Minimal hippocampal width relates to plasma homocysteine in community-dwelling older people

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Abstract

Background: the hippocampus is important for memory. Hippocampal atrophy and higher levels of homocysteine may both predict cognitive dysfunction in community-dwelling older people. We tested if higher homocysteine relates to hippocampal thinning in this group.

Subjects: 156 community-dwelling volunteers without clinical memory problems.

Method: we measured minimal hippocampal widths on magnetic resonance images and homocysteine in plasma.

Results: minimal hippocampal widths related inversely to homocysteine levels.

Conclusions: our results indicate that, even in healthy older people, homocysteine may damage the hippocampus. Reducing homocysteine levels in healthy older people may help to prevent Alzheimer’s disease.

Keywords: ageing, hippocampus, homocysteine, magnetic resonance imaging, body mass index

Introduction

The hippocampus has a pivotal role in memory [1, 2]. Even in community-dwelling older people, hippocampal size declines with age [3–6]. These changes are important, since pre-clinical cognitive impairments correlate with smaller hippocampal size [7–12]. Moreover, in longitudinal studies early thinning of the medial temporal lobe (MTL) structures predicts cognitive dysfunction [3, 10, 13, 14].

Homocysteine levels rise with age [15–17]. In community-dwelling cohorts of older people, cognitive impairments relate to higher homocysteine [18–22, but see 23]. As with hippocampal size [3, 10, 13, 14], in longitudinal studies, higher homocysteine levels predict cognitive dysfunction [22, 24]. These convergent findings suggest that homocysteine may damage the hippocampus. Consistent with this, higher homocysteine levels predicted greater decline of medial temporal lobe widths in patients with Alzheimer’s disease [25]. However, the convergence of the findings relating hippocampal size and homocysteine to memory suggests that homocysteine may damage the hippocampus even in community-dwelling older people, with no clinical memory impairment. Our study tested this by analyzing whether such people show an inverse relation between homocysteine levels and hippocampal size.

We tested whether minimal hippocampal widths relate to homocysteine levels in community-dwelling volunteers, aged 65 or over. Our previous studies have shown that minimal temporal lobe widths are easy to measure [5]. Hippocampal width and homocysteine levels depend not only on age (see above), but also relate to body mass index (BMI) [17, 26, 27]. We therefore adjusted hippocampal widths for age and BMI, to test if they depend upon homocysteine, independently of these confounders.

Methods

Subjects

We recruited 156 community-dwelling volunteers aged 61–91 [20]. Exclusion criteria were a score of 80 or less on the CamCog [28], or less than 25 on the MMSE [29] or significant progressive subjective memory complaints. All except three participants (2 men) underwent brain magnetic resonance imaging (MRI), as follows:
All MRI scans were acquired on a Siemens 1.5T Magnetom Vision scanner (Siemens Medizinische Technik, Erlangen, Germany). A T1-weighted, volumetric sequence was performed (MPRAGE, TR 15 ms, TE 7 ms, FOV 220 by 165 mm, matrix 256 by 192). Images were acquired in the standard axial plane, parallel to the anterior commissure/posterior commissure line, with a slice thickness of 2 mm. The data were transferred to a workstation (Nuclear Diagnostics Hermes, Sweden). Images were viewed in a sagittal orientation and realigned along the plane of the body of the hippocampus. This produced a set of temporal lobe angulated axial images. The hippocampi are easily visualized as areas of grey matter signal bounded medially by the cerebrospinal fluid of the basal cisterns and laterally by the temporal horn of the lateral ventricle, or in subjects with very little atrophy, the white matter of the stem of the temporal lobe. The image for minimal hippocampal width measurement was selected as the image midway between the base of the temporal horn of the lateral ventricle and the most superior image showing the medial temporal lobe separate from the pons (i.e. the first image inferior to the cerebral peduncle). The minimal hippocampal width was measured from this image at its narrowest point between the anterior and posterior margins of the pons. This is an adaptation of the method described for CT [30], but has the major advantage that the temporal lobe angulation is always correct, making the measurement easier to perform. Axial head width was measured as the coronal distance between the inner tables of the skull passing through the anterior commissure (Figure 1). The rater, who was blind to the participants’ characteristics, repeated 40 random hippocampal width measurements to assess intra-rater reliability. This was 'substantial' (weighted Cohen's Kappa = 0.65 for the left MTL, 0.67 for the right MTL) [31, 32]. The coefficient of repeatability was 2.1 mm [33, 34].

