

**Variability in the macro- and microstructure of the
human brain and its importance to the investigation of
neurological disorders**

PhD thesis summary

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Original publications related to the thesis

- I.** **András Király**, Nikoletta Szabó, Eszter Tóth, Csete, Péter Faragó, Krisztián Kocsis, Anita Must, László Vécsei, Zsigmond Tamás Kincses: *Male brain ages faster: the age and gender dependence of subcortical volumes*.
Brain imaging and behavior. 2016 Sep; 10(3): 901-10.
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- II.** **András Király**, Nikoletta Szabó, Árpád Párdutz, Eszter Tóth, János Tajti, Gergő Csete, Péter Faragó, Péter Bodnár, Délia Szok, Bernadett Tuka, Éva Pálinkás, Csaba Ertsey, László Vécsei, Zsigmond Tamás Kincses: *Macro- and microstructural alterations of the subcortical structures in episodic cluster headache*.
Cephalalgia. 2018 Apr; 38(4):662-673. Epub 2017 Apr 20.
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- III.** **András Király**, Zsigmond Tamás Kincses, Nikoletta Szabó, Eszter Tóth, Gergő Csete, Péter Faragó, László Vécsei: Gray matter atrophy in presymptomatic Huntington's patients.
Ideggyógyászati szemle. 2016 Jul 30; 69(7-8):261-7.
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- IV.** Zsigmond Tamás Kincses, Nikoletta Szabó, István Valálik, Zsolt Kopniczky, Lívია Dézsi, Péter Klivényi, Mark Jenkinson, **András Király**, Magor Babos, Erika Vörös, Pál Barzó, László Vécsei: *Target identification for stereotactic thalamotomy using diffusion tractography*.
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Abbreviations

AD	Axial diffusivity
BET	Brain Extraction Tool
CH	Cluster headache
DOF	Degrees of freedom
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
FAST	FMRIB's Automated Segmentation Tool
FDT	FMRIB's Diffusion Toolbox
FIRST	FMRIB's Integrated Registration and Segmentation Tool
FLIRT	FMRIB's Linear Registration Tool
FSL	FMRIB's Software Library
GLM	General linear model
MD	Mean diffusivity
MRI	Magnetic resonance imaging
RD	Radial diffusivity
ROI	Region-of-interest
SIENAX	Structural Image Evaluation, using Normalization, of Atrophy (single-time-point estimation)
SPSS	Statistical Package for Social Sciences
TFCE	Threshold free cluster enhancing approach
VBM	Voxel-based morphometry
Vim	Ventral intermedial nucleus of the thalamus
Vop	Ventral oral posterior nucleus of the thalamus

1. Introduction

The exact shape of every human brain – including its micro- and macroscopic features – is as unique as a human fingerprint, resulting in inter-individual anatomical variability. In the past two decades, the understanding of this variability advanced dramatically not only at the level of sulcal/gyral patterns, anatomical features (e.g. cortical thickness, volume and shape) and extent of cytoarchitectonic areas defined at the microscopic level, but also in the anatomical and functional connectivity of the brain. The core concept within the field of brain mapping is the use of a standardized 3D coordinate frame for data analysis and reporting of findings from neuroimaging experiments. This simple construct allows brain researchers to combine (even structural or functional) data from many subjects to create group-averaged signals. Also, where the signal is robust enough to be detected in individuals, it allows for the exploration of inter-individual variance in the location of that signal. Spatial standardization requires two basic components: (i) the specification of the 3D standard coordinate space, and (ii) a mapping function that transforms a 3D brain image from “native” space to that standard space. The first component is usually expressed by the choice of a representative 3D MR image that serves as target (template or atlas). The native image is re-sampled to standard space under the mapping function that may have few or many degrees of freedom, depending upon the experimental design. The optimal choice of atlas template and mapping function depends upon considerations of age, gender, hemispheric asymmetry, anatomical correspondence, spatial normalization methodology and disease-specificity.

In our studies we investigated some of these aspects, e.g. 1) how gender and normal aging influences brain morphology, 2) how normal hemispheric asymmetry plays a role in lateralized neurological diseases, such as cluster headache, 3) how progressive neurodegenerative disorders, such as Huntington’s disease affect the brain structure, or 4) how we can deal with inter-individual variability in case of neurosurgical interventions, such as thalamotomy in the therapy of medication resistant tremor.

1.1. *Gender differences*

Several normal functions and disorders of neuropsychiatric and developmental origin manifest differently in males and females. Because cognitive processes are rooted in neuronal architecture, the identification of neurological structures underlying sexually dimorphism may provide important insight into disease etiology and potential targets for treatment.

Whereas studies are converging upon males having larger absolute cerebral volume and head size and females generally have a thicker cortex in several regions of the brain. There is much less evidence on the sexual dimorphism of subcortical gray matter (GM) structures including the amygdala, caudate nucleus, accumbens, hippocampus, amygdala, pallidum, putamen and thalamus. Considering that basal ganglia nuclei possess a high density of sex steroid receptors, the effect of gender on the volume of these structures might be crucial.

