UNIVERSITY OF PÉCS

Doctoral School of Biology

Examining brain volumetry and morphometry in relation to body mass index and coffee consumption: magnetic resonance imaging approaches

PhD thesis

Gergely Orsi

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Examining excessive energy intake or coffee consumption associated brain volumetric and morphometric changes with magnetic resonance imaging methods

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Supervisors:

Róbert Gábriel, D.Sc.	**József Janszky, D.Sc.**
Institute of Biology	Department of Neurology
Faculty of Sciences	Clinical Centre
University of Pécs	University of Pécs

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Supervisor's signature   Supervisor's signature

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Head of the Doctoral School

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1. ABBREVIATIONS

BMI – Body Mass Index

CICR – Calcium Induced Calcium Release

CSF – Cerebrospinal fluid

CT – Computed Tomography (X-ray computed tomography)

DTI – Diffusion Tensor Imaging

FSL – FMRIB Software Library

GLM – General Linear Model

GLIM – Generalized Linear Model

GM – Gray Matter

GPU – Graphical Processing Unit

GRF – Gaussian Random Field Theory

IP3 – Inositol 1,4,5-trisphosphate

LTP – Long Term Potentiation

MR – Magnetic Resonance

MRI – Magnetic Resonance Imaging

PDF – Probability Distribution Factor

PEPSI – Turbo Proton Echo Planar Spectroscopic Imaging

RyR – Ryanodine Receptor

SAR – Specific Absorption Rate

TFCE – Threshold-Free Cluster Enhancement

TBSS – Tract-Based Spatial Statistics

VBM – Voxel-Based Morphometry

WM – White Matter

2. INTRODUCTION

2.1 General introduction

Several invasive or non-invasive methods are available to assess the possible functions of different brain areas in human. Before the era of modern imaging and electrophysiology, the possible ways to study the functions of different brain areas were mostly lesional models or direct electric stimulation of the cortex during neurosurgery (Penfield and Boldrey, 1937). The number of available methods and methodologies has grown rapidly and by now, the non-invasive and minimal-invasive methods have more-or-less replaced the invasive ones. In this thesis, I will review two non-invasive magnetic resonance imaging (MRI) based methods - and present our novel findings based on these methods – which are suitable to track or compare brain volumetric or morphometric alterations. These methods are automated MRI volumetry (henceforth volumetry) and voxel based morphometry (VBM).

It is undebatable, that volumetry is the older one of these methods. The first MR volumetric examination /as tumor volumetry/ appeared in the literature at the end of the 80's (Hofmann et al., 1988). The first brain MR volumetry paper came up a year later (Mann et al., 1989), in which alcohol dependent individuals' cerebrospinal fluid volume were studied. The first article using automated MR volumetry was published in 1995 (Friedlinger et al., 1995); previous studies used manual segmentation, where the examined structure was manually labeled by the investigator, practically this meant outlining the given structure, in every slice, by hand. This methodology significantly limited the usefulness, because it is highly time consuming, especially for large cohort studies, where several hundred subjects must be evaluated. It was usually "solved" by incorporating several investigators, even several MR centers to speed up the process, but unfortunately this introduced a new and serious confounding factor, the investigator dependency, because the "between-investigator" error is always higher than the "within-investigator" one. These shortcomings are eliminated by the use of automated (but always controlled by the investigator) volumetric methods. The sensitivity and reproducibility of the method

have greatly improved, thanks to the non-declining development of the last 16 years. Numerous studies were published comparing the automated methods to manual segmentation (Akhondi-Asl et al., 2011; Deeley et al., 2011; Dewey et al., 2010; Doring et al., 2011).

VBM appeared in 1999, but the method itself was published in details a year later, in 2000 (Ashburner and Friston, 2000). The method faced heavy criticism in the beginning (Bookstein, 2001; Thacker, 2003), that contributed to the development of an optimized method (Good et al., 2001) and continuous improvements. Moreover, the constant development and improvement of registration and segmentation algorithms, along with the creation of more accurate and detailed atlases further mitigated other shortcomings significantly. By now, this method is considered mature, elaborated enough to be used in basic and clinical research reliably. Its shortcomings mainly arise from the (mis)interpretation of the results or the poor quality input datasets and not from the methodology itself.

2.2 Technical background

2.2.1 MRI Volumetry

There are a few software tools available (both commercial and freeware) to automatically process and evaluate MRI volumetry. Today, FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) is one of the most often used software packages (set of software tools) to study the cortical and subcortical anatomy. It consists of a surface based and a volume based stream. In the first one, the applied automatic tools construct models of the boundaries between cortical white and gray matter and pial surface as well. Once we assessed the 3 main surfaces in every slice creating 3 separate surfaces in the three dimensional space, an array of anatomical measurements becomes possible, including cortical thickness, surface area, curvature, and surface normal at each point on the cortex. The software offers the possibility to inflate and/or flatten the surfaces for improved visualization. In addition, a cortical surface-based atlas has been defined and is supplied with the package, based on average folding patterns mapped to a sphere. The surfaces from individuals can be aligned with this standard atlas with a high-dimensional nonlinear registration algorithm. The registration directly aligns the anatomy by aligning the cortical folding patterns, instead of aligning image intensities. This spherical atlas naturally forms a coordinate system in which a point-to-point correspondence between subjects can be achieved. This coordinate system is usually used to create group maps (this is similar to how Talairach space is used for volumetric measurements). The main advantage of FreeSurfer is that while this package runs reliably on good quality datasets, most parts of its pipeline are automated, which makes it ideal for use on large data sets.

2.2.1.1 The Surface-based Stream

The surface-based stream consists of several stages, fully described in (Dale et al., 1999, Fischl et al., 1999a). First, using an affine registration, the volume is registered with the Talairach atlas (Talairach and Tournoux, 1988). This allows the software to compute seed points in later stages of the stream. B1 bias field is estimated automatically by measuring the variation in the white matter intensity. The field is estimated using the main body of the white matter across the entire volume. Likely white matter points are chosen based on their locations in the Talairach space as well as on their intensity and the intensities of local neighboring voxels. Next, a bias field correction is applied by dividing the intensity of each voxel by the estimated bias field at that location in order to remove the bias field effect. The next step is the skull strip, using a deformable template model (Segonne et al., 2004). In the next step, basic segmentation starts by classifying voxels as white matter or something other than white matter based on their intensity and neighbor properties. The hemispheres get splitted by proper cutting planes while brain stem and cerebellum are also detached from the cerebral hemispheres. The position of the cutting planes are based on multiple attributes, first the expected Talairach location of the corpus callosum and pons, as well as several other algorithms that encode the expected shape of these structures. Based on the previous data, the software builds an initial surface for each hemisphere by tiling the outside of the white matter mass. This initial surface is then refined by the intensity gradients between the white and gray matter (henceforth, this is referred to as the white surface). In the next step, the white surface is nudged to follow the intensity gradients between the gray matter and CSF (this will be the pial surface). One example, where the white and pial surfaces are overlaid on the original T1- image is shown in Figure 1.
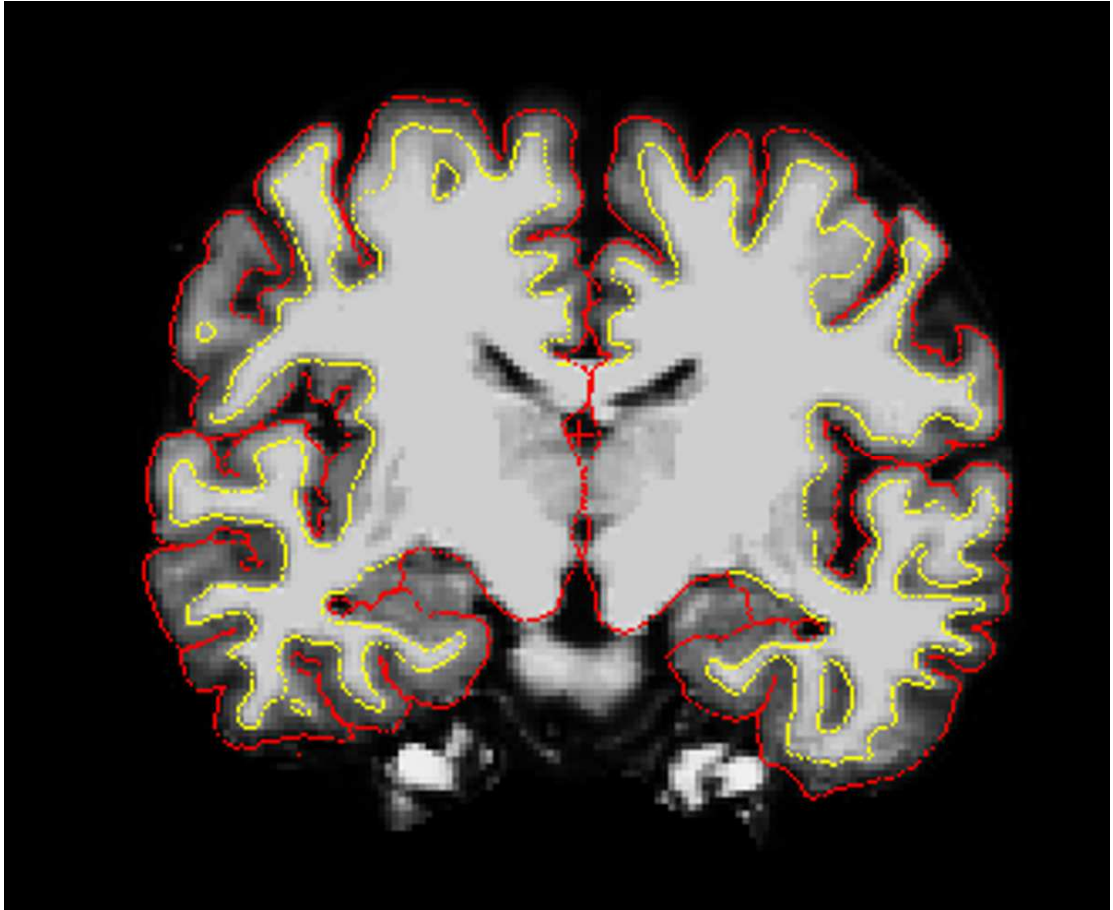
Figure 1. The WM and pial surfaces overlaid on the original T1 weighted image. Note that the tissues outside the pial surface will not affect the surface-based measurements of cortical morphometry, similarly, as the volumes are calculated by the volume based stream the pial surface cutting the hippocampus will not affect the measured subcortical volumes.

The distance measured between the white and the pial surfaces gives the cortical thickness at each location (Fischl and Dale, 2000). The local curvature and surface area can also be calculated. The pial surface can be inflated to show the areas in the sulci, shown in Figure 2.

Figure 2. The 3D model of the left hemisphere and its corresponding flattened form, on the right. The blue represents the gyri, while the green color depicts the sulci.

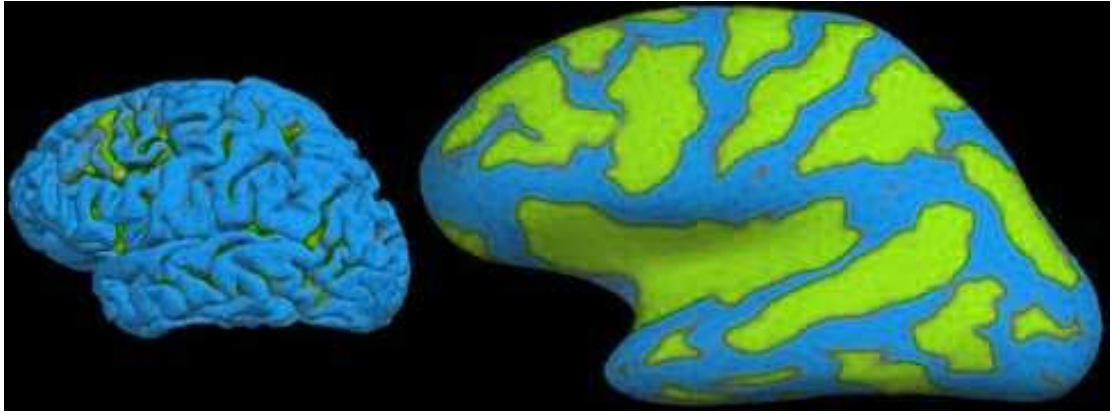This surface can then be registered to the spherical atlas based on the folding patterns (Fischl et al., 1999). For surfaced-based labeling, the measured value is the curvature in each of the principal directions at that vertex. General Linear Model (GLM) analysis on the surface provides an easy-to-use tool to test models of how any surface-based measure (cortical thickness, surface area, curvature, or surface normal) might change as a function of genetic or demographic variables, group membership (e.g. patient or normal) etc..

2.2.1.2 The Volume-based (Subcortical) Stream

The volume-based stream has two main purposes, (i) to preprocess MRI volumes and (ii) to label subcortical tissue classes. The stream has five stages (fully described in (Fischl et al., 2002), Fischl et al., 2004).

- 1. Registration to Talairach space (affine registration, designed to be insensitive to pathology and to maximize the accuracy of the final segmentation). This is a different procedure than the one employed by the surface-based stream.

- 2. Initial volumetric labeling.

- 3. B1 bias field correction to compensate the variation in intensity due to field inhomogeneity (using a different algorithm than the surface-based stream).

- 4. High dimensional nonlinear volumetric alignment to the Talairach atlas.

- 5. The final volume labeling (see below in more details).

The volume-based stream only depends upon the skull stripping to create a mask of the brain in which the labeling is performed. The last stage of the volume based stream, labeling the volumes, is described below.

The cortical (Fischl et al., 2004) and the subcortical (Fischl et al., 2002) labeling use the same basic algorithm. The final segmentation is based on both subject-independent probabilistic atlas and subject-specific measured values. The probabilistic atlas is built from a training set, (subjects whose brains including surfaces and volumes have been labeled manually). These labels are then mapped into a common space (Talairach space for volumes and spherical one for surfaces) to achieve point-to-point correspondence for all subjects, where a "point" is a voxel in the volume or a vertex on the surface. At each point in space, there exists the label that was assigned to each subject and the measured value (or values) for each subject. In the next step, three types of probabilities are then computed at each point.

In the first step, the probability that the point belongs to each of the label classes is computed automatically.

