrepancies between these studies in terms of Sex × Age interactions for cortical and subcortical volumes could arise from differences in terms of educational levels, hypertension, or

nutrition status. Since we do not have that information available for all subjects of our sample (these parameters are only available for the elderly participants) we cannot explicitly examine whether these aspects might have changed our results. However, Lemaitre et al. reported that even after controlling for educational level and hypertension did not change their results and there was still no $\text{Sex} \times \text{Age}$ interaction.

Corpus Callosum

Some special notes are mandatory for the CC. The CC is a brain structure, which has frequently been studied in the context of questions related to aging, addiction, intelligence, hemispheric asymmetry, and sex differences [Sullivan et al., 2010]. As Christine Leonard and her colleagues [2008] pointed out "the corpus callosum probably holds the record for reported sex differences." One of the reasons that this brain area has received that amount of attention is partly due to the fact that it is relatively easy to measure in MRI and postmortem brains. As in our study nearly all studies have found that the CC increases with brain size in both sexes. Some authors, however, have claimed that women would have CCs that are larger than expected for brain size [Allen et al., 1991; Davatzikos and Resnick, 1998; DeLacoste-Utamsing and Holloway, 1982; Steinmetz et al., 1992; Witelson, 1989]. Thus, women would show a kind of overproportional betweenhemisphere connectivity. The popular press has used this claim to promote the idea of female superiority in multitasking. This advantage in multitasking is thought to depend on the better and more efficient interhemispheric wiring via the CC in women, which should be indicated by larger mid-sagittal CC areas or larger CC volumes. This idea has received some support in a recent article using DTI and graph-theoretical analyses. On the basis of this analysis, the authors identified that males had greater within-hemispheric connectivity whereas betweenhemispheric connectivity was superior in females [Ingalhalikar et al., 2014]. However, a major problem of this article is that the analyses of Sex differences were not controlled for brain size. The authors did not take into account that only 15-20% of women have brains of the same size as the average male brain. Conversely approximately the same amount of men show brains of the same size as the average female brain. Thus, it is most likely that when controlling for brain size Sex differences will disappear or at least substantially shrink. With respect to morphological CC measures it has repeatedly been demonstrated that when brain size is controlled for any Sex difference in CC size or volume disappear or decrease substantially [Jancke et al., 1997; Leonard et al., 2008]. In our study, there was no Sex influence on CC, there was however a small to moderate strong brain size and Age influence on CC volume. The larger the brain the larger is the CC volume and the older the brain the smaller is the CC volume. Thus, these findings correspond to many studies, which have focused on CC morphology [Sullivan et al., 2010].

Limitations

This study has some limitations that are worth mentioning. First, this is a cross-sectional study comparing young and old subjects differing in age on average 43 years. Thus, cohort effects could possibly have influenced morphological features. The nutritional status, education, health, and social interactions have substantially changed within the last 40-60 years. Meanwhile there is ample evidence available demonstrating that the aforementioned factors influence brain anatomy [de Bruin et al., 2005; He et al., in press; Pannacciulli et al., 2006; Taki et al., 2006, 2004). Thus, we cannot exclude that our results are at least to some degree influenced by these factors. Longitudinal studies would be helpful to control for these cohort effects. However, it will be nearly impossible to conduct a longitudinal MRI study of that size covering the age range of our study. Second, the subjects of our sample are not hired on a random basis. They were rather recruited by public announcements or in the context of other studies. Thus, there may be some selection biases because only those subjects will participate in these studies that are really interested in the study topic and who are healthy enough, mobile, and motivated to participate. Third, we cannot rule out the possibility of misclassification during tissue segmentation, such as the classification of WM hyperintensities as GM. WM hyperintensities are quite common in older subjects [Meyer et al., 1992], thus misclassifications could lead to an overestimation of GM volume resulting in an diminished age-related decline in GM volume. Fourth, the age distribution in our sample does not allow to study the age-related trajectories of volume loss using polynomial regression analyses or to categorize age in more than two groups. For example, in the Ziegler et al. study [Ziegler et al., 2012] the authors have used seven age categories with 55 to 123 subjects per age category with the exception of the oldest group (older than 80 years) only containing eight subjects. The sample we are using for this article contains too few subjects in the middle ages compared to the large number of subjects being older than 65 and younger than 45. Thus, we decided to split our group into two groups (young vs. old). Despite these limitations, we believe that the fully automated segmentation of the MR images is actually a strength in dealing with the large quantity of data in an objective, reproducible, and efficient way.

