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Changes of individual *BrainAGE* during the course of the menstrual cycle

Katja Franke [a,b,*], Georg Hagemann [c], Ekkehard Schleussner [d], Christian Gaser [a,b]

[a] Structural Brain Mapping Group, Department of Psychiatry, University Hospital Jena, Jena, Germany
[b] Department of Neurology, University Hospital Jena, Jena, Germany
[c] HELIOS Klinik Berlin-Buch, Dept. of Neurology, Berlin, Germany
[d] Department of Obstetrics, Friedrich-Schiller-University, Jena, Germany

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ABSTRACT

Brain morphology varies during the course of the menstrual cycle, with increases in individual gray matter volume at the time of ovulation. This study implemented our previously presented *BrainAGE* framework to analyze short-term neuroanatomical changes in healthy young women due to hormonal changes during the menstrual cycle. The *BrainAGE* approach determines the complex multidimensional aging pattern within the whole brain by applying established kernel regression methods to anatomical brain MRIs. The "*Brain Age Gap Estimation*" (i.e., *BrainAGE*) score is then calculated as the difference between chronological age and estimated brain age. Eight women (21–31 years) completed three to four MRI scans during their menstrual cycle (i.e., at (t1) menses, (t2) time of ovulation, (t3) midluteal phase, (t4) next menses). Serum levels of estradiol and progesterone were evaluated at each scanning session.
Individual *BrainAGE* scores significantly differed during the course of the menstrual cycle ($p < 0.05$), with a significant decrease of −1.3 years at ovulation ($p < 0.05$). Moreover, higher estradiol levels significantly correlated with lower *BrainAGE* scores ($r = -0.42$, $p < 0.05$). In future, the *BrainAGE* approach may serve as a sensitive as well as easily implementable tool to further explore the short-term and maybe long-term effects of hormones on brain plasticity and its modulating effects in lifestyle-related diseases and dementia.

Introduction

Animal research suggests that hippocampal synaptic density changes rapidly during the menstrual cycle, mediated by estrogen (Woolley and McEwen, 1992; Gould et al., 2000; Yankova et al., 2001). Recently, it was demonstrated that brain morphology varies across the menstrual cycle also in humans. At the time of ovulation, significant volume increase of about 1.8% in gray matter (GM) as well as corresponding significant volume decrease in cerebro-spinal fluid (CSF) of 4.4% was shown (Hagemann et al., 2011). Studies comparing regional volumes in women during different phases of the menstrual cycle found increased GM in the right anterior hippocampus and decreased GM in the right dorsal basal ganglia (globus pallidus/putamen) in the postmenstrual, high-estrogen, late-follicular phase (Days 10–12 after onset of menses; Protopopescu et al., 2008) as well as larger GM volumes in the right fusiform/parahippocampal gyrus during the early follicular phase (i.e., between onset of menstruation to 5 days before ovulation; Pletzer et al., 2010). Thus and amongst others, individual brain structure in adulthood is influenced by hormonal factors (Breedlove and Jordan, 2001; Melcangi and Panzica, 2006; Balu and Lucki, 2009). Furthermore, a neuroprotective role for estrogen on age-related brain atrophy was previously suggested (Eberling et al., 2003), whereas long-term decline in brain volume was observed in postmenopausal women and in women receiving antiestrogens (Eberling et al., 2004; Erickson et al., 2005).

This study analyzed the short-term effects of hormonal changes during the menstrual cycle on individual *BrainAGE* estimations. Based on the widespread but well-ordered brain tissue loss that occurs with healthy aging into senescence (Good et al., 2001), we previously proposed a modeling approach to identify abnormal aging-related brain atrophy that may precede the onset of cognitive decline and clinical symptoms. The novel *BrainAGE* approach (Franke et al., 2010; Franke et al., 2012b) is based on a database of structural magnetic resonance imaging (MRI) data that aggregates the complex, multidimensional aging patterns across the whole brain to one single value, i.e. the estimated brain age. The difference between estimated and true chronological age will reveal the individual *brain age gap estimation* (*BrainAGE*) score. Consequently, the *BrainAGE* score directly quantifies subtle deviations in "normal" age-related brain atrophy by analyzing only one standard MRI per subject, with positive *BrainAGE* scores indicating accelerated structural brain aging and negative *BrainAGE* scores indicating attenuated structural brain aging. The *BrainAGE* framework has been shown to accurately and reliably estimate the age of individual brains

* Corresponding author at: Structural Brain Mapping Group, Department of Psychiatry, University of Jena Jahnstr. 3, D-07743 Jena, Germany. Tel.: +49 3641 934751; fax: +49 3641 934755.
E-mail address: katja.franke@uni-jena.de (K. Franke).

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with minimal preprocessing and parameter optimization using anatomical MRI scans (Franke et al., 2010; Franke et al., 2012a). Additionally, this method demonstrated its potential to identify pathological brain aging on an individual level, with increasing *BrainAGE* scores being related to measures of clinical disease severity in patients with Alzheimer's disease (AD) and prospective decline in cognitive functioning (Franke et al., 2012a), conversion to AD (Gaser et al., 2013), as well as diabetes mellitus type 2 (Franke et al., 2013).