The set of probability value is derived from the spatial configuration of labels that exist in the training set, which is termed: "the neighborhood function". The neighborhood function is the probability that a given point in space belongs to a label given the classification of its neighboring points. The neighborhood function is important because it helps to prevent the formation of mis-labeled structure islands of a given structure in another one at the borderline between them.

The last one is the probability distribution function (PDF) of the measured value. It is estimated separately for each label at each point. In case of volume-based labeling, the measured value is the intensity at each voxel. The PDF is modeled as a

normal distribution, so we only need to estimate the mean and variance for each label at each point in space. Figure 3 shows the result of the segmentation in a single subject.
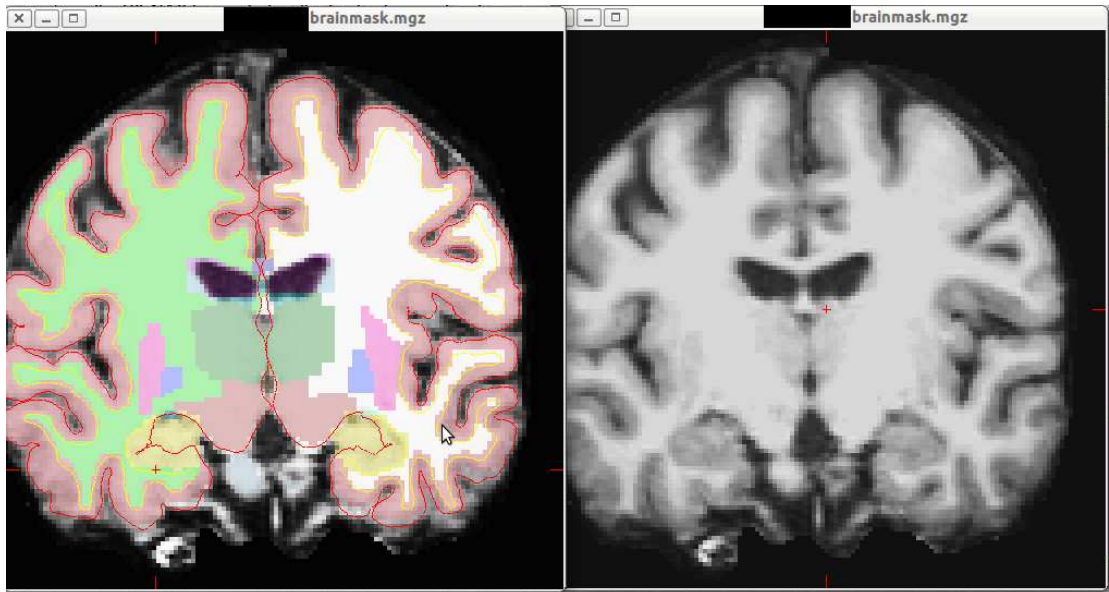
Figure 3. The brain extracted T1 weighted original image is on the right, while the segmented and labeled result can be seen on the left. The different colors represent different anatomical structures.

To extend the analytical capabilities of the suite, a longitudinal processing stream is also available in Freesurfer. An unbiased study-specific within-subject template space and average image (Reuter and Fischl, 2011) is created using robust, inverse consistent registration (Reuter et al., 2010). This subject template (similar to the one created in the optimized VBM protocol) is used to initialize the longitudinal image processing in several locations to increase repeatability and statistical power (Freesurfer-Wiki). The complete analysis pipeline overview is freely available at (Freesurfer-Wiki).

2.2.2 Voxel Based Morphometry

There are several VBM implementations, which may differ in some steps, while identical in others. I would like to present a general view on how the "optimized" VBM protocol (Good et al., 2001) is used in practice. The steps I will present are the ones used by the FSL-VBM script a member of FSL tools (Smith et al., 2004), available in the FSL software library (http://www.fmrib.ox.ac.uk/fsl).

In the first step, the input images are sorted in a new subdirectory, and get an extra extension in their filenames *_struc. The next step is to run the brain extraction on the images using BET2 (Smith, 2002). In case of having excessive neck tissue on the structural images (generally common if the 3D structural image was acquired in the sagittal plane) the –N option is mandatory; this option provides an extensive neck clean-up.. In other case, the –b option will result in default BET behavior. The remaining neck tissues on the brain extracted images will most likely confound the BET preprocessing, thus it should be avoided.

The second step of the FSL-VBM protocol is to create the study-specific gray-matter template. For this, the brain-extracted images of every subject are segmented into gray-matter (GM), white-matter (WM) and cerebrospinal fluid (CSF). The respective mirror images are also calculated. GM images (marked *_struc_GM) and their mirror images (*_struc_GM_xflipped) are registered to the GM ICBM-152 template atlas.

As the result of this registration a 4D image is generated containing the registered GM images and their mirror images called "template_GM_4D". This is averaged to create the study-specific GM template at the isotropic resolution of $2x2x2mm^3$ in standard space.

In case of different populations (e.g. patients and controls), they all should be represented in the template. The ideal case if the template is generated from equal number of group members, to avoid introducing any bias in the evaluation. In case of such bias, the registration quality would favor one of the groups, and the result would be hard to interpret, as the results may have originated from the better registration accuracy of one group compared to the other. In other words, the results showing

differences in the GM volume distribution between the two groups, may be the consequence of the examined disease or purely registration-related!

FSL-VBM offers two options for the study-specific template generation. The –a option will result in template generation based on an affine registration of GM images to the GM ICBM-152 template, while the –n option offers the possibility of non-linear registration.

In the next step, the script will non-linearly register all the GM images to the study-specific template. The result will be concatenated in a single 4D image file (GM_merg). After this registration, as initially recommended by Good and colleagues (Good et al., 2001) a modulation step is also introduced to compensate for the reduction/enlargement due to the non-linear style transformation: each voxel of each registered grey matter image is divided by the Jacobian of the warp field. The new, modulated 4D merged image is then saved as "GM_mod_merg" in the stats directory and then smoothed with a series of Gaussian kernels; sigma = 2, 3, 4mm. This is approximately from FWHM = 4.6mm to 9mm, and saved as "GM_mod_merg_sx", where x represents the given sigma value (2, 3 or 4).

Finally, as the last step, a permutation-based non-parametric inference should be initialized, using our own design, the GM mask and the 4D multi-subject concatenated, modulated and smoothed data. First, we start randomise without generating p-value maps. In this case, we will quickly get the raw t-stat maps, which can help us to choose the proper sigma value for smoothing, than we feed the data to a full run of randomize with at least 5000 permutations. We can choose between cluster-based thresholding (option –c) or the Threshold-Free Cluster Enhancement (TFCE) option (-T) instead of the cluster-based thresholding.

TFCE is a new method for finding "clusters" in your data without having to define clusters in a binary way (Smith and Nichols, 2009). Cluster-like structures are enhanced but the image remains fundamentally voxelwise. In case of VBM analysis, TFCE based tresholding is generally recommended instead of cluster based tresholding.

It is strongly recommend to use randomise (permutation testing) for inference in VBM-style analysis. The Gaussian random field theory (GRF) is a widespread approach and would be the first option to implement in such case, but the underlying

approximations are not generally appropriate in such analyses (FMRIB_Software_Library).

2.3 Usability and clinical relevance of new MR imaging techniques

Diagnostic decisions in clinical imaging currently rely mostly on visual image interpretation. However modern imaging modalities like volumetry, VBM, Functional MRI (fMRI) and Diffusion Tensor Imaging (DTI) are gaining importance in today's advanced diagnostics and helping differential diagnosis. The promise is that these methods can reduce the problem of observer dependence, while simultaneously increase diagnostic accuracy. Relaying purely on the visual analysis of structural images can also lead to uncertainty in some cases. One of the best example is dementia, where some of the changes resemble those of normal ageing (Heckemann et al., 2008).

Volumetry can also serve the diagnosis in several other diseases and provide a useful tool for further studies, e.g. Alzheimer's Disease (Kantarci et al., 2002, Heckemann et al., 2008, Palesi et al., 2012) epilepsy (Lai et al., 2010), mild cognitive impairment (Kantarci et al., 2002), chronic alcohol consumption (Agartz et al., 2003), mild traumatic brain injury (Cohen et al., 2007), etc. The list is by far not complete, and strictly limited to brain volumetry. Voxel-based morphometry was proven to be useful in the diagnosis of the following diseases: fronto-temporal lobar degeneration (Chang et al., 2005), amyotrophic lateral sclerosis (Ellis et al., 2001, Abrahams et al., 2005, Chang et al., 2005, Grosskreutz et al., 2006, Wang et al., 2009) and epilepsy (Guimarães et al., 2007). Diffusion tensor imaging and functional magnetic resonance imaging can be used for surgical planning, where the planned preservation of eloquent brain areas (localized for example by fMRI) along with their major tracts (visualized by DTI) around the tumorous tissue is of foremost importance. Moreover, DTI data can be further processed for tract based spatial statistics (TBSS) (Smith et al., 2006, Smith et al., 2007), which can provide a reliable and solid tool for the exploration of white matter associated differences between different groups. Diffusion imaging techniques can support the diagnosis in epilepsy (Lai et al., 2010) and Alzheimer's Disease (Palesi et al., 2012), multiply system atrophy (Shiga et al., 2005), optic neuritis (Hickman et al., 2005), while may be helpful in early differentiation between amyotrophic and primary lateral sclerosis

(Ciccarelli et al., 2009), and those with sporadic versus familiar forms of ALS (Blain et al., 2005). Furthermore, all these methods are able to assess information on brain development and normal aging that can be useful in clinical practice to differentiate between the normal and pathological variations.

2.4 Background literature review

The new MRI methods described in Chapter 2 will be applied in this thesis, where we demonstrate the relationship between brain structure and caffeine consumption or excessive energy intake. For these reasons, in the following chapter, I try to give a general background review concentrating on caffeine and body weight.

2.4.1 The effects of Caffeine

Caffeine's main action – unlike other psychoactive compounds – is evoked via the adenosine receptors. Drury and Albert Szent-Györgyi were the first ones who demonstrated the cardiac effects caffeine in 1929 (Drury and Szent-Gyorgyi, 1929). Since then, it is well-known that the adenosine receptors play a crucial role in cardiac and brain functions.

Caffeine's main mechanism of action is blocking A_1 and A_{2A} adenosine receptors (Fredholm et al., 1999b).

Adenosine receptors are prevalent throughout the whole body and are important in many biological processes like neuronal functioning, inflammation signaling, cell proliferation, etc. (Nakav et al., 2008; Ohana et al., 2001; Sebastiao and Ribeiro, 2009). Adenosine receptors (or P1 receptors) are G-protein coupled purinergic receptors with adenosine as the endogenous ligand. There are four types of adenosine receptors in humans: A_1, A_{2A}, A_{2B} and A_3 encoded by ADORA1,

ADORA2A, ADORA2B and ADORA3 genes respectively (Fredholm et al., 2001; Fredholm et al., 2011).

The main function of the A_1 and A_3 subtypes are to reduce the production of cAMP from ATP via G_i protein, while A_{2B} and A_{2B} subtypes increase the cAMP production by activating adenylate cyclase via Gs alpha subunit. Additionally, A_1 receptors couple to G_o also, which mediate adenosine inhibition of Ca^{2+} conductance, while A_{2B} and A_3 receptors can also couple to G_q and stimulate phospholipase activity (Hasko and Cronstein, 2004). Beside adenosine, the common agonist, adenosine receptors have common antagonists also, these are theophylline and caffeine. Figure 4 below shows the concentration dependent effects of caffeine.
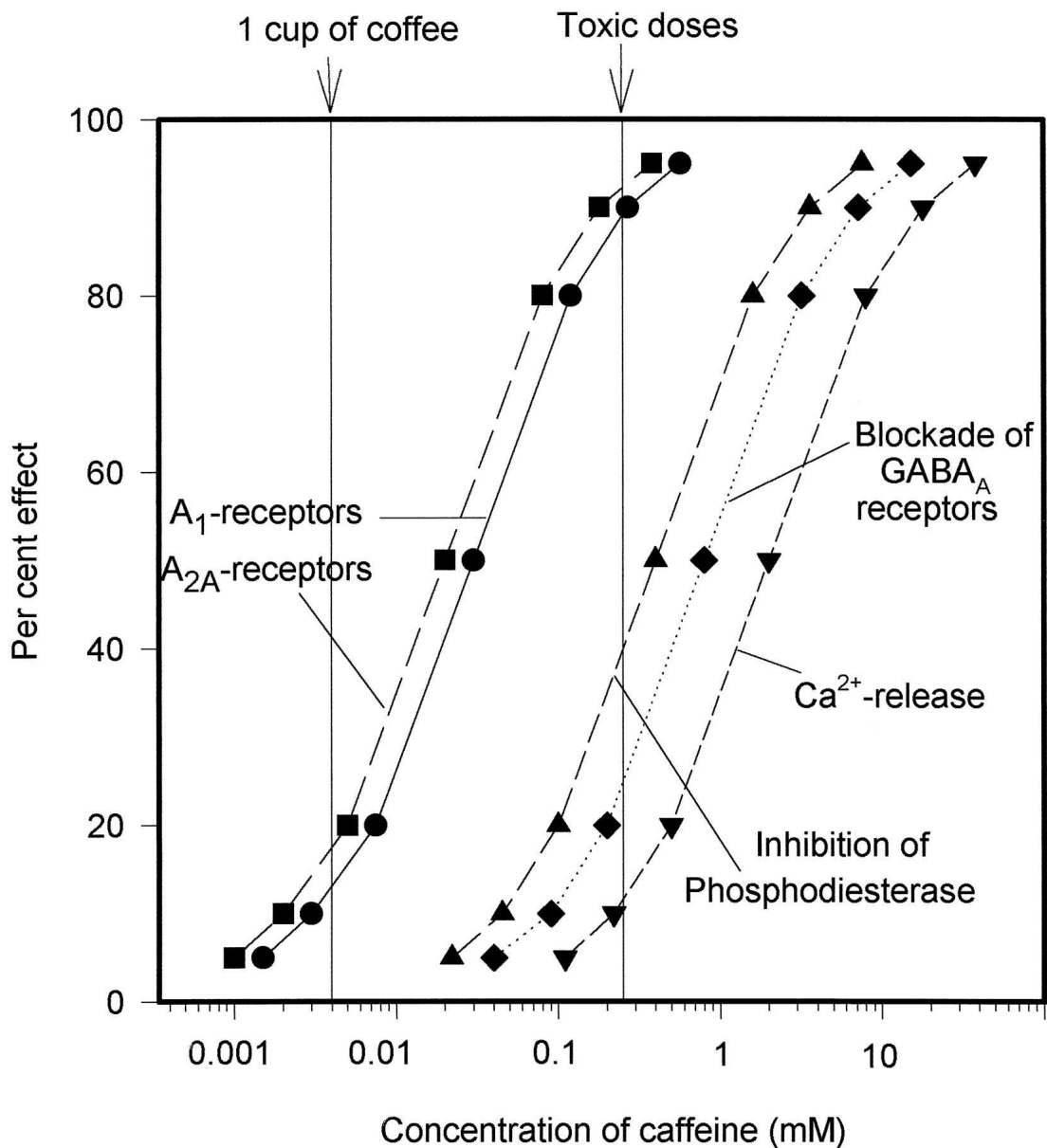
Figure 4. The Effect of caffeine on different biochemical targets in relation to its levels in humans. It is clearly visible that caffeine is able to significantly block adenosine effects on A_1 and A_{2A} receptors already at the low concentrations (achieved after a single cup of coffee). Reprinted from Bertil B. Fredholm, Karl Bättig, Janet Holmén, Astrid Nehlig, and Edwin E. Zvartau. Actions of Caffeine in the Brain with Special Reference to Factors That Contribute to Its Widespread Use, Pharmacol Rev March 1, 1999 51:83-133 with the permission of the corresponding author and the Journal's Director, find the permission letter attached in the Appendices section (Fredholm et al., 1999a).