Conclusions

In a large sample of young and old subjects, we examined the influence of brain size on cortical, cerebellar, and subcortical compartmental volumes. In addition, we examined whether Sex differences and Sex \times Age

interactions for compartmental volumes shrink or even disappear when controlling for brain size. In fact, we identified that brain size exerts strong statistical influences on nearly all cortical, cerebellar, and subcortical compartmental volumes. Controlling for brain size resulted practically in an elimination (or at least substantial decrease) of Sex effects on compartmental volumes. Thus, brain size is obviously more important than Sex in determining interindividual differences in cerebral volumes. In addition, the relative proportion of the compartmental volumes varies with brain size. Larger brains are associated with relatively small compartmental volumes than smaller brains. Most interestingly, larger brains are associated with larger relative cerebral WM volumes reflecting the need for efficient information propagation in larger brains. We also identified no substantial Sex × Age interactions for the compartmental volumes. Finally, Age turned out (as expected) to be strongly related with nearly all compartmental volumes. Older brains demonstrate smaller volumes for nearly all compartmental volumes compared to young subjects. CSF increased substantially in older brains reflecting the brain tissue loss associated with aging.

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REFERENCES

- Allen JS, Bruss J, Brown CK, Damasio H (2005): Normal neuroanatomical variation due to age: The major lobes and a parcellation of the temporal region. Neurobiol Aging 26:1245–1282.
- Allen LS, Richey MF, Chai YM, Gorski RA (1991): Sex differences in the corpus callosum of the living human being. J Neurosci 11:933–942.
- Annett M (1970): A classification of hand preference by association analysis. Br J Psychol 61:303–321.
- Baur V, Hänggi J, Langer N, Jäncke L (2013): Resting-state functional and structural connectivity within an insula-amygdala route specifically index state and trait anxiety. Biol Psychiatry 73:85–92.
- Bishop KM, Wahlsten D (1997): Sex differences in the human corpus callosum: myth or reality? Neurosci Biobehav Rev 21:581–601.
- Bourque M, Dluzen DE, Di Paolo T (2009): Neuroprotective actions of sex steroids in Parkinson's disease. Front Neuroendocrinol 30:142–157.