1.2. *Aging*

Even in a cognitively normal, „healthy” brain, a variety of underlying neurodegenerative processes may be present, which may cause small, but detectable differences on MRI that could suggest e.g. increased risk for later cognitive decline. Detecting these subtle differences and relating them to physical or cognitive (dys) functions during the aging process will allow us to understand the biological basis of differences in normal aging of the brain. Numerous cross-sectional MRI studies have characterized age-related differences in regional brain volumes that differ with structure and tissue type. In general, recent research evidences are consistent in reporting that “normal” aging is associated with the enlargement of cerebrospinal fluid (CSF) filled structures and decreased whole-brain and gray matter volumes, as well as thinner cortical gray matter in several regions (mainly in the frontal and temporal lobe). However, age-related shrinkage of selective subcortical structures is more controversial. Studies investigating the effects of aging on white matter (WM) volume are also inconsistent.

The combined effects of age and gender on the human brain have been assessed, suggesting a more profound decline in GM volume in males. Discrepancies between studies could be due to using different age ranges or different sample sizes, as well as using different image processing and statistical (mostly univariate) methods. While age, gender and head size (intracranial volume) are the most commonly included “nuisance” variables when performing neuroimaging analysis, studies vary as to which of these variables are included and which method is used for correction.

In our first study, we investigated the effect of aging and gender on the size of the subcortical structures with special attention to the interactions of those factors.

1.3. *Lateralization*

The asymmetry of inter-regional structural connectivity (even large-scale connection patterns or small-world attributes) in the two brain hemispheres is an important topic in the study of the neural basis of brain functional asymmetries. We know that in some neurological disorders symptoms occur only on one side of the body or start on one side and propagate to the other with time. A typical example for the first case is cluster headache and Parkinson's disease best represents the second. In neuroimaging studies investigating unilateral processes - to boost the number of observations - it is common to flip the data about the midsagittal axis to have hemispheres/structures aligned according to the affected side. This approach is used despite evidence that there is a normal asymmetry in white matter diffusion parameters. Previously, asymmetry was most consistently found in the arcuate fasciculus and in the cingulum. Similarly, the diffusion parameters of subcortical structures were reported to be asymmetric in healthy subjects.

In our second study, we investigated the normal and altered lateralization in the diffusion data of subcortical gray matter structures through episodic cluster headache (CH), which is a primary headache disorder with prominent features including extremely severe unilateral, periorbital headache attacks accompanied by ipsilateral autonomic symptoms and occurring in clusters usually lasting some weeks, followed by much longer headache-free periods.

1.4. *Huntington's disease*

Huntington's disease (HD) is a neurodegenerative disorder with autosomal dominant inheritance. The expansion of CAG triplets in the Huntingtin gene (IT15), which is coded on the 4th chromosome, causes loss of neurons mainly in the striatum. Although, the exact behavior of the mutated Huntingtin protein is not completely understood, it seems that toxic (or ineffective defensive) mechanisms, which may already start to operate in the intrauterine life, are in the background of this cell loss. Early symptoms are attributable to the function and connections of the striatum (movement control, mood, higher cognitive functions). Symptom onset, which is heavily dependent on the number of CAG repeats, usually happens during mid-adulthood, but neurodegenerative changes begin years earlier. Diagnosis is based on the symptoms and the family history confirmed by genetic testing. The length of CAG repeats accounts for 60% of the variation in the time of symptom onset and the rate of progression. A longer repeat results in an earlier age of onset and a faster progression of symptoms. The remaining variation is due to environmental factors and other genes that

influence the mechanism of the disease. With medical imaging techniques, such as CT and MRI, atrophy of the caudate nuclei in the early, and cerebral atrophy in the advanced stages of the disease can be seen. However information about the pattern of the atrophy is very sparse.

In our third study, we investigated the rate of brain atrophy over 24 months in presymptomatic Huntington's patients.

1.5. *Inter-individual variability*

Replacing selective stereotactic lesioning the electrical stimulation of the ventral intermedial nucleus of the thalamus (*Vim*) is used in the treatment of medication resistant tremor nowadays. In the ideal case, this procedure is performed under local anesthesia, with the patient awake. As part of the intervention a temperature-controlled electrode is inserted into the thalamus. If no unwanted effect occurs during the speech, language, coordination and tremor testing, the probe is heated to create an about 3-mm permanent lesion. Ideally, the lesion causes the tremor to permanently disappear without disrupting sensory or motor control. Precise targeting within the brain is of crucial importance for successful surgical intervention. Targeting the desired thalamic nucleus is usually carried out by using stereotactic coordinates, specified in relation to a point on the anterior commissure – posterior commissure (AC-PC) line. However, this method does not incorporate properly the individual anatomical variations. Recently, probabilistic tractography was successfully used to investigate the connectivity profile of two major thalamic target nuclei for functional neurosurgery: the ventral intermedial (*Vim*) and ventral oral posterior nuclei (*Vop*). In addition, it is possible to segment thalamic nuclei based on connectivity patterns defined by MR diffusion tractography.

In our fourth study, we investigated the normal spatial variability of thalamic nuclei targets for functional neurosurgery.