Highly selective ligands and validated antibodies are available to precisely localize the A_1 and A_{2A} receptors in the brain (Fredholm et al., 2005). A_{2A} receptors are mainly located in the basal ganglia, caudate putamen, tuberculum olfactorium, olfactory bulb, nucleus accumbens and hippocampus, but at lower levels they are also expressed in the rest of the brain (Fredholm et al., 2000, Fredholm et al., 2005a, Rebola et al., 2005a), while A_1 receptors are present in almost all parts of the brain, but can be primarily found in the hippocampus, cerebellum and cerebral cortex (Mahan et al., 1991, Reppert et al., 1991, Fredholm et al., 2005b). The mechanisms of the psychostimulant effects of caffeine is the ability to release brakes that adenosine imposes on dopaminergic neurotransmission (both pre- and post-synaptically) by acting on different adenosine receptor heteromers localized in different elements of the striatal spine module (Ferré, 2008). Available data of both animal (Cunha and Agostinho, 2010; Duarte et al., 2009) and human (Arendash and Cao, 2010, Eskelinen and Kivipelto, 2010, Ritchie et al., 2010) studies suggest that caffeine intake may induce long-term and short-term functional and morphological changes in specific parts of the brain. A systematic review and meta-analysis on caffeine intake and dementia by Santos and colleges gives a general overview on effect of coffee consumption on Alzheimer's disease, dementia, cognitive impairment and cognitive decline (Santos et al., 2010). In fetal and neonatal rats the adenosinergic system can indicate physiological and structural changes. The activation of A_1 adenosine receptors can profoundly influence the brain formation in the neonatal period (Rivkees et al., 2001). Large reductions in the subcortical and hippocampal white matter volumes are observed when neonatal rats are treated with A_1 adenosine receptor agonist (Turner et al., 2002). Numerous studies have

investigated the effect of coffee consumption on various health conditions, especially in brain disorders affecting the hippocampus-related memory functions. Hippocampal function is traditionally related to learning and memory as it underlies the ability to recall specific personal experiences (Squire, 1992, Vargha-Khadem et al., 1997, Tulving and Markowitsch, 1998). Today this approach became extended by other critical functions like prediction and imagination (Buckner, 2010), but the role of the hippocampus in memory retrieving remained. Recent studies found that there is a modest inverse association between coffee and all-cause mortality and this association can be mainly explained by a reduction in deaths due to cardiovascular disorders (Lopez-Garcia et al., 2008, Mukamal et al., 2009). The association between lower mortality and higher coffee consumption is stronger in women than men (Lopez-Garcia et al., 2008). There is an association between higher coffee consumption and lower risk for stroke (Lopez-Garcia et al., 2009), Parkinson disorder (Petzer et al., 2009), Alzheimer's disease, and dementia (Eskelinen and Kivipelto, 2010). Also, dysfunction of memory performance is normalized by chronic caffeine consumption, which is documented in both epidemiological (Ritchie et al., 2007; Santos et al., 2010) as well as in animal studies (Cunha and Agostinho, 2010). Thus, coffee seems to have beneficial effects on cardiovascular health, and it may have a role in treating or preventing some brain disorders and memory impairment.

Caffeine does not only affect adenosine receptors, but it also has important effect on Ca^{2+} currents. The mobilization of intracellular Ca^{2+} stores, localized in the endoplasmic reticulum takes place through receptors sensitive to ryanodine or inositol 1,4,5-trisphosphate (IP3). They play a key role in generation of the calcium signal (Ehrlich et al., 1994; Kostyuk et al., 1995; Mody and MacDonald, 1995; Ogawa, 1994; Simpson et al., 1995; Sitsapesan and Williams, 1990). In the hippocampal formation of the rat brain, ryanodine receptors (RyRs) are mainly expressed in the neurons of the dentate gyrus, while IP3 receptors (IP3Rs) are almost exclusively present in CAI neurons of the Ammon's Horn (Alaraj et al., 1998; Sharp et al., 1993; Worley et al., 1989). Using the microdialysis technique in rat gyrus dentatus *in vivo*, combined with radio-labeling of endogenous Ca^{2+} and measurement of Ca^{2+} efflux, Mohd Alaraj and colleagues demonstrated the NMDA - evoked Ca^{2+} release to the dialysate (Alaraj et al., 1998). Pharmacological characteristics of this

phenomenon correspond to calcium induced calcium release (CICR) throughout RyRs (Lazarewicz et al., 1998). Indeed, RyR-mediated CICR regulates numerous neuronal processes, including synaptic plasticity and LTP (Kohda et al., 1995), neurotransmitter release and exocytosis (Peng, 1996; Smith and Cunnane, 1996), and differentiation along with neurite outgrowth (Gomez et al., 1995; Holliday et al., 1991).

2.4.1.1 Research possibilities

The above discussed properties of caffeine and its beneficial effects carry the question of weather caffeine itself is associated with any kind of visible and measurable difference in brain morphology or not. Although the required methodologies are available to "extract" this information from structural MRI images, there were no previous studies trying to establish such association.

So the question is, how could someone search for the missing association?

Recently developed automated-software based brain segmentation methods allowed us to use semi-automatic MR volumetry in user-independent manner by assessing the volumes of subcortical brain structures in healthy people and patients (Fischl et al., 2002, Auer et al., 2008, Morey et al., 2009, Shen et al., 2010).

Using voxel-based morphometry, it is possible analyze the relationship between coffee consumption and the cortical architecture, because small alterations in cortical morphology can be more accurately demonstrated by this technique (Good et al., 2001). Although caffeine is the most often used substance acting on the central nervous system (Johnson-Greene et al., 1988), associated with lower risk of some brain disorders and accompanied by neuroplastic changes in experimental conditions, to our knowledge, no studies have been conducted investigating the relationship between coffee consumption and human brain structure. In order to avoid confounding effects of sex-specific physiological differences in the brain structure (Witte et al., 2010), in mechanism of caffeine action (Ritchie et al., 2007, Lopez-Garcia et al., 2008, Noschang et al., 2009), in caffeine consumption habits (Von Post-

Skagegård et al., 2002), as well as effects of pathological conditions associated with coffee consumption (nicotine and alcohol abuse) (Hewlett and Smith, 2006; Istvan and Matarazzo, 1984) or ageing (neurovascular and neurodegenerative disorders), the included sample must be as homogenous as possible.

One such way is to exclusively include healthy, young, non-smoking women. Caffeine, - the most prominent bioactive component of coffee - is the most widely used psychoactive substance in the world; about 90% of the population (including children) in the United States regularly consume caffeine-containing beverages or foods (Frary et al., 2005). If we take the beneficial effects of caffeine in various pathological conditions mentioned above (cardiovascular disorders, stroke, Parkinson disorder, Alzheimer's disease, dementia) and it's prevalence in our daily life, investigating the effect of coffee consumption on human brain may have not only theoretical, but also highly relevant clinical importance.

2.4.2 Obesity and the Body Mass Index

38 years ago, an editorial was published in the Lancet that named obesity as "the most important nutritional disease in the affluent countries of the world" (Lancet_Editorial, 1974), (see figure 5 for more details).
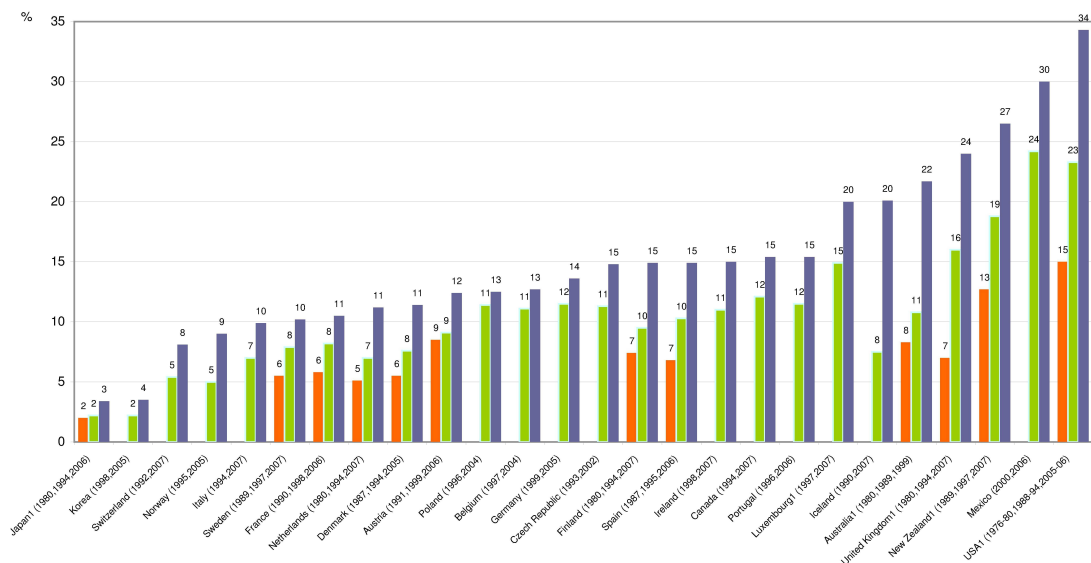

Figure 5. Increasing obesity rates among adults in OECD countries

1. Australia, Czech Republic (2005), Japan, Luxembourg, New Zealand, Slovak Republic (2007), United Kingdom and United States figures are based on health examination surveys, rather than health interview surveys. Source: (OECD_Health_Data, 2009)

Obesity is simply defined as a condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired (World_health_statistics_annual, 1995). Obesity has numerous health consequences ranging from the increased risk of premature death to many non-fatal but serious debilitating issues that have adverse effect on life quality. Obesity is the major risk factor for several non-communicable diseases such as non-insulin dependant diabetes mellitus, cardiovascular diseases, cancer, etc. and associated with various psychosocial problems in industrialized countries (WHO_TRS_894, 2000).

The body mass index (BMI), or Quetelet index, is a heuristic proxy for human body fat based on an individual's weight and height. The quest for a practical index of relative body weight that began shortly after actuaries reported the increased mortality of their overweight policyholders culminated after World War II, when the relationship between weight and cardiovascular disease became the subject of epidemiological studies. It became evident then that the best index was the ratio of the weight in kilograms divided by the square of the height in meters. It was invented by Adolphe Quetelet (1796–1874) a Belgian mathematician, astronomer and statistician, who developed a passionate interest in probability calculus that he applied to study human physical characteristics and social aptitudes (Eknoyan, 2008). During the century-long usage of the index, studies based on large populations and long follow-up durations have demonstrated a relationship between elevated BMI and mortality from all causes, especially from vascular disorders (Hoffmans et al., 1988, Manson et al., 1990, Winter et al., 2008). Obesity is a risk factor for many brain disorders including cerebrovascular (Lapidus et al., 1984, Larsson et al., 1984, Haapaniemi et al., 1997), Parkinson's (Chen et al., 2004) and Alzheimer's diseases (Gustafson et al., 2003). These brain disorders as well as hypertension alone are associated with structural brain abnormalities even in the pre-clinical phases (Karas et al., 2003, Raz et al., 2003, Taki et al., 2004). There are many functional neuroimaging studies which have analyzed the neural mechanism of human food intake, body weight regulation, and the pathomechanism of obesity

(Holsen et al., 2005, Holsen et al., 2006, Passamonti et al., 2009, Stoeckel et al., 2009, Tomasi et al., 2009, Wang et al., 2009, Grabenhorst et al., 2010). There are also six studies investigating the morphological changes associated with BMI (see Table 1).