- Bruhl AB, Hanggi J, Baur V, Rufer M, Delsignore A, Weidt S, Jancke L, Herwig U (2013): Increased cortical thickness in a frontoparietal network in social anxiety disorder. Hum Brain Mapp 35:2966–2977.
- Cahill L, Babinsky R, Markowitsch HJ, McGaugh JL (1995): The amygdala and emotional memory. Nature 377:295–296.
- Chung SC, Tack GR, Yi JH, Lee B, Choi MH, Lee BY, Lee SY (2006): Effects of gender, age, and body parameters on the ventricular volume of Korean people. Neurosci Lett 395:155–158.
- Coffey CE, Lucke JF, Saxton JA, Ratcliff G, Unitas LJ, Billig B, Bryan RN (1998): Sex differences in brain aging: a quantitative magnetic resonance imaging study. Arch Neurol 55:169–179.
- Cohen J (1992): A power primer. Psychol Bull 112:155-159.
- Colom R, Stein JL, Rajagopalan P, Martínez K, Hermel D, Wang Y, Álvarez-Linera J, Burgaleta M, Quiroga MÁ, Shih PC, Thompson PM (2013): Hippocampal structure and human cognition: Key role of spatial processing and evidence supporting the efficiency hypothesis in females. Intelligence 41(2):129–140.
- Courchesne E, Chisum HJ, Townsend J, Cowles A, Covington J, Egaas B, Harwood M, Hinds S, Press GA (2000): Normal brain development and aging: Quantitative analysis at in vivo MR imaging in healthy volunteers. Radiology 216:672–682.
- Cowell PE, Turetsky BI, Gur RC, Grossman RI, Shtasel DL, Gur RE (1994): Sex differences in aging of the human frontal and temporal lobes. J Neurosci 14:4748–4755.
- Davatzikos C, Resnick SM (1998): Sex differences in anatomic measures of interhemispheric connectivity: Correlations with cognition in women but not men. Cereb Cortex 8:635–640.
- de Bruin EA, Hulshoff Pol HE, Bijl S, Schnack HG, Fluitman S, Bocker KB, Kenemans JL, Kahn RS, Verbaten MN (2005): Associations between alcohol intake and brain volumes in male and female moderate drinkers. Alcohol Clin Exp Res 29:656– 663
- DeLacoste-Utamsing C, Holloway RL (1982): Sexual dimorphism in the human corpus callosum. Science 216:1431–1432.
- Driscoll I, Davatzikos C, An Y, Wu X, Shen D, Kraut M, Resnick SM (2009): Longitudinal pattern of regional brain volume change differentiates normal aging from MCI. Neurology 72: 1906–1913.
- Drobetz R, Hänggi J, Maercker A, Kaufmann K, Jäncke L, Forstmeier S (2014): Structural brain correlates of delay of gratification in the elderly. Behav Neurosci 128:134–145.
- Du AT, Schuff N, Chao LL, Kornak J, Jagust WJ, Kramer JH, Reed BR, Miller BL, Norman D, Chui HC, Weiner MW (2006): Age effects on atrophy rates of entorhinal cortex and hippocampus. Neurobiol Aging 27:733–740.
- Elmer S, Hanggi J, Meyer M, Jancke L (2011): Differential language expertise related to white matter architecture in regions subserving sensory-motor coupling, articulation, and interhemispheric transfer. Hum Brain Mapp 32:2064–2074.
- Elmer S, Hänggi J, Meyer M, Jäncke L (2013): Increased cortical surface area of the left planum temporale in musicians facilitates the categorization of phonetic and temporal speech sounds. Cortex 49:2812–2821.
- Filipek PA, Richelme C, Kennedy DN, Caviness VS (1994): The young adult human brain: An MRI-based morphometric analysis. Cereb Cortex 4:344–360.
- Fischl B, Dale AM (2000): Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci USA 97:11050–11055.
- Fischl B, Liu A, Dale AM (2001): Automated manifold surgery: constructing geometrically accurate and topologically correct

- models of the human cerebral cortex. IEEE Trans Med Imaging 20:70–80.
- Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002): Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355.
- Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM (2004): Automatically parcellating the human cerebral cortex. Cereb Cortex 14: 11–22.
- Fjell AM, Walhovd KB (2010): Structural brain changes in aging: Courses, causes and cognitive consequences. Rev Neurosci 21: 187–221.
- Fjell AM, Westlye LT, Amlien I, Espeseth T, Reinvang I, Raz N, Agartz I, Salat DH, Greve DN, Fischl B, Dale AM, Walhovd KB (2009a): High consistency of regional cortical thinning in aging across multiple samples. Cereb Cortex 19:2001–2012.
- Fjell AM, Westlye LT, Amlien I, Espeseth T, Reinvang I, Raz N, Agartz I, Salat DH, Greve DN, Fischl B, Dale AM, Walhovd KB (2009b): Minute effects of sex on the aging brain: a multisample magnetic resonance imaging study of healthy aging and Alzheimer's disease. J Neurosci 29:8774–8783.
- Folstein MF, Robins LN, Helzer JE (1983): The Mini-Mental State Examination. Arch Gen Psychiatry 40:812.
- Fotenos AF, Snyder AZ, Girton LE, Morris JC, Buckner RL (2005): Normative estimates of cross-sectional and longitudinal brain volume decline in aging and AD. Neurology 64:1032–1039.
- Francis AN, Bhojraj TS, Prasad KM, Kulkarni S, Montrose DM, Eack SM, Keshavan MS (2011): Abnormalities of the corpus callosum in non-psychotic high-risk offspring of schizophrenia patients. Psychiatry Res 191:9–15.
- Gershon J (2002): A meta-analytic review of gender differences in ADHD. J Atten Disord 5:143–154.
- Geschwind N, Galaburda AM (1985): Cerebral lateralization. Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. Arch Neurol 42:428–459.
- Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS (2001a): A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14:21– 36.
- Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS (2001b): A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14:21– 36.
- Greenberg DL, Messer DF, Payne ME, Macfall JR, Provenzale JM, Steffens DC, Krishnan RR (2008): Aging, gender, and the elderly adult brain: an examination of analytical strategies. Neurobiol Aging 29:290–302.
- Grieve SM, Clark CR, Williams LM, Peduto AJ, Gordon E (2005): Preservation of limbic and paralimbic structures in aging. Hum Brain Mapp 25:391–401.
- Gronenschild EHBM, Habets P, Jacobs HIL, Mengelers R, Rozendaal N, van Os J, Marcelis M (2012): The effects of Free-Surfer version, workstation type, and Macintosh operating system version on anatomical volume and cortical thickness measurements. PLoS One 7:e38234.
- Gur RC, Gunning-Dixon FM, Turetsky BI, Bilker WB, Gur RE (2002a): Brain region and sex differences in age association with brain volume: A quantitative MRI study of healthy young adults. Am J Geriatr Psychiatry10:72–80.

