Neuroscience Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Are there any gender differences in the hippocampus volume after head-size correction? A volumetric and voxel-based morphometric study

Gabor Perlaki[a,b,*], Gergely Orsi[a,b], Eniko Plozer[c], Anna Altbacker[c], Gergely Darnai[c], Szilvia Anett Nagy[b], Reka Horvath[c], Arnold Toth[c,d], Norbert Kovacs[a,c], Peter Bogner[b], Attila Schwarcz[a,d], Jozsef Janszky[a,c]

[a] MTA-PTE Clinical Neuroscience MR Research Group, Ret u. 2, 7623 Pecs, Hungary
[b] Pecs Diagnostic Centre, Ret u. 2, 7623 Pecs, Hungary
[c] Department of Neurology, University of Pecs, Ret u. 2, 7623 Pecs, Hungary
[d] Department of Neurosurgery, University of Pecs, Ret u. 2, 7623 Pecs, Hungary

ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form 24 March 2014
Accepted 9 April 2014

Keywords:
Head-size correction
MRI
VBM
Volumetry
Gender difference
Hippocampus

ABSTRACT

Previous findings on normal sexual dimorphism in hippocampal volume have not always been consistent. This study investigated gender differences in hippocampal volume using different head-size correction strategies. T1-weighted MR images were collected in 99 healthy, Caucasian, university students (66 female subjects; mean age: 23.1 ± 2.3, range: 19–31 years). Sexual dimorphism in hippocampus was investigated by automated MRI volumetry and voxel-based morphometry (VBM) using both general linear model (GLM) and proportion head-size correction strategies. Absolute hippocampal volumes were larger in men than women. After adjusting for head-size, the proportion method indicated larger hippocampi in women than men, while no gender differences were found using the GLM approach. Investigating absolute hippocampal volumes in 15 head-size matched pairs of males and females indicated no gender differences. We suggest that there is no sexual dimorphism in hippocampal size and the apparent gender differences found by the proportion method may have more to do with head-size than with sex. The GLM and proportion head-size correction strategies are not interchangeable and may yield different results. The importance of the present findings is mostly related to scientific reproducibility across MRI volumetry or VBM studies.

1. Introduction

The hippocampus is a prime focus of MRI-based volumetric analysis in healthy subjects, cognitive disorders and neuropsychiatric conditions. Altered hippocampal volumes were reported in epilepsy, Alzheimer's disease, schizophrenia and post-traumatic stress disorder [1,2]. Hippocampal volume measurements can assist the diagnosis of Alzheimer's disease [3] and epilepsy [4].

Hippocampal volume changes were found to differ in some neuropsychiatric conditions as a function of gender [5,6]. Investigating gender difference in hippocampal volumes of normal subjects is essential to understand why male and female hippocampi differ in their predisposition for volumetric changes in certain conditions and to distinguish disease patterns from normal ones. Previous findings on sexual dimorphism in hippocampal volume have not always been consistent. For example, larger hippocampal volumes in females [7,8] and no difference in hippocampal volumes between males and females [9–11] were also reported after correcting for head-size differences. Interestingly, the above studies reporting gender difference used the proportion method [7,8], while the studies describing no gender difference used the general linear model (GLM) approach [9,10] or an extension of that [11] to adjust for head-size. Based on this observation, we hypothesized that part of the discrepancy in results may be attributed to different types of head-size correction used.

Volumetric measures tend to be associated with normal variation in head-size; people with larger heads typically have larger brain structures [12]. To account for this irrelevant variance, volumetric measures are often "normalized" with respect to intracranial

* Corresponding author at: MTA-PTE Clinical Neuroscience MR Research Group, Ret u. 2, 7623 Pecs, Hungary. Tel.: +36 72535900; fax: +36 72 535911.
E-mail address: petzinger.gabor@gmail.com (G. Perlaki).

http://dx.doi.org/10.1016/j.neulet.2014.04.013
0304-3940/

volume (ICV) [13]. The strong effect of ICV on voxel-wise volume information was also demonstrated, suggesting that head-size correction should also be considered in voxel-based morphometry (VBM) [10,14]. *GLM and proportion* approaches are two common methods to correct for ICV [13,15].

(i) The GLM approach corrects for head-size in a linear regression model, including ICV as a covariate of no interest.
(ii) The proportion method normalizes for head-size directly by dividing the volumetric measure by the ICV (generating relative measures).

Although there are a few critical evaluations about the advantages/disadvantages of the GLM and proportion methods, there is no consensus regarding the most accurate way to adjust for head-size in statistical analyses [12,13,15–17].

The aim of the present study was twofold: (1) to re-examine the gender effect on hippocampal size using GLM and proportion head-size correction strategies. (2) To compare GLM and proportion approaches in hippocampal volumetry and VBM, using the same anatomical images from a set of normal control subjects. Our working hypothesis is that these two normalization methods may yield different results when comparing groups unmatched for head-size or when testing for inter-individual differences in a population with considerable variation in head-size.