Table 1. Principal characteristics of MR studies investigating the association between the body weight and brain structures

Investigators	MR method	Investigated population	Age (ys)	Investigated regions	Results	Controlled Confounder Factors
Haltia, et al	VBM	Group 1. 30 obese (before and after weight lose due to diet) Group 2. 16 lean subjects	Group 1. Mean age: 37 Group 2. Mean age: 37	Whole brain	Obese subjects had *greater* relative WM volumes in: superior, middle, and inferior T gyri, fusiform gyrus, parahipp.gyrus, brain stem, cerebellum A *positive* correlation was seen between WM volume and waist to hip ratio in the obese group in T. lobes, brain stem, and cerebellum, occ lobe.	Sex Total Intracranial volume
Gazdzinski, et al.	MRS, MR volumetry in the four lobes	50 healthy, middle-aged (33 men, 17 women)	Mean age: 41.7	Whole brain	Greater BMI was associated with *lower* NAA concentrations in Fr, P, and T WM, Fr GM, and *lower* Cho confrontation in Fr WM.	age, sex, smoking Total intracranial volume
Panacciulli, et al.	VBM	Group 1. 24 obese (11 men, 13 women) Group 2. 36 lean (25 men, 11 women)	Group 1. Mean age: 32 Group 2. Mean age: 33	Whole brain	Obese individuals had *lower* GM density in the post-central gyrus, Fr operculum, putamen, and middle Fr gyrus. BMI was *negatively* associated with GM density of the left post-central gyrus in obese but not lean subjects.	Sex, age
Ward, et al.	Global brain volume	114 middle-aged healthy subjects	mean age: 54	Whole brain	*decreased* whole brain volume	Sex, age

Investigators	MR method	Investigated population	Age (ys)	Investigated regions	Results	Controlled Confounder Factors
Taki, et al.	VBM	1428 healthy individuals	mean age in men: 44.5	Whole brain	*Negative* correlation in men but not in women: between BMI and the whole GM. *Negative* correlation in men between BMI and relative GM volume in medial T lobes, anterior cerebellum, superior P lobule, Occ lobe, inferior and superior Fr gyri, precuneus, and midbrain.	age, sex, alcohol, hypertension, diabetes
			mean age in women: 46.4		*Positive* correlations with BMI: posterior cerebellum, inferior and superior Fr gyri, inferior, middle, and superior T gyri, thalamus, precentral gyrus, and caudate heads	
Walther, et al.	VBM	95 community-dwelling older females	Range: 52-92 ys	Whole brain	Higher BMI was associated with *decreased* GM volumes in the OFC, inferior Fr, precentral, parahippocampal, fusiform, and lingual gyri, right cerebellar regions, as well as with *increased* WM volumes in the Fr, T, P lobes	Hypertension

One of these studies (Ward et al., 2005) found that obesity in middle-aged subjects is associated with brain atrophy. Gazdzinski et al. performed a magnetic resonance spectroscopy study in middle-aged healthy subjects and found that a greater BMI was associated with lower N- acethly-aspartate (NAA) concentration in grey-matter and lower choline and NAA concentration in white matter (Gazdzinski et al., 2008). Others (Haltia et al., 2007) performed an MR study in middle-aged subjects using voxel-based morphometry (VBM) and found no differences in obese vs. non-obese people concerning the grey matter but there were white-matter abnormalities in various parts of the brain (see Table 1). Using VBM-MR, Panacciulli found (Pannacciulli et al., 2006) that obese middle-aged individuals had a lower grey-matter density in the post-central gyrus, frontal operculum, putamen, and middle frontal gyrus. Using VBM-MR, Walther et al. found that higher BMI was associated with decreased grey- matter and increased white-matter volumes in various parts of the brain in the elderly (see Table 1, (Walther et al., 2010)). Using VBM-MR, Taki et al. showed a negative correlation between BMI and the whole grey-matter volume in middle-aged men but not in women. They found that in men the regional grey-matter volume of the medial temporal lobes, cerebellum, occipital lobe, frontal lobe, precuneus, and midbrain showed significant negative correlations with BMI, while volumes of the inferior frontal gyri, posterior cerebellum, frontal and temporal lobes, thalami, and caudate heads showed positive correlations with BMI (Taki et al., 2008). The major drawback of all of these studies is that they include middle-aged subjects (the mean age was >32 years in all studies; see Table 1), in whom obesity-associated disorders can be present even without clinical signs. The huge variability of regions found to be associated with BMI in these studies and the numerous contradictory results might indicate that the cause/effect between BMI and brain structure, as well as the effect of obesity- associated brain disorders may confound the data in these studies. For example, the grey- matter density in superior and inferior frontal gyri showed both positive as well as negative association with the BMI even within one study (Taki et al., 2008). Table 1 demonstrates that in these studies the analysis of an association between brain structure and BMI was controlled only for a few confounding factors.

2.4.2.1 Research possibilities

It was shown that numerous studies with several different MRI techniques were conducted to establish associations between BMI and different properties of the human brain, including metabolite ratios, volume or gray matter density (see Table 1 for further details). However, the results are controversial and it is clear that these controversies are probably the direct results of the numerous confounding factors.

Yet, it is clear that investigating this relationship is of utmost importance, as obesity represents a major public health problem in industrialized as well as in developing countries. Based on previous studies shown above, it is reasonable to assume that a long-term change in body weight is associated with altered brain structure via obesity-associated brain disorders. BMI (body mass index) is one of the most commonly used indices of obesity, that makes BMI an ideal measure to be associated with the morphological changes. Indeed, MR volumetric studies showed a relationship between BMI and cerebral atrophy (Lapidus et al., 1984, Gustafson et al., 2004, Ward et al., 2005). Thus, due to the possible bidirectional relationship between brain structure and body weight, if someone intended to investigate the relationship between body weight and brain structure in humans, it is difficult to separate the cause/effect relationships between the observed parameters. In my opinion, regarding middle-aged or elderly subjects, it is almost impossible to control all confounders because many conditions may lead to obesity and obesity is a risk factor for numerous disorders (see above).

Therefore, the ideal experimental set-up would be the investigation of a homogenous group with young people and controlled for all possible confounding factors. The involvement of young subjects is also necessary because the roots of obesity originate in childhood (Mietus-Snyder and Lustig, 2008). Moreover, none of the previous studies have used brain-segmentation MR techniques for automated parcellation of anatomic structures which are one of the most reliable methods for investigating the subcortical structures (Fischl et al., 2002) and removes all user-related bias from the evaluation.

These subcortical structures play the pivotal role in the pathomechanism of obesity. According to animal and human experiments, different structures in the

reward system of the brain, especially amygdale (Lénárd and Hahn, 1982, Lénárd et al., 1982, Karádi et al., 1998, Holsen et al., 2005, Abraham et al., 2009, Grabenhorst et al., 2010), hippocampus (Holsen et al., 2006), orbitofrontal cortex (Grabenhorst et al., 2010; Lukáts et al., 2005), accumbens region (Abraham et al., 2009), caudate nucleus, putamen, and the hypothalamus (Druce and Bloom, 2003, Mietus-Snyder and Lustig, 2008, Stice et al., 2008, Stoeckel et al., 2008, Passamonti et al., 2009, Stoeckel et al., 2009) play a significant role in the body weight regulation.

It is reasonable to assume that differences in these brain structures may be associated with differences in food/energy intake regulation due to different activity of the reward system. This may lead to differences in body weight. The hyperactivity of the reward system may result in excessive food intake and chronic positive energy imbalance. This may be the primary cause of human obesity (Nielsen et al., 2002, Stoeckel et al., 2008).

3. AIMS

The main goal of this thesis is to give a detailed overview on two possible MRI methods to identify gray or white matter changes/differences in different groups. It is demonstrated with two original studies, using different approaches to assess the presumed differences. Thus, the thesis has two set of aims, one for each study.

3.1 Caffeine

In the present study, we aimed to investigate the relationship between coffee consumption habits and brain morphology characterized by the volume of total brain, neocortex, and subcortical brain structures (basal ganglia, hippocampus, accumbens region) where caffeine is supposed to act.

3.2 Body Mass Index

We aimed to investigate the relationship between BMI (body mass index) and the volumes of the structures within the reward system (hippocampus, amygdala, accumbens, caudatum, putamen, and orbitofrontal cortex) showing a prominent role in the food/energy intake regulation.

4. MATERIALS AND METHODS

The following sections (Materials and methods, Results and the first part of the Discussion) will be split in two, as the two demonstrative studies had different methods, subjects and results to be discussed. Yet, the methods have the same purpose, to identify differences among groups in gray/white matter morphology attributable to the studied extrinsic or intrinsic factor.

4.1 Caffeine

Subjects

Based on an advertisement placed on notice boards across the University of Pécs, 45 (aged 23.2±2.7 years) healthy right-handed, Caucasian, female, graduate or postgraduate university students without history of brain disorders, smoking, or drug/alcohol abuse between the age of 19 and 30 were recruited and included. Each of them completed the same questionnaire regarding caffeine consumption (coffee, cola, tea, caffeine tablets, chocolate, and energy drinks), smoking, alcohol consumption, medications and health issues. The questionnaires were evaluated and the results were summed in SPSS 17.0 (SPSS Inc., Chicago, IL) for further processing. Daily caffeine intake was calculated based on the following assumptions for caffeine content: Coca Cola (regular, light and zero): 13.5 mg/100 ml; Pepsi Cola: 11 mg/100 ml; Pepsi light: 10.5 mg/100 ml; Coffee: 120 mg/cup; Coffee (decaffeinated) 2 mg/mug; Tea (green) 40 mg/mug; Tea (black) 70 mg/mug; Chocolate (dark) 62 mg/100 g; Chocolate (milk) 25 mg/100 g; Energy Drinks: 115 mg/can. (Cup= 50 ml; Mug=275 ml; Can=250 ml). According to coffee consumption, we divided the 45 subjects into three groups: (i) 20 subjects with low coffee consumption who drink no coffee or drink <1 cup of coffee in a day, (ii) 14 subjects with moderate coffee consumption who drink 1–3 cups of coffee in a day,

(iii) 11 subjects with high coffee consumption who drink ≥4 cups/day. The total caffeine intake was calculated from the consumption of coffee and the other food products containing caffeine. According to the total caffeine intake, we divided subjects into three groups proposed by previous studies (Webb et al., 1996): (i) 11 subjects with high caffeine intake (>400 mg/day), (ii) 23 subjects with moderate caffeine intake (100–400 mg/day), (iii) 11 subjects with low caffeine intake (<100 mg caffeine/day). All caffeine-related questions were focused on the last 12 months. Asking for self-reported coffee consumption is a reliable way to assess the real coffee consumption and generally used to estimate the chronic coffee intake in most coffee-related studies (Lopez-Garcia et al., 2008). This study was approved by the Ethics Committee of the University Pécs and all subjects gave written informed consent before each examination.

Magnetic resonance examinations and visual analysis

All measurements were performed on a 3 T Magnetom TRIO human whole-body MRI scanner (Siemens AG, Erlangen, Germany) with a 12-channel head coil. The total measurement time was approximately 7 min. For volumetric analysis, a T1-weighted axial MPRAGE sequence was used to measure with the following parameters: TR/TE/
TI:1900/3.41/900 ms, FOV: 240 mm, 256×256 matrix, slice thickness: 0.94 mm, (0.94×0.94 mm in plane resolution), slice number: 160, FA: 9°, bandwidth: 180 Hz/pixel, FOV Phase: 87.5%. For standardized and accurate axial slice positioning the anterior and posterior commissural line (ACPC line) was used as a reference determined by a T2-weighted turbo spin echo sequence measured in the sagittal plane. There were no brain abnormalities according to the visual analysis of the MRI images.

MR data processing evaluation

We evaluated the data with two different post-processing methods. Semi-automatic MR volumetry was used based on automated brain segmentation for assessing volumes of the subcortical structures. The cortical microstructure was evaluated by voxel-based morphometry.

Automated MR volumetry

Freesurfer 4.4.0 (http://surfer.nmr.mgh.harvard.edu) was used for the whole evaluation. This software provides one of the most reliable automated brain segmentation methods for subcortical structures and allows us to assess the volume of the pre-defined brain structures in a large amount of subjects (Fischl et al., 2002, Morey et al., 2009). Freesurfer's semi-automatic anatomical processing scripts (autorecon1, 2 and 3) were executed on all data. Manual verifications were performed after each script, and manual adjustments were applied where it was necessary, based on the recommended reconstruction workflow of the software, freely accessible at the FreesurferWiki website (Freesurfer-Wiki_II). The investigators who manually verified the evaluation were blinded to experimental data. The volume-based stream is designed to preprocess MRI volumes and label subcortical tissue classes. The stream consists of several stages fully described by Fischl and colleagues. (Fischl et al., 2002). The final segmentation is based on both a subject independent probabilistic atlas and subject-specific measured values. Figure 6 shows the representative labels of hippocampi on a single subject resulting from semiautomatic MR volumetry. To avoid bias due to different head size, the volumes of right-sided and left-sided brain structures were summed and divided by total intracranial volume. Thus, except for the total brain volume and total intracranial volume, we used relative bilateral volumes of the investigated structures (neocortex, n. caudatus, pallidum, putamen, hippocampus, accumbens area).
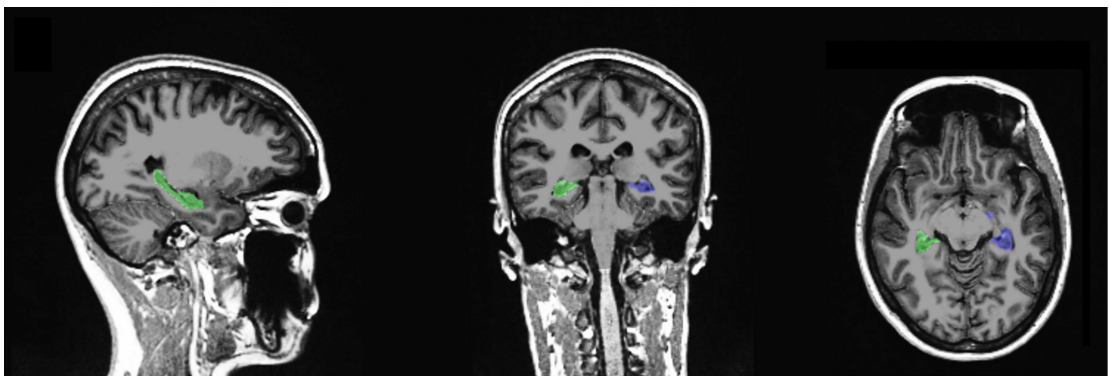

Figure 6. Representative labels of hippocampi on a single subject resulting from semi-automatic MR volumetry

Statistical analyses were performed using SPSS 17.0 software (SPSS Inc, Chicago, IL). Observing distributions of all volumes of the analyzed brain structures, we found no evidence against the normal distribution by Kolmogorov-Smirnov test ($p > 0.05$). We used a generalized linear model (GLIM) in order to define whether the volumes of predefined structures were associated with coffee consumption habits and estimated caffeine intake. Considering that we investigated a relatively homogenous group (non-smoking healthy young women), - considering age related volume differences across multiple samples of the investigated brain regions (Pakkenberg and Gundersen, 1997, Walhovd et al., 2011) - we controlled our model only for age and sleep complaints (only one patient reported relatively serious sleep complaints). Controlling for sleep habits was necessary because sleep habits may influence brain structure (Riemann et al., 2007, Neylan et al., 2010) and caffeine intake may be associated with sleep disorders (Levy and Zylber-Katz, 1983).