- Gur RC, Gunning-Dixon FM, Turetsky BI, Bilker WB, Gur RE (2002b): Brain region and sex differences in age association with brain volume: A quantitative MRI study of healthy young adults. Am J Geriatr Psychiatry 10:72–80.
- Gur RC, Turetsky BI, Matsui M, Yan M, Bilker W, Hughett P, Gur RE (1999): Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. J Neurosci 19:4065–4072.
- Hanggi J, Koeneke S, Bezzola L, Jancke L (2010): Structural neuroplasticity in the sensorimotor network of professional female ballet dancers. Hum Brain Mapp 31:1196–1206.
- He Q, Chen C, Dong Q, Xue G, Chen C, Lu ZL, Bechara A: Gray and white matter structures in the midcingulate cortex region contribute to body mass index in Chinese young adults (in press).
- Heymsfield SB, Gallagher D, Mayer L, Beetsch J, Pietrobelli A (2007): Scaling of human body composition to stature: New insights into body mass index. Am J Clin Nutr 86:82–91.
- Holm S (1979): A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70.
- Imfeld A, Oechslin MS, Meyer M, Loenneker T, Jancke L (2009): White matter plasticity in the corticospinal tract of musicians: a diffusion tensor imaging study. Neuroimage 46:600–607.
- Ingalhalikar M, Smith A, Parker D, Satterthwaite TD, Elliott MA, Ruparel K, Hakonarson H, Gur RE, Gur RC, Verma R (2014): Sex differences in the structural connectome of the human brain. Proc Natl Acad Sci USA 111:823–828.
- Jancke L (2009): The plastic human brain. Restor Neurol Neurosci 27:521–538.
- Jancke L, Beeli G, Eulig C, Hanggi J (2009): The neuroanatomy of grapheme-color synesthesia. Eur J Neurosci 29:1287–1293.
- Jäncke L, Preis S, Steinmetz H (1999): The relation between forebrain volume and midsagittal size of the corpus callosum in children. Neuroreport 10:2981–2985.
- Jancke L, Staiger JF, Schlaug G, Huang Y, Steinmetz H (1997): The relationship between corpus callosum size and forebrain volume. Cereb Cortex 7:48–56.
- Jernigan TL, Archibald SL, Fennema-Notestine C, Gamst AC, Stout JC, Bonner J, Hesselink JR (2001): Effects of age on tissues and regions of the cerebrum and cerebellum. Neurobiol Aging 22:581–594.
- Khan IN. 2012. Sex differences and the effects of sex hormones on the structure of the corpus callosum, Ph.D. thesis. Nottingham: University of Nottingham.
- Klauschen F, Goldman A, Barra V, Meyer-Lindenberg A, Lundervold A (2009): Evaluation of automated brain MR image segmentation and volumetry methods. Hum Brain Mapp 30:1310–1327.
- Koh I, Lee MS, Lee NJ, Park KW, Kim KH, Kim H, Rhyu IJ (2005): Body size effect on brain volume in Korean youth. Neuroreport 16:2029–2032.
- Langer N, Hanggi J, Muller NA, Simmen HP, Jancke L (2012): Effects of limb immobilization on brain plasticity. Neurology 78:182–188.
- Lemaître H, Crivello F, Grassiot B, Alpérovitch A, Tzourio C, Mazoyer B (2005): Age- and sex-related effects on the neuroanatomy of healthy elderly. Neuroimage 26:900–911.
- Leonard CM, Towler S, Welcome S, Halderman LK, Otto R, Eckert MA, Chiarello C (2008): Size matters: cerebral volume influences sex differences in neuroanatomy. Cereb Cortex 18: 2920–2931.