Homocysteine measurement

We estimated non-fasting homocysteine in plasma. We collected blood into EDTA tubes, refrigerated it immediately at 4°C, centrifuged it and stored aliquots of the plasma at −70°C. Total time from blood sampling to storage at −70°C was always less than 2 hours. We estimated homocysteine using an immunoassay (Abbott Imx, Axis-shield, Oslo, Norway) [35].

Statistics

Initial analyses used Spearman’s robust rank correlation. Subsequently, we used multiple linear regression to assess the relationship between hippocampal width and homocysteine independently of potential confounders. The regression weighted the dependent variable (mean minimal hippocampal width) for the number of observations that contributed to each mean. BMI data were missing for three participants. Finally, we checked the results of the linear regression using robust regression [36].

Results

The cohort included 80 men and 76 women (overall mean age 74.1 years, SD 6.1, range 60.8–90.6). Their mean CamCog score was 98.0 (SD 4.2, range 84–105), and mean blood pressure was 153/81 mmHg (SD 20 mmHg systolic, 9 mmHg diastolic). The mean plasma homocysteine concentration was 12.6 μmol/l (SD 3.8, range 6.1–25.9 μmol/l). The mean axial head width at the level of the anterior commissure was 132.7 mm (SD 5.8, range 118.6–150.4 mm) and the overall mean minimal hippocampal widths were 14.5 (SD 2.0 mm, range 9.8–19.7 mm).

Our initial analysis supported the hypothesis that higher homocysteine levels relate to smaller hippocampal widths ($r=0.18$, $N=153$, $p=0.027$) (Figure 2). As expected, minimal hippocampal widths also related inversely to age ($r=0.33$, $N=156$, $p<0.001$), but positively to BMI ($r=0.18$, $N=153$, $p=0.026$). Also, as expected, homocysteine related directly to age ($r=0.39$, $N=153$, $p<0.001$) and (in males only) to BMI ($r=0.20$, $N=77$, $p=0.05$, 1-tailed).

Multiple regression showed that higher homocysteine levels related to smaller hippocampal widths independently.
Participants with homocysteine levels greater than 20 μmol/l increment in homocysteine is equivalent to the loss from a decade of aging. This relationship was reliable, since it remained significant in the robust regression that excluded participants with homocysteine levels above 20 μmol/l. However, in view of its small size, its theoretical significance outweighs its practical utility for predicting hippocampal width in individuals. Nevertheless, even small effects may be important for large populations. Together with previous work [9, 14, 18–21, 40, 41], our results suggest a need for studies to test whether measures to lower homocysteine levels may help to prevent the development of cognitive dysfunction.

The main result of the present study extends previous findings of smaller hippocampal widths in patients with Alzheimer’s disease [25]. It is also consistent with reports that either higher homocysteine or smaller size of medial temporal structures relates to poorer cognitive performance in older people [9, 14, 18–21, but see 23, 40, 41]. The secondary findings of the present study, that hippocampal widths and homocysteine related to both BMI and age, are also consistent with previous findings [15–17, 26, 27, 42]. This consistency confers construct validity on our findings. Moreover, the inverse relation of minimal hippocampal widths to homocysteine levels was statistically independent of any confounding relationships with age, BMI or axial head width. This independence strengthens the possibility that higher homocysteine levels may damage the hippocampus.

Our findings provide independent confirmation of our previous CT-based observations that hippocampal width declines with age in cognitively intact older people [43]. In this, our linear measures are consistent with volumetric analyses [6, 44]. Linear measures have the advantage of speed and simplicity, but may yield less precise estimates of hippocampal size. So, our linear measures may under-estimate the strength of the relation between homocysteine and hippocampal size.

The main limitation of our study is its cross-sectional nature. However, findings from longitudinal studies make the alternative interpretation of our data, that hippocampal width controls homocysteine levels, unlikely. This is because longitudinal studies have shown that higher homocysteine predicts greater declines in cognitive performance over time in healthy older people [22, 24]. The convergence of these previous findings with our present results supports the hypothesis under test, that higher homocysteine levels may damage the MTL and so contribute to the development of cognitive dysfunction. Further analyses of long-term longitudinal data will be necessary to confirm this hypothesis [45].

**Key points**

- High homocysteine relates inversely to hippocampal width in community-dwelling older people.
• This is important, since high homocysteine and reduced hippocampal size both predict cognitive dysfunction.
• Measures to reduce homocysteine levels in healthy older people may help to prevent hippocampal atrophy.

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