We performed the following GLIM models for each pre-defined brain structure: Dependent variable volume of one of the pre-defined brain structures (total brain volume, total intracranial volume, relative brain volumes of the neocortex, basal ganglia, hippocampus, and accumbens area). Independent variables (i) coffee consumption habits, (ii) age, and (iii) bad sleep quality. The same models were repeated when the total daily caffeine intake was included as an independent variable, instead of coffee consumption.

Voxel-based morphometry

Voxel based morphometry (VBM) was performed with FSL-VBM (http://www.fmrib.ox.ac.uk/fsl). An 'optimised' VBM protocol (Good et al., 2001) was carried out with FSL tools (Smith et al., 2004). First, structural images were brain-extracted using BET (Smith, 2002). Next, tissue-type segmentation was carried out using FAST (Zhang et al., 2001). The resulting grey-matter partial volume images were then aligned to MNI152 standard space using affine registration (Rueckert et al., 1999). The resulting images were averaged to create a study-specific template, to which the native grey matter images were then nonlinearly re-registered.

The registered partial volume images were then modulated (to correct for local expansion or contraction owing to the non-linear component of the transformation) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxelwise GLM was applied using permutation-based non-parametric testing (randomise with 5000 permutations), correcting for multiple comparisons across space. Results were considered significant for $P < 0.05$, corrected for multiple comparisons using "threshold-free cluster enhancement" (TFCE), which avoids making an arbitrary choice of the cluster-forming threshold, while preserving the sensitivity benefits of cluster-wise correction (Smith and Nichols, 2009). Age was included as nuisance variable for grey matter density comparisons.

ROI-based VBM analysis was carried out using the hippocampal label from the 2 mm isotropic Harvard-Oxford Subcortical Atlas with 25% probabilistic threshold (HarvardOxford-sub-maxprob-thr25-2mm). To get the ROI based results, a new randomise script was executed with the "–m mask_file_name" switch. Results were considered significant for $P < 0.05$, corrected for multiple comparisons using "threshold-free cluster enhancement" (TFCE), identically to whole-brain VBM analyses.

4.2 Body Mass Index

Subjects

Based on an advertisement placed on notice boards at the University of Pécs, 103 (44 male and 59 female, aged 23.34±2.67 years) healthy, right-handed, Caucasian university students without history of brain and/or eating disorders, or drug/alcohol abuse, aged between 19 and 30 were recruited. All subjects were asked about health issues and diet. Three subjects were excluded because they were on a special (total-vegetarian) diet. This study was approved by the Ethics Committee of

the University of Pécs and all subjects gave written informed consent before each examination.

MR examinations

All measurements were performed on a 3T Magnetom TRIO human whole-body MRI scanner (Siemens AG, Erlangen, Germany) with a 12-channel head coil. The total measurement time was approximately 7 min. For volumetric analysis, T1-weighted axial MPRAGE sequence was used to measure with the following parameters: TR/TE/TI:1900/3.41/900 ms, FOV: 240 mm, 256×256 matrix, slice thickness:0.94 mm, (0.94×0.94 mm in plane resolution), slice number: 160, FA: 9°, bandwidth: 180 Hz/pixel, FOV Phase: 87.5%. For standardized and accurate axial slice positioning the anterior and posterior commissural line (AC-PC line) was used as a reference determined by the analysis of a T2-weighted turbo spin echo sequence measured in the sagittal plane.

MR data processing evaluation

Freesurfer 4.4.0 (http://surfer.nmr.mgh.harvard.edu) was used for the whole evaluation. This software provides one of the most reliable automated brain segmentation methods for subcortical structures and allows us to assess the volumes of the pre-defined brain structures in a large number of subjects (Fischl et al., 2002, Morey et al., 2009, Pardoe et al., 2009). Freesurfer's semi-automatic anatomical processing scripts (autorecon1, 2 and 3) were executed on all subjects' data. Manual verifications were performed after each script, and manual adjustments were applied where it was indicated. The volume-based stream is designed to preprocess MRI volumes and label subcortical tissue classes. The stream consists of several stages fully described by (Fischl et al., 2002). The final segmentation is based on both a subject independent probabilistic atlas and subject-specific measured values. Data of 8 subjects were excluded due to excessive head movements (bad image quality).

Statistical analysis

Statistical analyses were performed using SPSS 17.0 software (SPSS Inc, Chicago, IL). A multivariate general linear model (GLM) analysis was performed. In this model, the investigated structures (Table 4) were included as dependent variables while BMI served as an independent variable. Since the volume of brain structures may be influenced by sex and head size (total intracranial volume), they were included as covariates into our GLM model, in order to control the potential confounders. We also checked the interactions between BMI and covariates. Distributions of all volumes of the analyzed brain structures and the BMI were not different from normal distribution per the Kolmogorov-Smirnov test (p>0.05).

5. RESULTS

5.1 Caffeine

The GLIM analyses revealed that among the analyzed brain structures by semiautomatic MR volumetry, only the volume of the hippocampus was associated with coffee consumption (p for test for linear trend=0.012). Because we observed a U-shape curve by exploring the data, therefore, through the analyses we tested also for quadratic trends (p for test for quadratic trend=0.001). Table 2 shows the descriptive statistics in the three groups.

Table 2. Association between coffee consumption, the relative volume of the investigated structures, and the intracranial volume

Coffee consumption	Hippocampus (mean±SE)	Neocortex	Accumbens region	Putamen	Pallidum	Caudatum	Total intracranial volume (mean±SE)
Low (<1 cup/day)	0.56%±0.03	32.1%±1.7	0.077%±0.016	0.69%±0.06	0.20%±0.01	0.47%±0.05	1.57 dm^3±0.09
Moderate (1-3 cups/day)	0.52%±0.03	31.4%±1.6	0.076%±0.008	0.69%±0.05	0.20%±0.02	0.46%±0.05	1.58 dm^3±0.16
High (≥ 4 cups/day)	0.56%±0.03	32.4%±1.7	0.079%±0.01	0.70%±0.06	0.20%±0.02	0.48%±0.04	1.56 dm^3±0.1

Relative volumes of the investigated structures are presented: To avoid bias due to different head size, the volumes of right-sided and left-sided structures were summed and divided by total intracranial volume.

Pairwise comparison corrected for age and sleep complaints showed that the difference between subjects with low vs. moderate coffee consumption (p=0.002) and subjects with high vs. moderate coffee consumption (p=0.013) was significant regarding the hippocampal volume. Thus, both high-level coffee consumption and coffee abstinence were associated with a larger hippocampus. We also repeated the GLIM analyses in the above described manner considering not only the coffee consumption but also the total daily caffeine intake. The performed GLIM analyses revealed that among these brain structures only the volume of the hippocampus was associated with caffeine intake (p=0.003). Table 3 shows the descriptive statistics in the three groups.

Table 3. Association between caffeine intake, the relative volume of the investigated structures, and the intracranial volume

Caffeine intake	Hippocampus (mean±SE)	Neocortex	Accumbens region	Putamen	Pallidum	Caudatum	Total intracranial volume (mean±SE)
Low (< 100 mg/day)	0.58%±0.03 *	32.1%±1.9	0.076%±0.016	0.70%±0.07	0.20%±0.01	0.47%±0.06	1.56 dm^3±0.06
Moderate (100-400 mg/day)	0.53%±0.03 *	31.7%±1.5	0.077%±0.012	0.69%±0.06	0.20%±0.02	0.47%±0.05	1.58 dm^3±0.14
High (≥ 400 mg/day)	0.56%±0.03 *	32.4%±1.7	0.079%±0.01	0.70%±0.06	0.20%±0.02	0.48%±0.04	1.56 dm^3±0.1

Pairwise comparison corrected for age and sleep complaints showed that the difference between subjects with low vs. moderate caffeine intake (p<0.001) and subjects with high vs. moderate caffeine intake (p=0.023) was significant regarding the hippocampal volume. Figures 7 and 8 demonstrate the U-Shape association between hippocampal volumes and coffee consumption as well as caffeine intake.
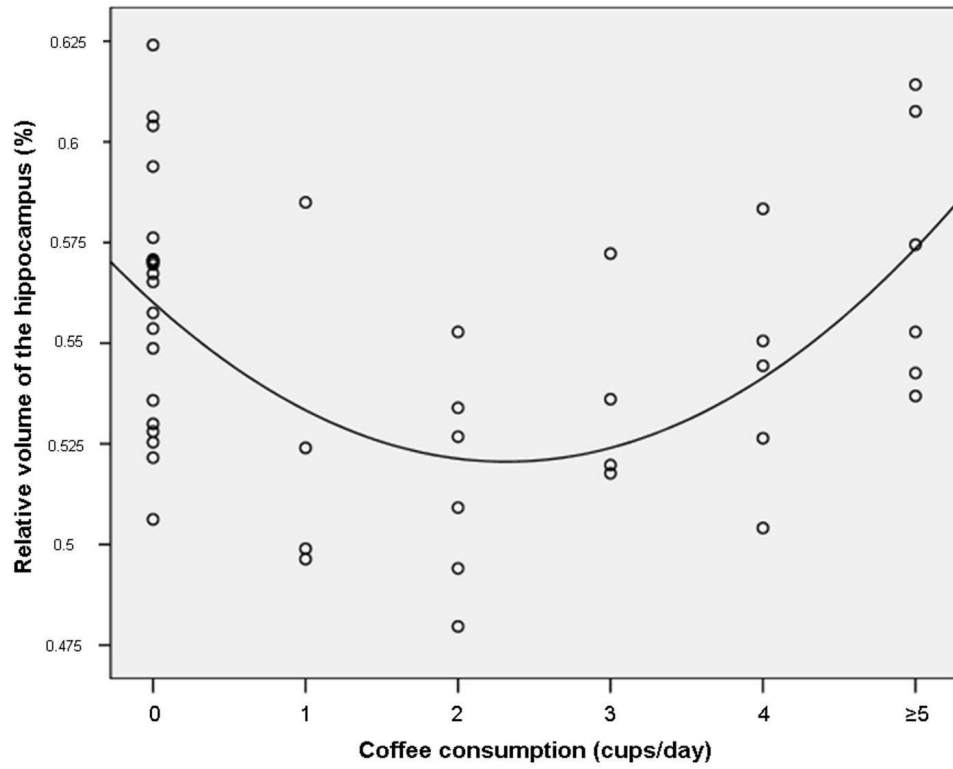
Figure 7. Scatter plots containing individual values of hippocampal volumes demonstrate the U-Shape association between hippocampal volumes and coffee consumption.
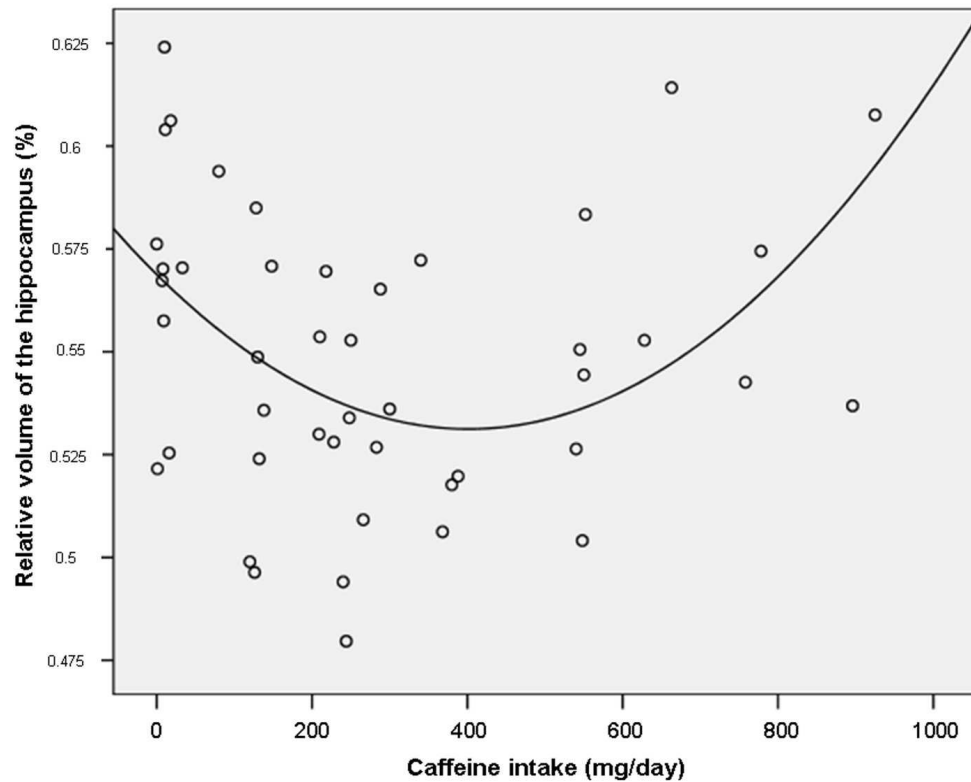
Figure 8. Scatter plots containing individual values of hippocampal volumes demonstrate the U-Shape association between hippocampal volumes and daily caffeine intake.

Using voxel-based morphometry for the whole brain, no significant differences could be demonstrated when testing for group differences between subjects according to coffee consumption or caffeine intake. However conducting VBM analysis only on the hippocampi have increased sensitivity to the accompanying grey matter changes, therefore VBM analysis was repeated limited to hippocampal regions as well.