- Levine TR, Hullett CR (2002): Eta squared, partial eta squared, and misreporting of effect size in communication research. Hum Commun Res 28:612–625.
- Li W, van Tol M-J, Li M, Miao W, Jiao Y, Heinze H-J, Bogerts B, He H, Walter M (2014): Regional specificity of sex effects on subcortical volumes across the lifespan in healthy aging. Hum Brain Mapp 35:238–247.
- Luders E, Gaser C, Narr KL, Toga A (2009): Why sex matters: Brain size independent differences in gray matter distributions between men and women. J Neurosci 29:14265–14270.
- Luders E, Narr KL, Thompson PM, Toga AW (2010): Neuroanatomical correlates of intelligence. Intelligence 37:156–163.
- Lüders E, Steinmetz H, Jäncke L (2002): Brain size and grey matter volume in the healthy human brain. Neuroreport 13:2371–2374.
- Luders E, Toga AW, Thompson PM (2014): Why size matters: differences in brain volume account for apparent sex differences in callosal anatomy: The sexual dimorphism of the corpus callosum. Neuroimage 84:820–824.
- May A (2011): Experience-dependent structural plasticity in the adult human brain. Trends Cogn Sci 15:475–482.
- Meyer JS, Kawamura J, Terayama Y (1992): White matter lesions in the elderly. J Neurol Sci 110:1–7.
- Meyer M, Liem F, Hirsiger S, Jancke L, Hanggi J: Cortical surface area and cortical thickness demonstrate differential structural asymmetry in auditory-related areas of the human cortex (in press).
- Murphy DG, DeCarli C, McIntosh AR, Daly E, Mentis MJ, Pietrini P, Szczepanik J, Schapiro MB, Grady CL, Horwitz B, Rapoport SI (1996): Sex differences in human brain morphometry and metabolism: An in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 53:585–594.
- Nopoulos P, Flaum M, O'Leary D, Andreasen NC (2000): Sexual dimorphism in the human brain: Evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. Psychiatry Res 98:1–13.
- Nunnemann S, Wohlschlager AM, Ilg R, Gaser C, Etgen T, Conrad B, Zimmer C, Muhlau M (2009): Accelerated aging of the putamen in men but not in women. Neurobiol Aging 30: 147–151.
- O'Brien LM, Ziegler Da, Deutsch CK, Frazier Ja, Herbert MR, Locascio JJ (2011): Statistical adjustments for brain size in volumetric neuroimaging studies: Some practical implications in methods. Psychiatry Res 193:113–122.
- Olejnik S, Algina J (2003): Generalized eta and omega squared statistics: Measures of effect size for some common research designs. Psychol Methods 8:434–447.
- Pannacciulli N, Del Parigi A, Chen K, Le DS, Reiman EM, Tataranni PA (2006): Brain abnormalities in human obesity: A voxel-based morphometric study. Neuroimage 31:1419–1425.
- Peters M, Jäncke L, Staiger JF, Schlaug G, Huang Y, Steinmetz H (1998): Unsolved problems in comparing brain sizes in Homo sapiens. Brain Cogn 37:254–285.
- Pfefferbaum A, Mathalon DH, Sullivan EV, Rawles JM, Zipursky RB, Lim KO (1994): A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. Arch Neurol 51:874–887.
- Postuma RB, Dagher A (2006): Basal ganglia functional connectivity based on a meta-analysis of 126 positron emission tomography and functional magnetic resonance imaging publications. Cereb Cortex 16:1508–1521.