2. Materials and methods

2.1. Subjects

Ninety-nine healthy, Caucasian, graduate or postgraduate university students (66 women and 33 men; mean age: 22.9 ± 2.2 and 23.5 ± 2.4, range: 19–31 and 20–30 years respectively) were included. Males and females did not differ in the distribution of education or age. All subjects got detailed information about the investigation and gave written informed consent prior to the examination. The study was approved by the local ethical committee and performed in accordance with the Declaration of Helsinki.

2.2. Magnetic resonance imaging

All measurements were performed on a 3T MRI scanner (MAGNETOM Trio, Siemens AG, Erlangen, Germany) with a 12-channel head coil. T1-weighted three-dimensional MPRAGE images were acquired with the following parameters: TR/TI/TE = 2530/1100/3.37 ms; flip angle = 7°; 176 sagittal slices; slice thickness = 1 mm; FOV = 256 mm × 256 mm; matrix size = 256 × 256; receiver bandwidth = 200 Hz/pixel.

2.3. Data analysis

Sexual dimorphism in hippocampal volume was investigated using two different concepts (MR volumetry and VBM) and two head-size correction strategies (GLM and proportion).

2.3.1. Intracranial volume measure

Since the ICV cannot be reliably measured on T1-weighted MR images [18], the reciprocal of volumetric scaling factor derived automatically from SIENAX, henceforth referred to as ICV_{sienax}, was used as a surrogate for ICV [19]. The validity of ICV_{sienax} as a predictor of ICV was demonstrated in an earlier study [18].

2.3.2. MR volumetry

Hippocampal volumes were estimated from T1-weighted MPRAGE images using the Freesurfer 4.5 image analysis suite (https://surfer.nmr.mgh.harvard.edu/). The technical details were described previously [20]. Quality control was performed throughout the automatic processing stream. Error correction was performed when necessary, based on the recommended reconstruction workflow (http://surfer.nmr.mgh.harvard.edu/fswiki/RecommendedReconstruction/).

Statistical analyses were performed using SPSS 20. Left and right hippocampal volumes were handled separately.

(i) Absolute hippocampal volumes were compared between males and females using unpaired *t*-test.
(ii) To correct for head-size through GLM, a multiple linear regression model was constructed with absolute hippocampal volume as dependent, while gender and ICV_{sienax} as independent variables.
(iii) To correct for head-size using the proportion method, the hippocampal volumes were divided by their corresponding ICV_{sienax}. The relative hippocampal volumes were then compared between males and females using unpaired *t*-test.
(iv) A subset of subjects with closely matched head-size was also analyzed. Males and females were paired based on an automated and objective method described earlier [21]. Briefly, the absolute differences in ICV_{sienax} were computed for every possible pairings of males and females and then one male and one female with the minimum absolute difference in ICV_{sienax} were paired and removed from the cohort. This procedure was repeated until there were no remaining pairs of males and females with relative difference in ICV_{sienax} less than 5%. The above method resulted in 15 matched pairs of males and females. This group was then tested for gender differences in absolute hippocampal volumes using non-parametric Wilcoxon signed-rank test.
(v) Both women and men were divided into two subgroups based on their median ICV_{sienax}. The hippocampal volumes of women (or men) with smaller ICV were compared to women (men) with larger ICV by using both GLM and proportion head-size correction strategies. When using the GLM method a multiple linear regression model was constructed with absolute hippocampal volume as dependent, while the subgroup membership and ICV_{sienax} as independent variables. In case of the proportion method the relative hippocampal volumes were compared between the subgroups by Mann–Whitney *U* test.

In order to control for the potential confounding effects of age, the above comparisons were repeated using multiple linear regression with age as an independent variable.

Results were considered significant at $P \leq 0.05$ for all statistical tests.

2.3.3. Voxel-based morphometry

Voxel-based morphometry was performed using FSL-VBM (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fslvbm/). First, all structural images were brain-extracted using BET [22]. Next, tissue-type segmentation was carried out using FAST [23] and the resulting gray matter partial volume images were non-linearly registered to MNI152 standard space [24]. The registered images of 33 men and 33 randomly chosen women were averaged together with their respective mirror images to create a left–right symmetric study-specific gray matter template (an equal number of subjects from both groups was used to avoid bias). All the 99 native gray matter images were non-linearly registered to this study-specific template and "modulated" to correct for volume changes due to spatial transformation.

Two different types of modulations were performed. First, voxel values in gray matter images were corrected for volume changes due to both affine and nonlinear components of the registration (*full_modulation*). Thus, the total amount of gray matter remains

Please cite this article in press as: G. Perlaki, et al., Are there any gender differences in the hippocampus volume after head-size correction? A volumetric and voxel-based morphometric study, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.04.013