This, ROI-based VBM of the hippocampi showed significantly increased gray matter density in subjects with low coffee consumption (and caffeine intake) compared to subjects with moderate coffee consumption (and caffeine intake), see Figure 9. High coffee consumption (and caffeine intake) seemed to be related to higher gray matter density in the hipoccampi compared to moderate coffee consumption (and caffeine intake), but it did not pass the significance test corrected for multiple comparisons across hippocampi.
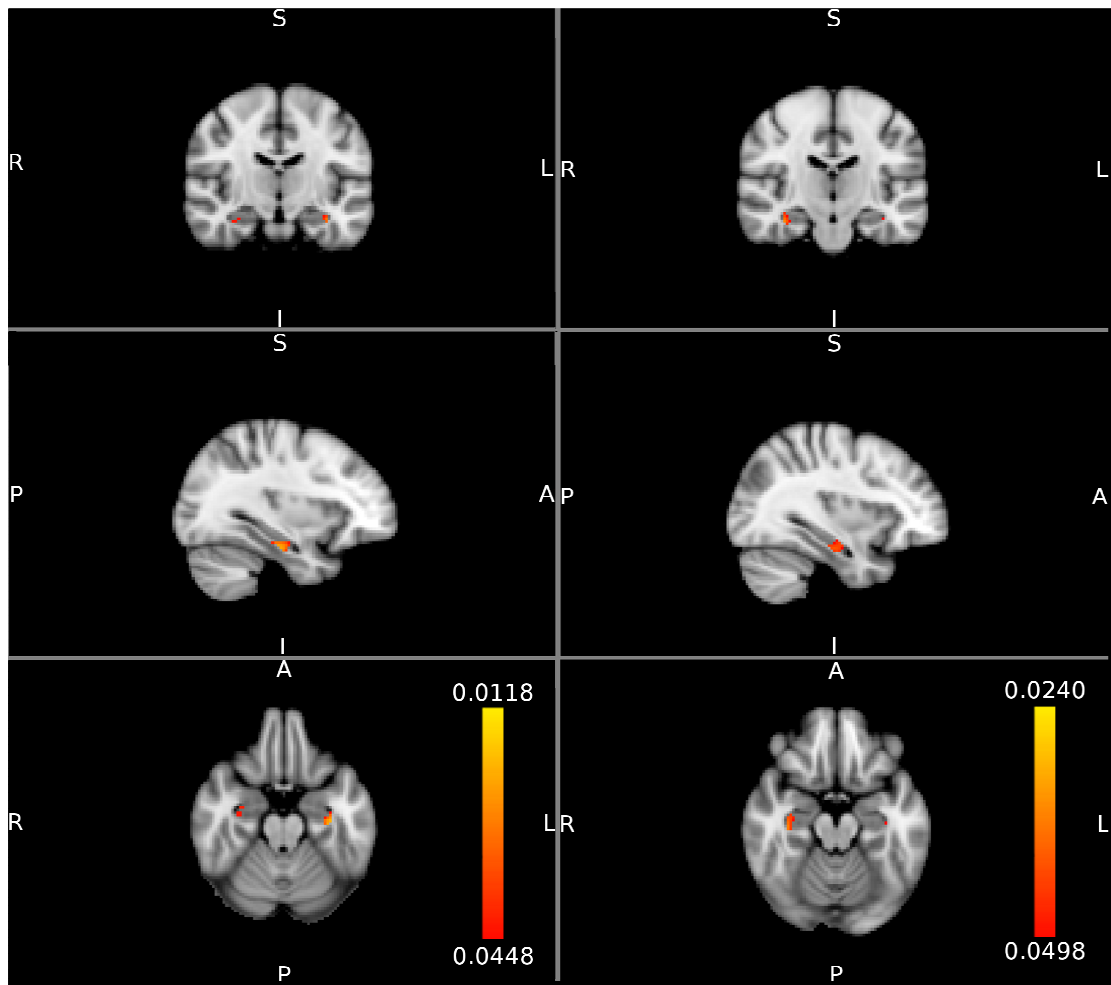
Figure 9. ROI-based VBM analyses shows significantly increased gray matter density in subjects with low coffee consumption compared to subjects with moderate coffee consumption (left column). Right column demonstrates significantly increased gray matter density in low caffeine intake compared to moderate caffeine intake. Results were considered significant for P<0.05, corrected for multiple comparisons using TFCE. Color bars indicate the corrected P values.

5.2 Body Mass Index

Ninety-two subjects (52 women) were included in the final analyses. Their mean age was 23.2±2.7 years (range: 19– 30 years), while mean BMI was 22.3±3.4 kg/m^2 (range: 16.4–33.9 kg/m^2). The histogram on Figure 10 shows the BMI distribution.
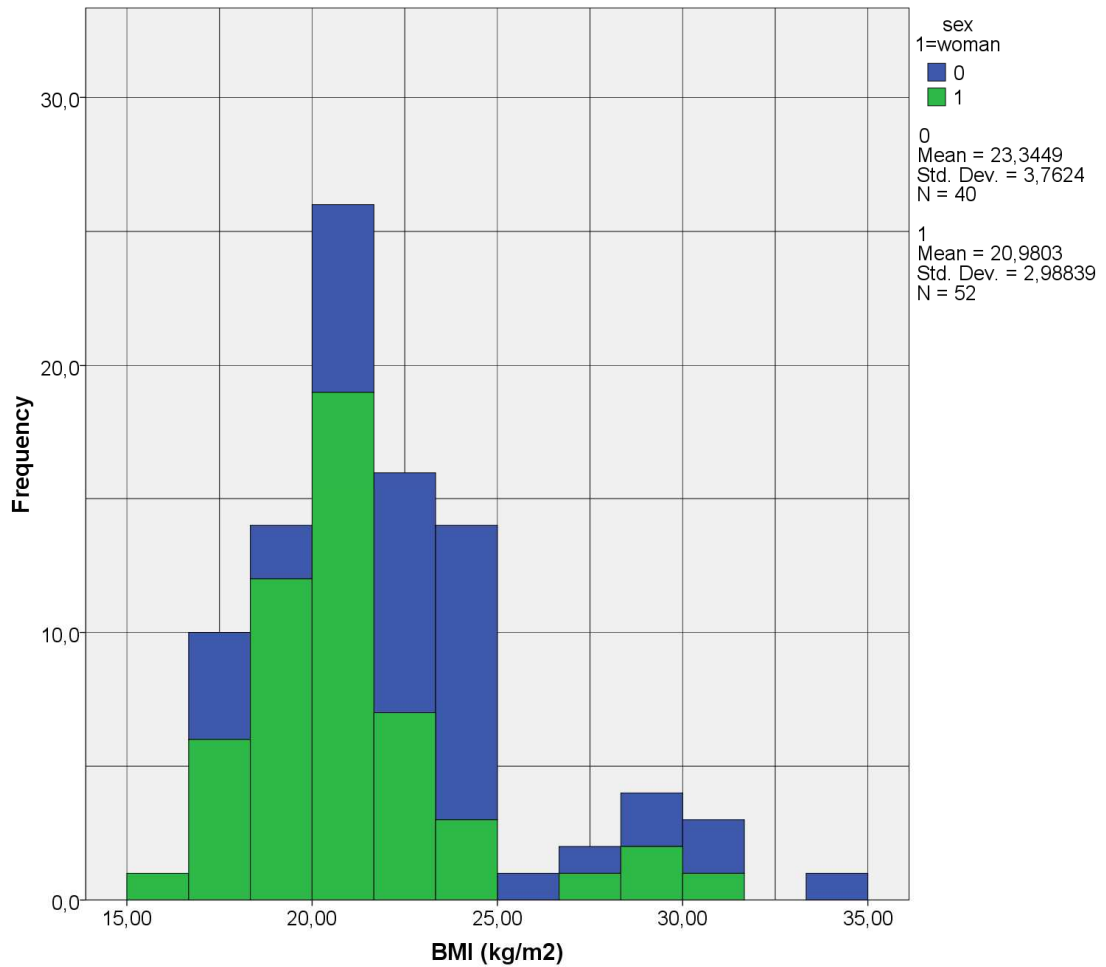
Figure 10. Histogram showing the distribution of the body mass index (BMI) in the examined population. Green shows the BMI distribution of women (1), while blue represents the BMI distribution of men (0). The columns are stacked, thus the BMI distribution of the whole population should be interpreted as blue + green in each columns.

Visual analysis of MR pictures showed no brain abnormalities. The results of the GLM analysis presented in Table 4 show that only the right amygdala had a significant association with BMI. There was an interaction between gender and BMI (p=0.007) for the volume of the right amygdala. In our subjects, the mean BMI of women was 21.0 ± 3.0 kg/m^2, while of men 24.0 ± 3.3 kg/m^2 (p<0.001).

Table 4 Association of the investigated brain structures with the BMI

Investigated brain structure:	P values*
Right-sided structures:	
Hippocampus	0.451
Amygdala	0.005
Accumbens region	0.07
Caudatum	0.39
Putamen	0.64
Orbitofrontal region	0.11
Left-sided structures:	
Hippocampus	0.72
Amygdala	0.06
Accumbens region	0.29
Caudatum	0.23
Putamen	0.93
Orbitofrontal region	0.58

* p-values are specific to the BMI term in the model

The gender differences in BMI and obesity among young people are well-known (CDC, Centers for disease and prevention., Sundquist and Johansson, 1998). Moreover, there are well-known gender differences among young adults in amygdala and other subcortical structures. Due to the gender differences and the interaction found by GLM, we further investigated the association between the relative volume of the right amygdala and BMI in both sexes separately. The relative volume of the right amygdala means that the absolute volume was divided by the total intracranial volume. Thus, we could demonstrate the relationship between the right amygdala and the BMI, taking into consideration that the gender and the head size may have an association with both amygdala volume and BMI. In women, there was no significant correlation between the relative volume of right amygdala and the BMI (Pearson's R=0.08, p=0.58).

Conversely, Figure 11 shows the highly significant positive association between the relative volume of the right amygdala and BMI in men (R=0.52, p=0.001).
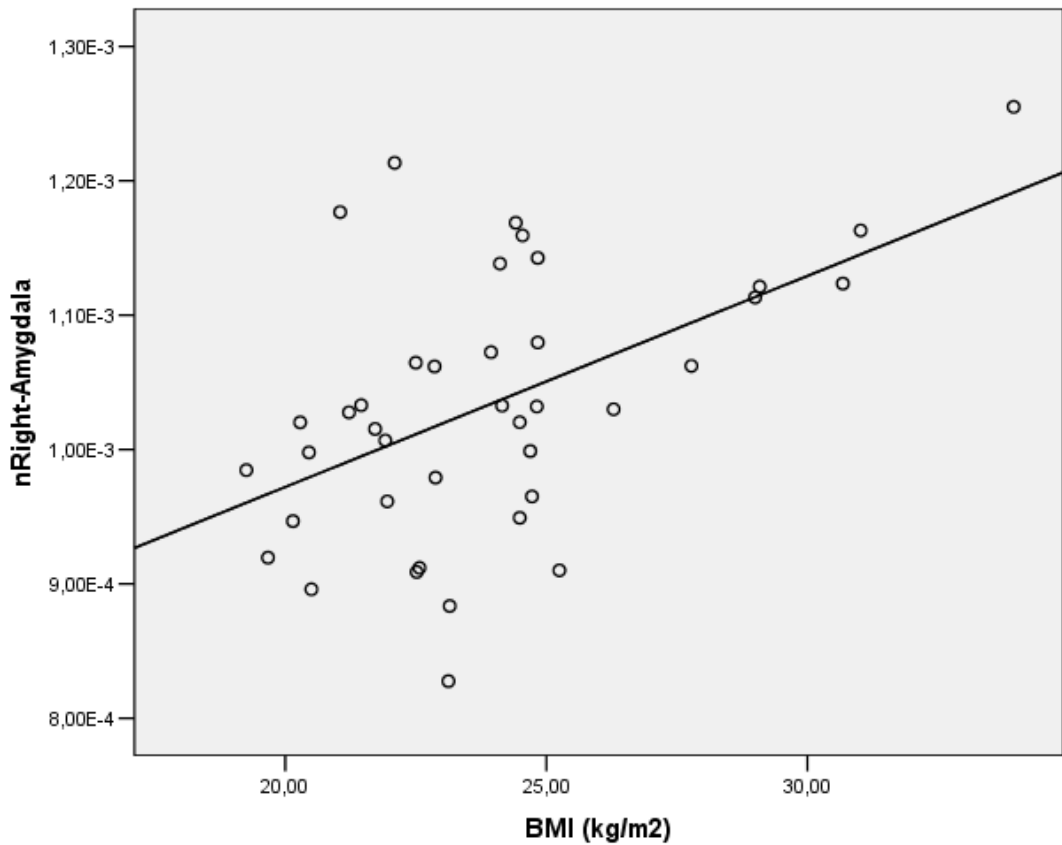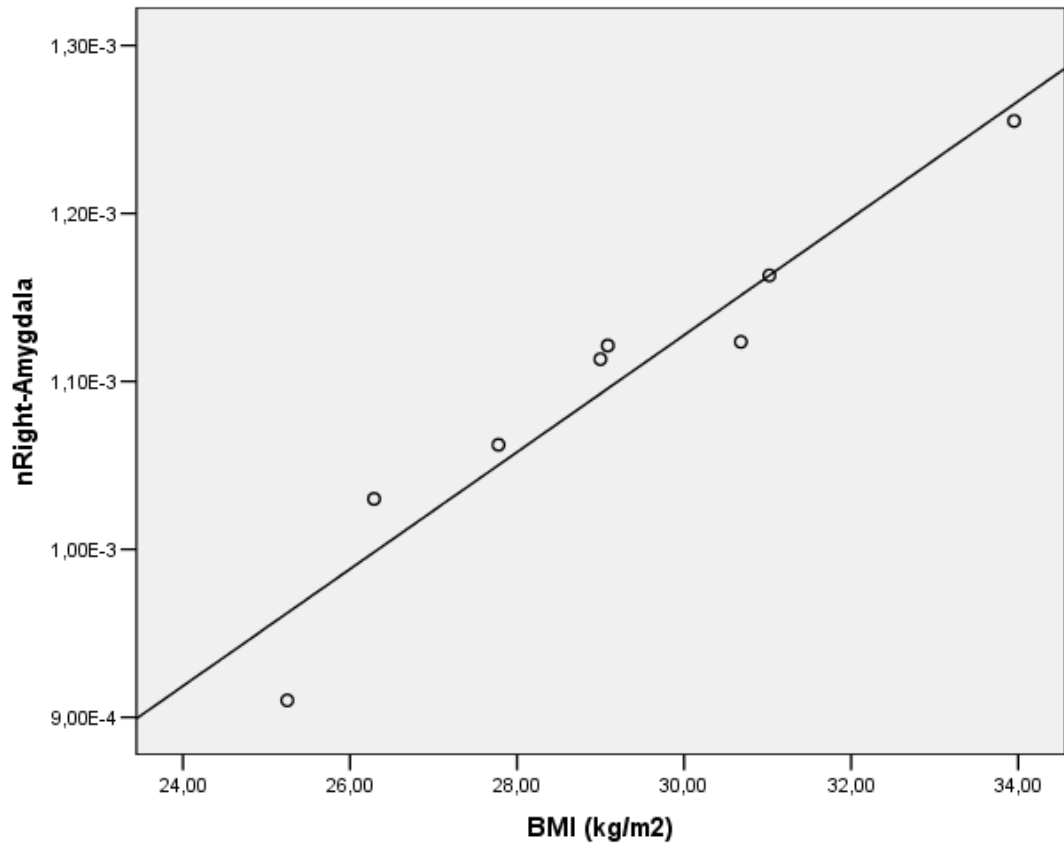

Figure 11. Bivariate correlation between the relative volume of the right amygdale and the body mass index in 40 men. Pearson's R=0.52, p=0.001.