1.6. *Methodological overview*

1.6.1. *Volume measurement*

During the last decades, magnetic resonance imaging (MRI) has become the method of choice for the examination of macroscopic neuroanatomy *in vivo*, due to the excellent image resolution and between-tissue contrast. In brain imaging, the precise and quantitative measurement of the volume and shape of brain compartments is important, as well as

measuring the temporal evolution of these parameters. However, achieving accurate and robust segmentation of cortical and subcortical areas is a great challenge for both manual and automated methods. Unlike manual quantitative region of interest methods, semi-automated and automated approaches do not require manual delineation of brain structures through determined number of MR sections. Although, these methods sometimes use different mathematical approaches for the same purpose, they also share some common features, such as various geometric parameters with reference to a stereotactic template for spatial normalization, and either a combination of between-tissue signal intensity differences and reference to a stereotactic template or just signal intensity differences for segmentation. These spatial transformations enable cohort comparisons given that homologous brain regions can be compared between brains, or between hemispheres in analyses of cerebral asymmetry.

In our studies we used the automated methods (FIRST, SIENAX and VBM) included in FSL (FMRIB's Software Library), because they are widely used, well documented, multiplatform and sufficiently accurate and robust. They also support parallel computing, and are freely available.

1.6.2. Diffusion tensor imaging

Diffusion-weighted imaging (DWI) is based on Brownian motion of water molecules. Diffusion-tensor imaging (DTI) is a recent application of diffusion imaging and is a non-invasive method for investigating the anatomical features of white matter tracts. With this approach, even though the spatial resolution of the image is in the millimeter range, we gain information about the tissue microstructure.

The ellipsoid surface that describes the diffusion has a principal long axis and two small axes that describe its width and depth. All three of these are perpendicular to each other and cross at the center point of the ellipsoid. The directions and the lengths of the axes can be estimated with a simple mathematical algorithm (singular value decomposition). We call the axes in this setting eigenvectors and their lengths are the eigenvalues. The lengths are symbolized by the Greek letter λ . Since the diffusion is not hindered significantly along the axons, the longest vector is parallel with the main fiber direction (λ_1 – axial/parallel diffusivity or AD). The two smaller vectors, perpendicular to the longest, will have lengths λ_2 and λ_3 (radial or perpendicular diffusivities). The diffusivities in the two minor axes are often averaged to produce a measure of radial diffusivity (λ_{\perp} or RD). Mean diffusivity (MD) or apparent diffusion coefficient (ADC) – which summarizes the total diffusivity – is the average of the 3

main diffusion directions. This measure is higher if water particles can diffuse in longer distances in every direction of the sphere (like diffusion in cortex compared to diffusion in cerebro-spinal fluid (CSF)). From the diffusion tensor, fractional anisotropy (FA) can be calculated, representing the local integrity of white matter. Fractional anisotropy (FA) is approximately 1 for anisotropic (ellipsoid, radial diffusivity relatively smaller than axial) and zero for isotropic (spherical, equal in all directions) diffusion.

1.6.3. Probabilistic tractography

As described above, diffusion has direction in well-structured tissues, such as the white matter. Since membranes hinder diffusion, its magnitude is relatively small perpendicular to the main fiber direction, but unaltered parallel to it. With diffusion MRI the main direction of the diffusion can be determined in each voxel as the largest diffusion that represents the main fiber direction. By tracing the vectors of the main diffusion direction, the white matter tracts can be mapped. Tractography methods can reconstruct an entire white matter pathway based on the alignment of the dominant orientation of local water diffusion, which represents the mean orientation of white matter fibers from voxel to voxel. There are various tractography algorithms, but probabilistic approaches are particularly attractive because they generate probabilistic maps of fiber connectivity between brain regions and can trace pathways into gray matter. An interesting application of probabilistic tractography is the connectivity-based segmentation of subcortical structures, which was first described Behrens and Johansen-Berg. In their approach the probability of connection of every thalamic voxel to 10 cortical target regions was estimated. The connectivity pattern of the thalamus corresponded well with its known microscopic internal structure.

2. Objectives

The aim of this thesis was to investigate the effects of age, gender, normal hemispherical lateralization and inter-individual variability on the structural properties of the brain through our neuroimaging studies about healthy subjects and patients with cluster headache, medication resistant tremor and Huntington's disease. We also present retrospective identification of the target thalamic nuclei in four patients who underwent stereotactic *Vim* and *Vop* thalamotomy.

3. Participants

3.1. *Brain atrophy in presymptomatic Huntington's disease*

Seven presymptomatic HD mutation carriers (mean age at baseline: 36.43±10.29) and ten healthy control subjects (mean age: 37.1±9.23) were recruited. Data from six patients were available for the longitudinal analysis. MRI measurements were repeated three times: baseline, 12 and 24 months. Patients had no motor symptoms (as measured with the motor section of the Unified Huntington's Disease Rating Scale) or cognitive disturbance over the period of the study (as measured by Mini Mental State Examination, Digit Span Test, Backward Digit Span, Listening Span Task, and Semantic Fluency Task). The controls had no neurological or psychiatric disorders.

3.2. *Subcortical gray matter structures in healthy subjects*

3.2.1. *Gender differences and Aging*

Fifty-three healthy males (mean age: 31.08±10.03 years) and fifty age-matched healthy females (mean age: 33.00±11.34 years) with no history of any neurological or psychiatric disorder were included in the study.

3.2.2. *Lateralization*

Ninety-four healthy individuals (mean age: 32.59±10.43, male: 50) were included in the study. Inclusion criteria for the controls were: 18-80 years of age, no history of neurological (including primary headaches, other pain conditions) or psychiatric diseases. All the participants were right handed.