- Pruessner JC, Collins DL, Pruessner M, Evans AC (2001): Age and gender predict volume decline in the anterior and posterior hippocampus in early adulthood. J Neurosci 21:194–200.
- Qiu A, Crocetti D, Adler M, Mahone EM, Denckla MB, Miller MI, Mostofsky SH (2009): Basal ganglia volume and shape in children with attention deficit hyperactivity disorder. Am J Psychiatry 166:74–82.
- Raz N, Gunning FM, Head D, Dupuis JH, McQuain J, Briggs SD, Loken WJ, Thornton AE, Acker JD (1997): Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. Cereb Cortex 7:268– 282
- Raz N, Gunning-Dixon F, Head D, Rodrigue KM, Williamson A, Acker JD (2004): Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: Replicability of regional differences in volume. Neurobiol Aging 25:377–396.
- Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, Dahle C, Gerstorf D, Acker JD (2005): Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. Cereb Cortex 15:1676–1689.
- Resnick SM, Goldszal aF, Davatzikos C, Golski S, Kraut Ma, Metter EJ, Bryan RN, Zonderman aB (2000): One-year age changes in MRI brain volumes in older adults. Cereb Cortex 10:464–472.
- Resnick SM, Pham DL, Kraut MA, Zonderman AB, Davatzikos C (2003): Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. J Neurosci 23:3295–3301.
- Richardson JTE (2011): Eta squared and partial eta squared as measures of effect size in educational research. Rev Educ Res 6:135–147.
- Riello R, Sabattoli F, Beltramello A, Bonetti M, Bono G, Falini A, Magnani G, Minonzio G, Piovan E, Alaimo G, Ettori M, Galluzzi S, Locatelli E, Noiszewska M, Testa C, Frisoni GB (2005): Brain volumes in healthy adults aged 40 years and over: A voxel-based morphometry study. Aging Clin Exp Res 17:329–336.
- Rijpkema M, Everaerd D, van der Pol C, Franke B, Tendolkar I, Fernández G (2012): Normal sexual dimorphism in the human basal ganglia. Hum Brain Mapp 33:1246–1252.
- Ruigrok ANV, Salimi-Khorshidi G, Lai M-C, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J (2014): A meta-analysis of sex differences in human brain structure. Neurosci Biobehav Rev 39:34–50.
- Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RS, Busa E, Morris JC, Dale AM, Fischl B (2004): Thinning of the cerebral cortex in aging. Cereb Cortex 14:721–730.
- Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN, Fox NC (2003): A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. Arch Neurol 60:989–994.
- Schlumpf YR, Nijenhuis ERS, Chalavi S, Weder EV, Zimmermann E, Luechinger R, La Marca R, Reinders aS, Jäncke L (2013): Dissociative part-dependent biopsychosocial reactions to backward masked angry and neutral faces: An fMRI study of dissociative identity disorder. Neuroimage Clin 3:54–64.
- Smith CD, Chebrolu H, Wekstein DR, Schmitt FA, Markesbery WR (2007): Age and gender effects on human brain anatomy: A voxel-based morphometric study in healthy elderly. Neurobiol Aging 28:1075–1087.
- Sorkin JD, Muller DC, Andres R (1999): Longitudinal change in height of men and women: Implications for interpretation of