Looking at Figure 11, we can see that the correlation between the volume of the amygdala and BMI in men was much more pronounced in the overweight male subpopulation. Thus, we investigated those 8 men with BMI>25 kg/m^2 separately. Figure 12 demonstrates this very strong positive correlation with high significance level (R=0.96, p<0.001).

Figure 12. Bivariate correlation between the relative volume of the right amygdale and the body mass index in 8 overweight men with BMI > 25. Pearson's R= 0.96, p<0.001.

The investigation of the remaining 32 non-overweight men have not revealed any significant correlations (R=0.27, p=0.13).

6. DISCUSSION

Computed Tomography (CT) and MRI, located within several research sites and most of today's radiology departments came to dominate the imaging of human anatomy in both research and clinical practice.

Out of the two demonstrated methods, VBM analyses are only available for MR images, while volumetry is applicable for both CT and MRI images. Automatic volumetric software packages are available for both modalities although for different purpose (i.e. structures). Yet, the modality itself limits the usability of CT and MR imaging for volumetric purpose.

CT is considered better for highly calcified elements, i.e. bones and organs where magnetic susceptibility artifact is prominent, e.g. the lungs. The general advantages of CT over MRI are availability, cost, speed, less susceptibility to distortions and that the level of required cooperation is lower. The resolution of a CT scan is adequate for volumetric evaluation, as a good resolution with isotropic $1mm^3$ voxels can be achieved.

However, the biggest disadvantage is the use of ionizing radiation that makes CT a generally unsuitable method for examining healthy controls in clinical or basic research, at least from the ethical point of view. A study indicated that radiation of CT scans is usually higher than cited, meaning that the 19,500 CT scans that were daily performed in the US (in 2007) were equivalent to 30 to 442 chest radiographs (X-rays) per scan, in terms of radiation. It has been estimated that CT radiation exposure will result in 29,000 new cancer cases just from the CT scans performed in 2007 (Nelson, 2009). Moreover, MRI is superior in many properties; MRI is capable of forming numerous different contrasts, by altering dozens of scanning parameters. It is very useful to differentiate different classes of soft tissues, which is beneficial in case of tissue segmentation. The intensity of a given voxel depends on a series of intrinsic parameters; these are spin-lattice relaxation time (T1), spin-spin relaxation time (T2), spin density, molecular diffusion and perfusion, chemical shift differences, susceptibility effects, etc. These made the MR images extremely rich in information content. Although MRI is slower than CT imaging, the MR scanner uses no ionizing

radiation, and the examination can be repeated several times without causing any harm to the subject, according to the current and generally accepted guideline on this topic (Coskun, 2011).

It is the flexibility in data acquisition and the rich contrast mechanisms of MRI that endow this technique with superior scientific and diagnostic values and prefer MRI over other imaging modalities.

The development of evaluation software packages have accelerated in the last decade, partly by the constant development of the scanner hardware, but the most remarkable impact on this development was due to the revolution in modern information technology. The sensitivity and reproducibility of both automated volumetry and voxel-based morphometry have greatly improved, thanks to the non-declining development of the last 10-15 years. By now, both methods are considered mature and evolved enough to be used in basic and clinical research reliably.

6.1 Caffeine

The main finding of our study is that high-level or low level coffee consumption (caffeine intake) was associated with a larger hippocampus compared to moderate-level coffee consumption. Other subcortical brain structures or cortical morphology showed no association with caffeine intake. Earlier studies found hippocampal volume changes related to various conditions such as Alzheimer's disease (Du et al., 2004, Hashimoto et al., 2005, Schuff et al., 2009), mild cognitive impairment (van de Pol et al., 2007), acute phase of major depression (Ahdidan et al., 2011), early onset depression (Gerritsen et al., 2011), environmental demands (Maguire et al., 2000), schizophrenia and bipolar disorder (Rimol et al., 2010). Despite the large number of the above mentioned volumetric studies and the fact that the effect of coffee consumption on human brain structure may have highly relevant clinical importance, the relationship between coffee consumption and the human brain morphology was not investigated before this study. The effects of adenosine

receptors on synaptic plasticity was previously reviewed (de Mendonça and Ribeiro, 2001), but only a few studies described the effects of caffeine on synaptic plasticity, and most of them did use very high concentrations of caffeine which were not related to physiological consumption of caffeine. A recent study suggests that relevant concentrations of caffeine inhibit the magnitude of frequency-induced long term potentiation (LTP) - possibly via A_{2A} receptors - in hippocampal slices in a similar way to selective adenosine A_{2A} receptor antagonists (Costenla et al., 2010). It would seem paradoxical that caffeine causes a decrease of LTP magnitude while restoring memory dysfunction, but the general assumption about the parallel relationship between the magnitude of LTP and memory performance is clearly incorrect. LTP is required for memory processing but there is no evidence that they are linearly related, moreover there are studies which suggest that in certain circumstances enhanced LTP can be coupled to decreased performance in memory-related tasks (Migaud et al., 1998, Sahún et al., 2007). At non-toxic, micromolar concentration, the only evident and documented action of caffeine is the antagonism of adenosine receptors. Namely the A_1, A_{2A} and A_{2B} receptors (Fredholm et al., 1999b). The role of the A_{2B} receptor in the nervous system is still unclear (Fredholm et al., 1999b). A previous animal study showed (Cognato et al., 2010), that chronic caffeine consumption or selective A_{2A} receptor antagonist KW6002 prevented the morphological change found in hippocampus of adult rats that were exposed to a convulsive period in their early life (caused by the administration of kainate, 2 mg/kg). This study also supported the involvement of synaptotoxicity in memory dysfunction, since A_{2A} receptors are predominantly localized in the hippocampus (Rebola et al., 2005a, Rebola et al., 2005b), and controlling the synaptotoxicity (Cunha, 2005; Silva et al., 2007). The action of caffeine causes plastic change in the synapse between mossy fibers and CA3 pyramidal cells. A_{2A} receptors are localized post-synaptically at the synapses between mossy fibers and pyramidal cells and are essential for NMDA-EPSC induced LTP, thus they can potentially affect information processing in CA3 neuronal networks and memory performance (Rebola et al., 2008). It seems the mossy fibre-CA3 synapse in the hippocampus is one of the possible places where caffeine can affect learning and memory. Age-dependent changes in the density of adenosine receptors have been reported in animal studies. There is a consistent decrease in the density of A_1 receptors (Cunha et al., 2001), whereas there is a strong increase in the density of A_{2A} receptors in the limbic cortex

(Cunha et al., 1995). The age-induced changes of the density and function of A_{2A} receptor might anticipate the efficiency of caffeine in age-related memory impairment (Cunha and Agostinho, 2010). Chronic consumption of caffeine mostly acts by antagonizing A_{2A} rather than A_1 receptors (Quarta et al., 2004, Ferré, 2008). During a stressful condition, an initial up-regulation of A_1 receptors can be observed first, then followed by an immediate down-regulation of A_1 receptors and up-regulation of A_{2A} receptors (von Arnim et al., 2000; Zhou et al., 2004). Based on the above, it is clear that chronic caffeine consumption might be particularly effective to prevent stress-related memory dysfunction by antagonizing the A_{2A} receptors; the ones responsible for the fine-tuning of NMDA receptors engagement (Cunha and Agostinho, 2010).

It was shown in streptozotocin induced, general type 1 diabetes model (Duarte et al., 2009), that the chronic consumption of caffeine in high doses (1 g/l in the drinking water) prevents the neurochemical modifications associated with memory impairment (Biessels et al., 1996) while the up-regulation of A_{2A} receptors in the hippocampus, and the down-regulation of A_1 receptors were also observed (Duarte et al., 2006, Duarte et al., 2009). The accompaniment of A_{2A} receptor blockade with robust neuroprotection was observed in other chronic noxious brain conditions also (Cunha, 2005; Rebola et al., 2005). In mouse, caffeine treatment in a dose of 1 g/l can cause an increase in A_1 agonist binding in the hippocampus and increase the number of A_{2A} receptors, suggesting that up-regulation of A_{2A} receptors may be an adaptive effect of caffeine intake (Johansson et al., 1997). These changes regarding A_1 and A_{2A} receptors cannot be seen at moderate caffeine doses (Johansson et al., 1996; Johansson et al., 1997). High-doses of caffeine causes an increase in the immediate early gene expression, suggesting that even a single, albeit high dose of caffeine can induce adaptive changes in the brain (Fredholm et al., 1999b). Conversely moderate doses of caffeine decrease the immediate early gene expression (Fredholm et al., 1999b; Svenningsson et al., 1995). Among brain regions, hippocampus is probably the structure showing the most prominent neuroplastic features. Neurogenesis continues through adulthood in the human hippocampus (Eriksson et al., 1998). Theoretically, caffeine might influence the neurogenesis in a U-shape manner because Wentz and Magavi and Han et al. (Han et al., 2007; Wentz and Magavi, 2009) demonstrated that moderate doses of caffeine depress cell

proliferation, while high doses increase proliferation of neuronal precursors in the rodent hippocampus. However, this is purely a hypothesis and no data is available yet to support it in human.

Apart from its neuroplastic features, the hippocampus is rich in A_1 and A_{2A} receptors and has a prominent role in learning and memory, moreover chronic caffeine consumption seems to be able to treat or prevent memory impairment (Cunha and Agostinho, 2010; Ritchie et al., 2007; Santos et al., 2010). Based on these findings, the hippocampus specific results were not surprising. We found that both low-level coffee consumption (and caffeine intake) and high-level coffee consumption (and caffeine intake) is associated with larger hippocampus compared to moderate coffee consumption (caffeine intake). It is hard to explain these associations and we can only provide some speculative theories. However, the non-linear ("U-shape") association between caffeine concentration and its effect is well described in some other studies (Ascherio et al., 2001; Eskelinen and Kivipelto, 2010; Lopez-Garcia et al., 2009). Theoretically, this might suggest that the U-shape association between caffeine intake and hippocampal volume found in our study might reflect a caffeine-modulated neurogenesis in the human hippocampus. Although neural plasticity is a well-known phenomenon in animal studies, the novel MR techniques changed our view regarding human neuroplasticity: it plays a much greater role in human than previously thought. Neuroplasticity can be demonstrated in representation of brain functions (Janszky et al., 2003; Janszky et al., 2004). Morphological or functional brain disturbance or even asymmetric hand usage can induce reorganization of higher cognitive functions (Janszky et al., 2004, Auer et al., 2009). Moreover, other MR studies indicate that functional changes in the behavior can lead to morphological neuroplastic changes in the adult human brain. A newly-learned skill can alter the brain regions that help regulate these skills (Maguire et al., 2000, Draganski et al., 2004).

It should also be briefly discussed, that constantly growing list of pharmacological tools modulating Ca^{2+} *in vitro* includes caffeine, which activates the most important RyR isoforms (Ehrlich et al., 1994; Simpson et al., 1995). Timothy R. Cheek and colleagues demonstrated that the effect of caffeine on CICR from endoplasmatic reticulum is not only concentration dependent, but shows similar dose dependence that we found in our study (Cheek et al., 1993).

However, at least 5 mM caffeine (at least 50 times its pharmacological concentration and more than hundred times the concentration introduced by daily caffeine intake) is necessary to produce Ca^{2+}-independent activation of RyR and to inhibit IP3R *in vitro* (Ehrlich et al., 1994; Nehlig et al., 1992; Sitsapesan and Williams, 1990). Therefore, cellular mechanisms of caffeine action in the central nervous system *in vivo* have been explained mostly by antagonism of adenosine receptors, and binding to benzodiazepine receptors, whereas *in vivo* caffeine effects on the intracellular mobilization of Ca^{2+} in the brain has been generally rejected (Nehlig et al., 1992). Another article from Mohd Alaraj and colleagues (Alaraj et al., 1998) had also concluded, that systemic administration of caffeine has no effect on the spontaneous and NMDA-induced Ca^{2+} release in rat gyrus dentatus, thus *in vivo* applied caffeine, even at high doses does not influence calcium induced calcium release in brain neurons.

To our knowledge this is one of the very first studies, exploring the association between brain morphology and coffee consumption or caffeine intake in humans using MR imaging. The most robust caffeine-induced changes in animals affect the hippocampus: the very same structure which was found to be altered in our study. Our study suggests that coffee consumption might cause morphological alterations in the human hippocampus (although causality can not be established). Some animal studies suggest that caffeine may influence neuroplasticity by synaptogenesis (Weaver, 1996), or neurogenesis (Wentz and Magavi, 2009), but currently there are no studies showing that caffeine consumption has a direct relation with plastic changes in the brain. Thus, we suppose that our study might potentially help in understanding function-related morphological changes in the human brain. Consequently, - although it is clearly a speculation - synaptogenesis, or neurogenesis might also play a role in function-related morphological changes not only due to caffeine intake, but also due to other functional/behavioral changes in human. One of the limitations of our study is that both coffee abstinence and high coffee consumption can be associated with polymorphisms of the adenosine A_{2A} receptor gene. ADORA2A genotype can reflect the caffeine consumption within a population (Cornelis et al., 2007), and this polymorphism may also be a potential confounder in our study. In order to avoid sex-, age-, and nicotine-related differences and thus confounding by these factors, we investigated exclusively healthy, young, non-

smoking women. Because of this, our findings are not directly generalizable to other populations. Moreover, our study was cross-sectional. Although coffee consumption is most probably not associated with personality traits (Hewlett and Smith, 2006), we cannot rule out that the association of coffee consumption and the volume of the hippocampus means that coffee consumption is determined by differences in brain structure. To address this question, longitudinal or prospective studies are needed. Another limitation is that we could assess the coffee consumption or an estimated caffeine intake through the consumption of all caffeine-rich food products. The contents of these foods is mixed and we cannot rule out that not only the main investigated component (the caffeine) of these foods have an impact on the brain structure but other food components may also have an influence on the brain morphology. This is why we assessed not only the coffee consumption (the main source of the caffeine) but also the total estimated caffeine intake. However; we cannot rule out and could not statistically control the hypothetic role of substances other than caffeine on our results. Although it is not a real limitation in our case, but must be noted that we performed multiple comparisons but did not correct the significance level accordingly and used an uncorrected statistical threshold $p<0.05$ (except for VBM analysis where "threshold-free cluster enhancement" (TFCE) was used to correct for multiple comparisons). This statistical method is widely accepted in case of an apriori analysis, where only predefined structures are examined (based on earlier studies) and having small number of variables, as in this case the correction for multiply comparisons is controversial (Bacchetti, 2002; Goodman, 1998; Perneger, 1998; Rothman, 1990).