3.2.3. *Inter-individual variability in the position of thalamic nuclei*

Nine healthy individuals, with no history of neurological or psychiatric diseases were included in the study (mean age: 28.36±7.09, male: 3). Furthermore, four patients were also included, who underwent stereotactic thalamotomy.

3.3. *Subcortical gray matter structures in cluster headache*

Twenty-two patients were recruited into the study (mean age: 38.10±11.33, male: 19). Inclusion criteria for the cluster headache (CH) patients were: 18-80 years of age, primary CH according to The International Headache Society diagnostic criteria, no interval therapy for the CH, no accompanying neurological (including other primary headache disorders and

pain conditions) or psychiatric disease, no regular neuro-psychiatric medication, negative routine MRI scan. Special attention was paid to the exclusion of depression, for which the Hamilton questionnaire was used (>16 points was the exclusion criterion). There were 12 left-headache-sided (*LHS-CH*) and 10 right-headache-sided patients (*RHS-CH*) in the CH group. All the participants were right-handed.

Clinical variables, such as disease duration, time between bouts and average length of bouts were recorded for all patients. Furthermore, cumulative number of headache days – that is the total number of days the patient had experienced cluster headache over his/her entire life – was estimated for all the patients.

Control group was the same as the one used for the investigation of lateralization in the subcortical gray matter structures.

All studies were approved by the Ethics Committee of the University of Szeged (authority number: 87/2009), and all subjects provided their written informed consent.

4. Image acquisition

Imaging was carried out with a 1.5 T GE Signa Excite MRI scanner. High-resolution axial T1-weighted and diffusion-weighted images were acquired from all the participants. In case of cluster headache patients, MR image acquisition took place at least one month after the end of the last headache bout. In case of patients with tremor undergoing thalamotomy the preoperative scans were obtained using identical acquisition sequences to those used for control subjects. Postoperative scans were carried out three months after the surgery. High-resolution-T1 weighted images (with parameters identical to the preoperative ones) and sagittal 3D FLAIR images were acquired to localize the thalamotomy lesion.

5. Image processing

Tools from the FMRIB Software Library (FSL, version 5.0; Oxford Centre for Functional MRI of the Brain (FMRIB), UK; www.fmrib.ox.ac.uk/fsl) were used for data processing.

5.1. *Partial brain volumes*

Total intracranial volume, the volumetric scaling factor (*v-scale*, to be used as normalization for head size) as well as the gray and white matter volumes was estimated by SIENAX. For brain and non-brain tissue segmentation, SIENAX uses a deformable model (spherical

tessellated surface) that evolves to fit the brain's surface by the application of a set of locally adaptive model forces, followed by intensity based tissue segmentation.

5.2. *Cortical thickness*

For analyzing voxel-wise differences in local gray matter volume/topography between populations we employed an “optimized” VBM-style protocol using FSL. After tissue type segmentation, the resulting gray matter partial volume images were registered to a standard space (MNI152). These images were averaged to create a study-specific template, to which the native gray matter images were then nonlinearly re-registered. Finally, voxel-wise General Linear Model (GLM) was applied using permutation-based non-parametric testing. The model coded group membership to identify Huntington's disease related brain atrophy. Thresholding was carried out by a novel method called threshold-free cluster enhancement (TFCE). This analysis requires no *a priori* information about the location of the possible differences.

5.3. *Volumes of the subcortical gray matter structures*

For comparison of subcortical structure volumes between groups, FIRST analysis was used. FIRST is a model-based segmentation/registration tool. This approach uses deformable surface meshes specific to subcortical structures (amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus). It allows probabilistic relationships between shape and intensity to be fully exploited. The model is trained for 15 different subcortical structures using 336 manually labeled T1-weighted MR images. The nucleus accumbens was not investigated due to inappropriate segmentation.

5.4. *Diffusion parameters of subcortical gray matter structures*

In order to evaluate the internal microstructure of subcortical gray matter structures, diffusion parameters were estimated for each and compared between two groups. Initially, raw diffusion data were corrected for eddy currents and motion artifacts with FDT by 12 degrees-of-freedom affine linear registration to the first non-diffusion-weighted reference image. Diffusion tensors were fitted at each voxel. Fractional anisotropy (FA), mean diffusivity (MD), and diffusivity parallel (AD, axial) and perpendicular (RD, radial) to the principal diffusion direction were computed in every voxel of the brain. Each subjects' binary masks of subcortical structures segmented by FIRST were registered to the subjects' own DWI images

with 6 degrees-of-freedom (rotations and translations only). The transformed masks were thresholded at 0.5 and binarized again to avoid size increment due to registration. All registered images then were then checked visually and corrected so as not to contain parts of ventricles or white matter tracts close to the subcortical structures. Average diffusion parameters were calculated under the masked areas.

5.5. *Probabilistic tractography*

The initial pre-processing steps for diffusion-weighted data are the same as described in section 5.3. The images were then skull stripped using the BET and the diffusion-weighted images were registered to the high-resolution T1-weighted image with a 6 degree-of-freedom linear registration using FLIRT. Probability distributions of fiber orientation were estimated for each brain voxel in the acquired diffusion space using a multi-fiber extension of the probabilistic tractography available in FDT.