- the body mass index: The Baltimore longitudinal study of aging. Am J Epidemiol 150(9):969–977.
- Sowell ER, Peterson BS, Kan E, Woods RP, Yoshii J, Bansal R, Xu D, Zhu H, Thompson PM, Toga AW (2007): Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age. Cereb Cortex 17:1550–1560.
- Steiger JH (1980): Test for comparing elements of a correlation matrix. Psychol Bull 87:245–251.
- Steinmetz H, Jäncke L, Kleinschmidt A, Schlaug G, Volkmann J, Huang Y (1992): Sex but no hand difference in the isthmus of the corpus callosum. Neurology 42:749–752.
- Striedter G. 2005. Principles of Brain Evolution. Sunderland, MA: Sinauer.
- Sullivan EV, Rohlfing T, Pfefferbaum A (2010): Longitudinal study of callosal microstructure in the normal adult aging brain using quantitative DTI fiber tracking. Dev Neuropsychol 35: 233–256.
- Sullivan EV, Rosenbloom M, Serventi KL, Pfefferbaum A (2004): Effects of age and sex on volumes of the thalamus, pons, and cortex. Neurobiol Aging 25:185–192.
- Taki Y, Goto R, Evans A, Zijdenbos A, Neelin P, Lerch J, Sato K, Ono S, Kinomura S, Nakagawa M, Sugiura M, Watanabe J, Kawashima R, Fukuda H (2004): Voxel-based morphometry of human brain with age and cerebrovascular risk factors. Neurobiol Aging 25:455–463.
- Taki Y, Kinomura S, Sato K, Goto R, Inoue K, Okada K, Ono S, Kawashima R, Fukuda H (2006): Both global gray matter volume and regional gray matter volume negatively correlate with lifetime alcohol intake in non-alcohol-dependent Japanese men: a volumetric analysis and a voxel-based morphometry. Alcohol Clin Exp Res 30:1045–1050.
- Taki Y, Kinomura S, Sato K, Goto R, Kawashima R, Fukuda H (2011a): A longitudinal study of gray matter volume decline with age and modifying factors. Neurobiol Aging 32:907–915.
- Taki Y, Thyreau B, Kinomura S, Sato K, Goto R, Kawashima R, Fukuda H (2011b): Correlations among brain gray matter volumes, age, gender, and hemisphere in healthy individuals. PLoS One 6:e22734.
- Tang T, Jiao Y, Wang X, Lu Z (2013): Gender versus brain size effects on subcortical gray matter volumes in the human brain. Neurosci Lett 556:79–83.

- Thambisetty M, Wan J, Carass A, An Y, Prince JL, Resnick SM (2010): Longitudinal changes in cortical thickness associated with normal aging. Neuroimage 52:1215–1223.
- Volkow ND, Wang GJ, Fowler JS, Tomasi D (2012): Addiction circuitry in the human brain. Annu Rev Pharmacol Toxicol 52: 321–336.
- Walhovd KB, Fjell AM, Reinvang I, Lundervold A, Dale AM, Eilertsen DE, Quinn BT, Salat D, Makris N, Fischl B (2005): Effects of age on volumes of cortex, white matter and subcortical structures. Neurobiol Aging 26:1261–1268.
- Walhovd KB, Westlye LT, Amlien I, Espeseth T, Reinvang I, Raz N, Agartz I, Salat DH, Greve DN, Fischl B, Dale AM, Fjell AM (2011): Consistent neuroanatomical age-related volume differences across multiple samples. Neurobiol Aging 32:916–932.
- Westlye LT, Walhovd KB, Dale AM, Bjornerud A, Due-Tonnessen P, Engvig A, Grydeland H, Tamnes CK, Ostby Y, Fjell AM (2010): Life-span changes of the human brain white matter: Diffusion tensor imaging (DTI) and volumetry. Cereb Cortex 20:2055–2068.
- Witelson SF (1989): Hand and sex differences in the isthmus and genu of the human corpus callosum. A postmortem morphological study. Brain 112:799–835.
- Witelson SF, Beresh H, Kigar DL (2006): Intelligence and brain size in 100 postmortem brains: sex, lateralization and age factors. Brain 129:386–398.
- Xu J, Kobayashi S, Yamaguchi S, Iijima K, Okada K, Yamashita K (2000): Gender effects on age-related changes in brain structure. AJNR Am J Neuroradiol 21:112–118.
- Zatorre RJ, Fields RD, Johansen-Berg H (2012): Plasticity in gray and white: neuroimaging changes in brain structure during learning. Nat Neurosci 15:528–536.
- Ziegler G, Dahnke R, Jäncke L, Yotter RA, May A, Gaser C (2012): Brain structural trajectories over the adult lifespan. Cereb Cortex 33:2377–2389.
- Zöllig J, Merrilat S, Eschen A, Röcke C, Martin M, Jäncke L (2011): Plasticity and imaging research in healthy aging: core ideas and profile of the International Normal Aging and Plasticity Imaging Center (INAPIC). Gerontology 57:190–192.