6.2 Body Mass Index

The main finding of our study is that the volume of the right amygdala showed a correlation with the BMI although it may be true only for the overweight male population. The volume of the right amygdala showed a positive correlation with the BMI in men, but not in women. We investigated a young and healthy population; therefore, it is very unlikely that our findings are the consequences of

obesity-associated brain disorders. Although our intention was to find an association between body weight and brain structure in a healthy, young population, unexpectedly, we found that the positive correlation between the volume of the amygdala and the BMI in men was more pronounced in the overweight subpopulation. In fact, we could not demonstrate a correlation between the BMI and the amygdala in the non-overweight subpopulation. Mesiotemporal structures play a pivotal role in food intake regulation (Druce and Bloom, 2003). Consequently, one of the possibilities is that the association of the BMI with enlarged amygdala in men is a marker of the reward system's activity. Conversely, our study was cross-sectional. Thus, we cannot rule out another possibility: larger BMI can cause functional and morphological alterations in these structures independently of obesity-related brain disorders.

Recent MR studies indicate that functional changes in behavior can lead to morphological changes in the brain. A newly-learned skill or exceeded use of a learned skill can alter the brain regions, which participate in regulating these skills (Maguire et al., 2000, Draganski et al., 2004). Furthermore, amygdala may even show a continued development in adulthood (Durston et al., 2001). Thus, it is also a plausible hypothesis that a change in body weight causes an altered functional organization of the reward system, leading to morphological changes demonstrated by our analyses of MR volumetry. To answer this cause/effect question, longitudinal studies should be performed. We found that larger amygdala was associated with larger BMI. The amygdala modulates the sense of taste, which is believed to guide eating behavior (Lénárd and Hahn, 1982). Conversely, the involvement of specific subdivisions of the amygdala in the regulation of food intake and body weight is different: experimental lesions in different parts of the amygdala can cause both hyperphagia/obesity (Lénárd et al., 1982; Yang et al., 2009) and hypophagia/underweight (Hajnal et al., 1992). Unfortunately, the resolution of MR imaging did not allow us to investigate the different nuclei of the amygdala, thus, we cannot answer which region of the amygdala showed the positive association with the BMI.

In accordance with our results, a previous MR volumetry study found that patients with anorexia nervosa had smaller hippocampo-amygdalar formation (Giordano et al., 2001). We found that the amygdalar volume showed correlation

with the BMI in men but not in women. The gender differences for BMI and obesity are well-known (CDC, Centers for disease and prevention., Sundquist and Johansson, 1998). There are also well-known gender differences in the structure and function of the amygdala (Pereno and Beltramino, 2009, Witte et al., 2010). Moreover, animal and human studies demonstrated gender differences in the neural mechanism of food intake and obesity (Clegg et al., 2003; Hahn et al., 1988; King et al., 1999; Wang et al., 2009; Woods et al., 2003). Thus, our study suggests further evidence to the fact that food intake, body weight regulation and the pathophysiology of obesity are different in the two sexes. We found correlation only between the right amygdala volume and the BMI, while no such association could be demonstrated concerning the left amygdala. We cannot fully explain this lateralization difference. However, Markowitsch concluded that there is definitely a hemisphere specific processing difference between the left and right amygdale (Markowitsch, 1998). For example, in rats the right amygdala has a greater involvement than the left one in fear conditioning (Baker and Kim, 2004). The ictal orgasm during human epileptic seizure and probably the physiological orgasm feeling are confined to the right amygdala (Janszky et al., 2002). Right amygdala activations may be associated with abnormalities of body image perception in eating disorders (Miyake et al., 2010). Markowitsch suggested that the left amygdala is more closely related to affective information encoding with a higher affinity to language and detailed feature extraction, while the right amygdala is related to affective information retrieval with a higher affinity to pictorial or image-related material (Markowitsch, 1998). In a meta-analysis, van der Laan et al. examined multiple functional neuroimaging studies which investigated the brain responses to visual food stimuli and concluded that hunger modulates the response to food pictures in the right amygdala and left lateral orbitofrontal cortex (van der Laan et al., 2011). In patients with anorexia nervosa, drinking energy-rich chocolate milk induces activations in the right amygdala and in the left medial temporal gyrus (Vocks et al., 2011). Thus, we may speculate that the enlargement of the right amygdala in an overweight man might reflect a sustained hyperactive reaction of the reward system to visual input of energy-rich food.

Our findings may suggest that an association between body weight and the morphological changes in the reward system can be demonstrated by MRI. However,

we could not demonstrate any relationship between reward area volumes in women and in healthy lean individuals. We found a potential relationship between BMI and the right amygdala only in heavier individuals. This however, needs further investigation to determine if the correlation withstands larger samples of both men and women including a large number of obese and severely obese patients. The small number of overweight people is the main limitation of this study, since the original goal of our study was to find an association between body weight and brain structure in a young, healthy population and not in pathological conditions. Thus, our study population was a non-selected sample of healthy, young university students showing a Gaussian distribution according to BMI (Fig. 10). Consequently, only the minority of subjects were overweight. Unexpectedly, we found that the association between the volume of the amygdala and BMI was more pronounced in the overweight – but otherwise healthy – subpopulation: concentrating only on the 8 men with BMI>25, we found a very strong correlation between the volume of amygdala and BMI (Fig. 11). Despite this unexpected finding, we did not expand our study and did not include more overweight people on the basis of our results: this would be a study violation regarding the methods and the original goal. The other limitation of the present study is that there are other reward-related regions which cannot be examined with the present methods. One of them is the hypothalamus, but unfortunately, the Freesurfer software has no automatic label for the hypothalamus.

Conversely, the fact that different brain structures correlated with BMI in men vs. women might indicate that there are not only gender differences in body weight regulation and obesity but also that different kinds of obesity may have different underlying neural mechanisms. This hypothesis should be confirmed by future studies. Not only the results but also the methods of our study may inspire new studies investigating body weight and food intake regulation. The short and non-invasive MR examination for volumetry using reliable automated software-based brain segmentation methods (Fischl et al., 2002; Morey et al., 2009; Pardoe et al., 2009) allow us to conduct further longitudinal and large-cohort MR volumetric studies to see whether the volume of amygdala can differentiate between different types of obesity, or predict the success of different weight loss therapies.

6.3 Current trends

There are several MRI methods to track suspected GM (cortical or subcortical) or WM changes. The most popular ones are volumetry, voxel/deformation/tensor-based morphometry, atrophy analysis /SIENA, SIENAX, SIENAr/ (Smith et al., 2001; Smith et al., 2002), TBSS, etc. Out of these, volumetry and voxel-based-morphometry were discussed in this thesis, and their usability was supported by two original investigations. These studies showed that both methodologies are capable of reliably approximate brain volumetric or morphometric changes attributable to an extrinsic or intrinsic factor, in this case caffeine intake or BMI. The applied methodology and the necessary software tools are freely availably, thus providing a universal and utilizable tool to assess brain volumetric or morphometric changes. Today, these methods are of utmost importance in the biomarker research. Although they can be effectively used in basic research, the discussed methods have emerging clinical importance (see Chapter 2.3). Moreover the required MRI measurement time is considerably low (around 10 minutes for a single subject) which makes these examination cost effective among MRI studies. Another advantage of the technique, that the structural images can also be obtained on lower magnetic field MRI scanners, namely at 1.5 Tesla, which is by far more widespread, compared to 3T machines, and have lower Specific Absorption Rate (SAR), that could be beneficial if the examined group have some kind of electronic implant (for e.g specific Deep Bran Stimulator) that can withstand the modest magnetic field of 1.5T and pre-specified low SAR values. One drawback is however the time consuming evaluation. Fortunately today's development also aims the reduction of the processing time, mainly by parallel /GPU- or Cloud-based/ processing techniques.

It is hard to foresee the future of clinical and research MRI usage. The move towards a large installed base of 3T scanners in the routine clinical field is the most predictable development, particularly for neurological and musculoskeletal imaging, because those are the fields, where speed and image quality are of preeminent importance. Although the clinical availability of 3T body imaging is constantly increasing, currently 1.5T scanners are more suitable for this purpose, as continuing

issues of image quality, power deposition (SAR) and higher cost of the stronger field limits its own spread. It is also clear that because of the rapid growth in MRI availability, CT system usage will decrease, and will be rationalized (used only for indicated cases e.g. acute traumatic or poly-traumatized patients, MRI incompatibility, skeletal examinations etc.). Nuclear medicine techniques such as SPECT and PET already offer selective and sensitive methods to map glucose metabolism, molecular pathways, receptor density, etc., via adequate radioligands but they both lack on decent level of spatial resolution offered by MRI. Thus the introduction of new hybrid systems (PET/MR; SPECT/MRI) will offer vast amount of new possibilities in both in research and clinical practice. In terms of MRI research, the move toward ultra-high field systems (above 3 Tesla) is the most predictable step. The number of installed 7 Tesla MR systems for human whole-body examination has increased steadily. In the last two years the number of installed 7 Tesla MR systems raised from 12 to about 40 worldwide. Apart from higher field systems, many other possibilities exist, where both clinical and research MRI applications are ready to evolve; these are pre- or hyperpolarized MRI, new RF-coils, parallel transmit technology, ultra-short echo time and targeted contrast agents, MR elastography and new magnetic resonance spectroscopy imaging techniques (e.g. turbo proton echo planar spectroscopic imaging (PEPSI)), just to mention some.

7. SUMMARY

Several methods are available to assess the possible functions of different brain areas, track their changes or compare/differentiate groups based on data resulting from these novel techniques. The number of available methods and methodologies has grown rapidly and by now, the non-invasive and minimal-invasive methods have gained exceptional popularity. In this thesis, two non-invasive magnetic resonance imaging based methods will be demonstrated, and their usability (meaning that they are suitable to track or compare brain volumetric or morphometric alterations) will be demonstrated by two original researches, both having novel findings. These methods are automated MRI Volumetry and Voxel-Based Morphometry.

These methods were used to investigate the relationship between caffeine intake (coffee consumption) or excessive energy intake (high Body Mass Index) and brain morphometry / volumetry.

It was found that both high-level and low-level caffeine intake was associated with a larger hippocampus compared to moderate-level caffeine intake, while other brain structures showed no association with coffee consumption or caffeine intake. The U-shape association between caffeine concentration and its effect has already been described in some experimental studies.

In case of chronic positive energy balance, we found that the volume of the right amygdala positively correlated with the Body Mass Index in men but not in women. It was shown that an association between body weight and the morphological variability of the reward system can be demonstrated by Magnetic Resonance Imaging. The study may have provided further evidence for a different body-weight regulation in the two sexes.

The described methods are widely accepted and freely available for non-commercial use. Although they can be effectively used in basic research, as demonstrated in the current thesis, the discussed methods also have an emerging clinical importance.

8. APPENDICES

May 7, 2012

Gergely Orsi
Diagnostic Centre of Pecs
Ret Str 2
Pecs, Baranya County 7623
Hungary

Email: gergo.orsi@gmail.com

Dear Gergely Orsi:

This is to grant you permission to reproduce the following figure in your thesis entitled "Examining brain volumetry and morphometry in relation to body mass index (BMI) and coffee consumption: magnetic resonance imaging approaches" for the University of Pecs, Faculty of Sciences:

> Figure 1 from Bertil B. Fredholm, Karl Bättig, Janet Holmén, Astrid Nehlig, and Edwin E. Zvartau, Actions of Caffeine in the Brain with Special Reference to Factors That Contribute to Its Widespread Use, *Pharmacol Rev* March 1, 1999 51:83-133

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Sincerely yours,

Richard Dodenhoff
Journals Director

American Society for Pharmacology and Experimental Therapeutics

9. PUBLICATIONS

Number of publications in peer-reviewed journals: 13

Cumulative impact factor excluding citable abstracts: 25.984

Cumulative impact factor including citable abstracts: 43.556

9.1 Peer-reviewed articles supporting the thesis

Perlaki G.*, Orsi G.*, Kovacs N., Schwarcz A., Pap Z., Kalmar Z., Plozer E., Csatho A., Gabriel R., Komoly S., Janszky I., Janszky J.

Coffee consumption may influence the hippocampus volume in young women.

BRAIN IMAGING AND BEHAVIOR (2011) 5:(4) pp. 274-284.

* Equal contribution in first authorship

IF: 1.661

Orsi G., Perlaki G., Kovacs N., Aradi M., Papp Z., Karadi K., Szalay C., Karadi Z., Lenard L., Tenyi T., Plozer E., Gabriel R., Nagy F., Doczi T., Komoly S., Jokeith H., Schwarcz A., Janszky J.

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BRAIN IMAGING AND BEHAVIOR (2011) 5:(2) pp. 149-157.

IF: 1.661

9.2 Oral- and Poster-presentations supporting the thesis

Orsi G., Perlaki G., Kovács N., Schwarcz A., Pap Z., Kalmár Z., Plozer E., Csatho Á., Gábriel R., Komoly S., Janszky I., Janszky J.
Coffee consumption may influence the hippocampus volume in young women.
ESMRMB Congress 2011, October 6-8 2011, Leipzig/DE

Orsi G., Plozer E., Kalmar Z., Sellyei E., Schwarcz A., Perlaki G., Karadi K., Janszky J. Elhízás, testtömeg-szabályozás és a központi idegrendszer: volumetriás MR vizsgálatok.
Magyar Pszichológiai Társaság XIX. Országos Tudományos Nagygyűlése. Pécs, May 27-29 2010.

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