Binary masks of the thalamus and the cortical targets were drawn manually for each subject. Probabilistic multi-fiber diffusion tractography was initiated 5000 times from every voxel inside the thalamic mask. Counters were increased every time an individual streamline reached the cortical target region. Hence, the values stored in thalamic voxels in one of the resulting images represent the probability of those voxels being connected to the particular target cortical mask assigned to that image. To investigate the inter-subject variability of the target nuclei, a specific distance reserving registration method was used with further steps to exclude possible registration bias between the DTI and T1-weighted images.

In case of the patients going through thalamotomy, post-operative FLAIR images were transformed to the preoperative T1-weighted structural image to overlay the lesion on the generated segmented thalamus to reveal correspondence between the location of the surgical lesion and the predicted *Vim* nucleus. Ideally, the operative lesion of the thalamus should have been situated in the region of the thalamus connecting to the premotor cortex with the highest probability.

6. Statistics

6.1. *Brain atrophy in presymptomatic Huntington's disease*

Voxel-wise General Linear Model (GLM) was applied using permutation-based non-parametric testing (5000 random permutations). The ANCOVA style GLM design used for VBM analysis coded for time point and gender. Thresholding was carried out by the method

of threshold-free cluster enhancing technique (TFCE). Results were corrected for multiple comparisons and $p < 0.05$ was chosen as the significance threshold.

6.2. *Subcortical gray matter structures in healthy subjects*

6.2.1. *Gender differences and aging*

Raw volumes, volumes normalized to head size, and the diffusion parameters of subcortical structures were compared between groups. Mean diffusion parameters were estimated for each segmented subcortical structure. Multiple univariate analysis of variance with age as covariant (MANCOVA) was applied for statistical analysis (IBM SPSS Statistics 20). Correlations between volumes of subcortical structures, gray/white matter ratio, partial brain volumes and age were calculated for both groups (IBM SPSS Statistics 20). The differences between groups in correlations with age were calculated with Fisher's r-to-z transformation. The results were Bonferroni-corrected $p < 0.05$ was chosen as the significance threshold.

6.2.2. *Lateralization*

Left-right ratios of the size and diffusion parameters of the subcortical structures were estimated as the ratio of the left and right side parameters. A ratio above 1 indicates larger, while below 1 indicates smaller structures or diffusion parameters on the left. One sample t-test was used to test lateralization. The results were tested with a bootstrapping method described below in section 6.3.

6.2.3. *Inter-individual variability in the position of thalamic nuclei*

The Euclidean distance was calculated for each subject between points representing the thalamic voxel with peak connection probability to the premotor cortex (for *Vop*), to the motor cortex (for *Vim*) and the standard space voxel with peak connectivity as indicated by the Oxford Thalamic Connectivity Map. The distance between the voxel with peak connection probability to the premotor (for *Vop*) and to the motor (for *Vim*) cortex was calculated between all possible pair of subjects and this inter-subject distance was averaged for *Vop* and *Vim* separately. To assess the similarity of (between subjects) positions, the overlap between each pair of thalamic region masks connected to the premotor or motor cortex were calculated according to the method proposed by Crum and colleagues. Overlap was measured by the Tanimoto Coefficient (TC), which is defined as the ratio of the number of voxels in the intersection of the two regions to the number of voxels in the union.

6.3. *Subcortical gray matter structures in cluster headache*

The group differences were evaluated by using a standard general linear model (GLM), where the model was encoding group membership, age and gender. The solution of the regression model was estimated by ordinary least squares approach. Since the number of subjects in the control and patient groups differed significantly a bootstrap approach was used to confirm the stability of the findings. A similar approach was used for the correlations between clinical variables, volumes and diffusion parameters.

7. Results

7.1. *Brain atrophy in presymptomatic Huntington's disease*

With VBM analysis, gradual gray matter atrophy was observed in the bilateral frontal regions, the temporal and insular cortices and in the anterior and posterior cingulate cortices during the two years of the investigation. With regards to the subcortical structures, the gray matter density of the head of the left caudate nucleus was reduced during the study period.

7.2. *Subcortical gray matter structures in healthy subjects*

7.2.1. *Gender differences*

MANCOVA (mean age of comparison = 32.02 years) revealed that all subcortical structures and all partial brain volumes without normalization for skull size were significantly larger in the male than in the female group. The gray/white matter ratio did not reveal a significant difference. No significant group difference was found between left/right volume ratios of the subcortical structures. However, it is noteworthy that volumes of the right caudate nucleus and the left thalamus were larger than those of the corresponding contralateral structures in the male group only.

After normalization for skull size, MANCOVA (mean age of comparison = 32.02 years) revealed significantly larger subcortical GM volumes for the left and right hippocampus in the female group. Strikingly, we found the total and the cortical GM to be larger in the female group as compared to males. In the male group, the volumes of the right caudate nucleus and the left thalamus were found to remain significantly larger than the contralateral pair of these structures.

There were no differences in the diffusion parameters of the subcortical structures between groups. Also, there were no differences found in the left/right ratios of the diffusion parameters of the subcortical structures between groups.

7.2.2. Aging (separately for genders, with and without normalization for skull size)

In the male group, total and cortical GM volumes, as well as the right thalamus showed a significant negative correlation with age when corrected for multiple comparisons. As for the volume of the left thalamus a tendency to a significant negative correlation with age was detected. In the female group, volumes of total and cortical GM, the right hippocampus, as well as the left and the right thalamus showed a significant negative correlation with age. The gray/white matter ratio was found to correlate negatively with age for both males and females. However, the age-related gray/white matter ratio was found to be higher for females. Interestingly, the left/right volume ratio of the hippocampus exhibited a significant positive correlation with age in the female group only.

In the male group, normalized total brain volume, total and cortical GM, left and right caudate nucleus volume, left and right putamen, as well as and right thalamus volume showed a significant negative correlation with age. In the female group a significant negative correlation with age was revealed for normalized total brain volume, total and cortical GM, left and right thalamus. Interestingly, the decline with age in normalized GM volume occurred at a faster pace in the group of males than for females.

The FA of the left putamen showed positive correlation with age only in the male group. Otherwise, there was no correlation between age and the diffusion parameters of subcortical gray matter structures neither in the male nor the female group. Also, there were no differences in the correlation found between age and the left/right ratio of the diffusion parameters of subcortical gray matter structures between the male and female groups.

7.2.3. Lateralization

The laterality in the size and diffusion parameters of subcortical structures was investigated in 94 healthy control subjects. Head size normalized volumes of the right caudate nucleus, left putamen and left thalamus were significantly higher than the contralateral pair of these structures. The FA of the left amygdala, caudate nucleus, putamen and right pallidum was higher than the contralateral pair of these structures. AD and MD of all right side structures – except thalamus – were higher than in the contralateral structures. RD of the right amygdala,

caudate nucleus, pallidum, putamen and the left hippocampus were higher than in the contralateral structures.

These results indicate that there is a significant lateralization of the size and diffusion parameters in healthy subjects.

7.2.4. Inter-individual variability in the position of thalamic nuclei

As found through manual comparison the shape and the position of the thalamus was not affected by EPI distortions. The largest misregistration was found along the anterior-posterior axis, but even that was minimal (for AC and PC 0.6 ± 0.5 mm). The width of the third ventricle was not different, and the position of the highest point of the corpus callosum differed only in case of a single subject with 1 mm. The premotor thalamus was consistently localized in all subjects, however, since the individual brain shapes differed, the exact location of the *Vop* and *Vim* nuclei varied substantially across subjects.

The mean distance of the peak connection probability of *Vop* from the peak probability indicated by the Oxford Thalamic Connectivity map was 5.08 mm. The equivalent distance was 6.26 mm for the *Vim*. The mean pair-wise inter-subject distance of the peak connectivity voxel of *Vop* was 7.33 ± 3.37 mm (range: 0–14.56 mm) and 7.42 ± 3.35 mm (range: 2–14.28 mm) for *Vim*. The mean distances of the tractography defined *Vop* and *Vim* coordinates from the stereotactic target point defined by Hyam's method were 7.19 ± 4.36 mm (range: 2.45–14.89 mm) for *Vim* and 9.58 ± 4.82 mm (range: 3.0–17.12 mm) for *Vop*. The mean pair-wise overlap for *Vop* as calculated by the Tanimoto Coefficient was 40.2% (range: 15.5%–66.2%) and 31.8% (range: 3.2%–66.2%) for *Vim*.

7.3. Subcortical gray matter structures in cluster headache

7.3.1. Volumes and diffusion parameters

Since our analysis showed that there is a significant lateralization of the volumes and the diffusion parameters of subcortical structures in normal healthy subjects, we treated the left- and the right-sided headache patients as separate groups and did not pool patients by flipping their brain along the midsagittal axis. Because of the relatively smaller group sizes bootstrap statistics were used to test the stability of our findings. The GLM analysis showed that the FA of the right amygdala was significantly higher in *CH* and *LHS-CH* patients than in healthy subjects. A similar tendency was found in case of the left amygdala in *RHS-CH* patients. MD and RD of the right amygdala were higher in healthy subjects compared to the *CH* and *LHS-*

CH patients. A similar tendency was found in *RHS-CH* patients. AD of the right caudate nucleus was higher in *CH*, *RHS-CH* and *LHS-CH* patients as compared with healthy subjects. A similar tendency was found with MD and RD in *CH* and *LHS-CH* patients. In case of the right pallidum FA was lower in *CH* and *LHS-CH* patients. RD of the right pallidum showed tendency to be higher in *CH* and *LHS-CH* patients than in healthy subjects. The head size normalized volume of the right pallidum was lower in *RHS-CH* patients than in healthy subjects. Total brain volume and gray and white matter volumes were not different between groups.

7.3.2. *Correlation with clinical parameters*

The head size normalized volume of the total brain and cortical gray matter showed positive correlation with the cumulative number of headache days in the *CH* patients. Similar correlation was found in the *LHS-CH* patients. The head size normalized volume of the total gray and white matter showed similar, but not significant tendency of correlation in the *CH* patients.

The head size normalized volume of the left and right hippocampus and right caudatus showed positive, the AD of the left and right thalamus, the AD, MD and RD of the left hippocampus showed negative correlation with the cumulative number of headache days in the *CH* patients. Head size normalized volume of the left pallidum, left and right thalamus showed positive, the AD of the left hippocampus, the MD of the left pallidum showed negative correlation with the cumulative number of headache days in the *RHS-CH* patients. The head size normalized volume of the left hippocampus showed positive correlation with the cumulative number of headache days in the *LHS-CH* patients.

7.4. *Case reports about thalamotomy results*

There were 4 patients who underwent stereotactic thalamotomy. The surgical lesions were – mostly - situated at the proper location (*Vim / Vop* nucleus of the thalamus) in all cases. However, there was some unwanted effect occurring after the intervention. The cortical connections of the lesions mapped with probabilistic tractography explained well these side effects.

8. Discussion

In our studies we aimed to identify effects of age, gender and lateralization on subcortical gray matter volumes and diffusion parameters, as well as to show the significance of inter-individual variability in the features of the human brain.

Hence, a complete set of analyses was run in three consecutive studies: A) automatized, deformable mesh based segmentation toolkit (FSL-FIRST) was used to extract subcortical structures, B) partial brain volumes were extracted with an intensity-based segmentation toolkit (FSL-SIENAX), C) cortical thickness was measured with voxel-based morphometry (VBM), D) diffusion tensor imaging (DTI, FSL-FDT) was used to get the diffusion parameters of the subcortical structures (which is defined by the internal microstructure), E) probabilistic tractography based segmentation of the thalamus (FSL-PROBTRACKX) was run to reveal inter-individual variability in the position of the thalamic nuclei *Vim* and *Vop*.

The most important results of our investigations can be summarized as follows:

(1) In general, male brains were found to be larger than females', with larger gray and white matter fractions, as well as subcortical structures. However, most of these differences disappeared after skull size was accounted for. Moreover, as a result of correction for total intracranial volume we found females to have larger cortical and subcortical GM volumes. Importantly, the volume of the hippocampus was found to be significantly larger in the female group as compared to males. We also detected a significant effect of hemisphere side in the male group only, with larger volumes of the right caudate and the left thalamus as compared to their contralateral structures.

(2) We found an age-dependent decrease in the volume of the subcortical gray matter. This remained significant in the caudate, putamen and thalamus bilaterally for males and the thalamus bilaterally for females after correction for skull size. Within the age range of 21 to 58 years we found a linear decrease in GM volume with age. Strikingly, this process proved to occur at a faster pace in males. Additionally, we found that FA values of the left putamen increased with age only in the male group.

(3) A significant lateralization of the size (caudate, putamen and thalamus) and diffusion parameters of the subcortical gray matter structures were found in healthy controls. By demonstrating this lateralization in a large cohort of healthy subjects, we showed that in

patients with unilateral symptoms, pooling of the data depending on the affected side to boost the number of observations is not recommended.

(4) We found that, compared to the size of the nucleus, the spatial variability of the position of thalamotomy target nuclei *Vim* and *Vop* is substantial. Our results call attention to the importance of defining such small target individually for which our approach is suitable and is frequently used since the publication. We also showed that the lesion in four successful thalamotomy cases was in fact in the target nuclei as defined by tractography based segmentation.

(5) We showed that there is a progressive gray matter loss in Huntington patients even before the appearance of clinical symptoms. This gray matter atrophy represents the progressive neurodegeneration caused by the genetic alteration responsible for the disease.

We propose that the usefulness and importance of our current findings are two-fold: (i) they present methodological considerations for the investigation of subcortical structures and (ii) they raise potential functional implications related to gender and gray matter decline with age.

8.1. *Methodological considerations*

While there is converging research evidence for total brain and GM volumes to decline with age, we propose that the inconsistency of findings about age and gender-related differences in subcortical GM volumes might arise from methodological differences and relatively small sample sizes. Most of the previous studies applied a VBM approach to identify gender differences in subcortical nuclei. While VBM is an excellent tool for the investigation of focal gray matter density differences, deformable surface model approaches such as FIRST are directly tuned for the volumetric analysis of the subcortical structures. Furthermore, in MRI studies, age, gender and head size (intracranial volume) are the most commonly included “nuisance” variables, though studies greatly vary as to which of the variables are included and which method is used for correction. Nevertheless, it is important to communicate transformation approach and group intracranial volume considerations when reporting structural findings of subcortical GM since it might carry several implications for interpretation of the results.

There are only a few studies about the diffusion parameters of subcortical deep gray matter structures. In our study, compared to previous ones, we used a larger cohort of healthy subjects and found asymmetries in various diffusion parameters estimated from diffusion measurements using 60 different directions.

Despite the accurate stereotactic atlases that guide the standard methods for targeting, significant inter-subject variability still exists. Our results also indicate significant variability of the position of the *Vop* and *Vim* nuclei, especially when the size of the nucleus and the surgical lesion is considered. It is also important to emphasize that in the current variability estimation only young, healthy individuals were included. Brain pathology may further increase the variability.

8.2. *(Patho) biological considerations*

8.2.1. *Brain atrophy in presymptomatic Huntington's disease*

In Huntington's disease, brain atrophy is more significant than during healthy aging. Although in our study we did not find correlation between cortical thickness and CAG repeat number, from previous studies we know that higher CAG repeat numbers are also associated with faster clinical progress and earlier onset of symptoms. In a larger cohort of patients, a CAG repeat number increase of 1 resulted in the increment of brain atrophy rates by 0.12% per year.

8.2.2. *Effect of age and gender on brain (micro) structure*

The aspect to be considered involves the underlying cellular, molecular and functional mechanisms of age and gender related differences of GM volume. Primarily, neuronal and synaptic pruning has been proposed to play a critical role. However, findings of post-mortem histological studies suggested, that it is rather the size than the number of the individual cells that explain age-related GM decline. Recent results also imply the impact of aging on GM/WM diffusion changes, explaining some cognitive variability and even decline.

The background of the disproportionate GM volume changes in males and females has not yet been elucidated, but differences in hormone levels and the consequent sensitivity of the brain to hormonal effects are most certainly involved. In addition to gender effects, recent evidence supports the influence of brain hemisphere side showing lateralization of structure-function relationships, as well as more specific relationships between individual structures (e.g., left hippocampus) and functions relevant to particular aptitudes (e.g. vocabulary). It can be hypothesized that this difference relates to handedness, however, we did not find such a

relationship. A recent study examining the deep gray matter of healthy adults by using magnetic susceptibility-weighted imaging did not reveal an association with handedness either.

The interpretation of age-related changes of DTI metrics in the basal ganglia might be different and more complicated than in the white matter. In contrast to the microstructure of the white matter, the basal ganglia consist predominantly of neurons and glia. Neurons in the caudate nucleus and putamen have spherical dendritic arborization, which is covered densely with dendritic spines. These allow water to diffuse freely in these structures. In contrast, neuronal dendrites in the globus pallidus are long, smooth, and sparsely branched. The fibers in the globus pallidus are covered by myelin, and some fibers are arranged in bundles. The diffusion of water is, therefore, more restricted and more directed in the globus pallidus. Volume reduction with age due to the loss of neurons in the striatum has already been reported. The concurrent gliosis and tissue compaction hinder water diffusion. The cell membranes of the remaining atrophic neurons become less fluid and more rigid with aging, also the vessel wall shows some hyaline degeneration during aging, it becomes thicker and the water diffusion through the wall becomes more difficult. Also, the contribution of the extracellular and intracellular water fraction to the diffusion signal is not even. A combination of diffusion parameter changes could be explained by the change of the ratio of the extra and intracellular water fractions (e.g.: global shrinkage of the structure or intracellular edema) or for example protein deposits limiting water diffusion. These kinds of age-related changes add to the hindrance of water diffusion. Additionally, the body of the striatum, especially the putamen, is a region with an abundant blood supply compared to other regions in the brain. Hence, the pseudo diffusion effect caused by blood motion can be more significant here.

8.2.3. Alteration of subcortical gray matter structures in cluster headache

Recent studies suggest that neurodegeneration could result in diffusion changes of related basal ganglia. In our study, we found increased FA of the bilateral amygdala. The amygdala is known for being an important center of emotional and affective aspects of pain. Also, it is an important hub in the processing of noxious stimuli and it was shown to have structural and functional connections to cortical and subcortical structures involved in pain processing. It was shown by several studies that there is significant pain related plasticity in the amygdala in chronic pain conditions and amygdalar plasticity was suggested to be the key factor in the establishment of fear memory.

8.2.4. *Segmentation of the subcortical structures to guide functional neurosurgery*

Despite the accurate stereotactic atlases that guide the standard methods for targeting, significant inter-subject variability still exists. Differences in gross thalamic morphology are already conspicuous: the medio-lateral aspect – as defined by the position of the internal capsule – and the height of the thalamus varies between subjects. The position of the pulvinar system even varies between subjects with the same AC-PC distance. Moreover, as emphasized by Morel, these variations are not homogenous even within the thalamus, particularly along the medio-lateral axis.

9. Limitations

These analyses have some essential drawbacks. First of all, these are cross-sectional assessments of the focal shrinkage or alteration of diffusion parameters in the subcortical gray matter structures. In order to acquire an in-depth understanding of the dynamics in brain atrophy and diffusion parameters longitudinal studies are needed, which are certainly difficult to carry out in a timeframe long enough to be useful in researching normal aging.

In case of cluster headache, we do not have exact information about the time elapsed from the last attack. A further drawback is the relatively low power of the secondary analyses (RHS-CH and LHS-CH) due to the limited number of patients. Given the central role of the hypothalamus in CH it would be crucial to investigate its size and microstructure. Unfortunately, segmenting the hypothalamus is limited by the low intensity contrast to the surrounding structures.

It has to be pointed out that in the reported thalamotomy cases we did not plan targeting with tractography based segmentation before the surgery. One possible caveat of our analysis that cannot be neglected is the misregistration when aligning the high resolution T1-weighted and diffusion weighted images. Diffusion-weighted images suffer significant distortions because of susceptibility artifacts; however, our analysis indicated that these distortions were minimal in the region of the thalamus. Extension of this study to compare the result of the stereotaxic neurosurgery with and without tractography based targeting is crucial. Further improvements might be expected if additional imaging modalities (e.g. relaxometry, fMRI) could also be utilized. It is also important to emphasize that in the current variability estimation only young, healthy individuals were included. Brain pathology may further increase the variability.

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