Here, the *BrainAGE* framework was implemented to identify and quantify individual short-term neuroanatomical changes in healthy young women due to hormonal changes during the menstrual cycle. Furthermore, the relations between individual hormone levels (i.e., estradiol and progesterone) and *BrainAGE* scores were explored.

Methods

Subjects

To train the age estimation framework, we used MRI data of 561 healthy subjects [250 male] from the publicly accessible IXI cohort (http://www.brain-development.org; data downloaded in September 2011) aged 20–86 years [mean (SD) = 48.6 (16.5)], which were collected on three different scanners (Philips 1.5 T, General Electric 1.5 T, Philips 3.0 T). For more sample details see Franke et al. (2010).

The current *BrainAGE* analyses were conducted using existing MR scans of 16 healthy volunteers (8 females, age range 21–31 years; 8 males, age range 23–37 years) that were already used in Hagemann et al. (2011). The study protocol was approved by the local ethics committee. All subjects gave written informed consent. Confounding comorbidity was excluded by an interview, with special emphasis on endocrine dysfunction and hypertension. No subjects were on medication, including hormonal contraceptives. All volunteers were asked to refrain from alcohol consumption the night before and coffee intake the morning before scanning. Each scanning session was scheduled early in the morning at the same time (7:30 am), with minimal food and fluid intake. Each male was paired with a female and scanned in one recording session at three time points during the respective menstrual cycle. Scanning took place during menses (t1), at time of ovulation (t2), in the midluteal phase (t3). Half of the women as well as their paired male subjects were scanned again at their next menses (t4). Only women known to have an ovulational cyclus were allowed for the scanning protocol. This was achieved by an intravaginal ultrasound performed by an experienced gynecologist. To ascertain an ovulational cyclus and the time point of ovulation (t2) during the month the actual MR-scanning took place, ultrasound was repeated and scanning at t2 took place the day after a follicle ready for ovulation was detected. Furthermore, in women, blood samples were taken at each scanning session and serum levels of estradiol and progesterone were evaluated, while excluding other hormonal alterations (thyroid function, testosterone, cortisol). For quantification of sex steroid hormones ElektroChemiLumineszenzImmunoAssay (ECLIA) was used. Assays were performed using the E170 Module (Roche E170 Modular Analytical System®).

MRI

Image data were acquired on a 1.5 T Siemens Vision using a standard birdcage head coil with a T1-weighted fl3d Gradient Echo Sequence (GRE, $T_R = 15$ ms, $T_E = 5$ ms, $\alpha = 30°$, 192 slices, sagittal orientation, voxel size $1 \times 1 \times 1$ mm^3). To increase the signal-to-noise ratio and to minimize the effects of different head positioning, subjects were scanned twice at each recording session with the subject exiting the scanner between scans. A longitudinal design with several repeated measures was used as this is much more powerful than a simple cross-sectional design with only one measure per subject (Lui and Cumberland, 1992; Vickers, 2003). Data were visually checked for artifacts and any structural pathology.

Preprocessing of MRI data and data reduction

Preprocessing of the T1-weighted images was done using the SPM8 package (http://www.fil.ion.ucl.ac.uk/spm) and the VBM8 toolbox (http://dbm.neuro.uni-jena.de), running under MATLAB. All T1-weighted images were corrected for bias-field inhomogeneities, then spatially normalized and segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) within the same generative model (Ashburner and Friston, 2005). The segmentation procedure was extended by accounting for partial volume effects (Tohka et al., 2004), by applying adaptive maximum a posteriori estimations (Rajapakse et al., 1997), and by using a hidden Markov random field model (Cuadra et al., 2005). The images were spatially normalized using an affine registration and smoothed with 8-mm full-width-at-half-maximum smoothing kernels. Spatial resolution was set to 8 mm. For further data reduction, principal component analysis (PCA) was performed on the training sample with subsequently applying the estimated transformation parameters to the test sample. PCA was done using the 'Matlab Toolbox for Dimensionality Reduction' (http://ict.ewi.tudelft.nl/~lvandermaaten/Home.html), running under MATLAB.

Age estimation framework

The *BrainAGE* framework utilizes a machine-learning pattern recognition method, namely relevance vector regression (RVR; Tipping, 2001). It was recently developed to estimate individual brain ages based on T1-weighted images (Franke et al., 2010). In general, the model is trained with preprocessed whole brain structural MRI data of the training sample (here: the IXI sample). Subsequently, the brain age of each test subject can be estimated using the individual tissue-classified MRI data, aggregating the complex, multidimensional aging pattern across the whole brain into one single value (Fig. 1A). The difference between estimated and true chronological age will reveal the individual *brain age gap estimation* (*BrainAGE*) score. Consequently, the *BrainAGE* score directly quantifies the amount of acceleration or deceleration of brain aging. For example, if a 70 year old individual has a *BrainAGE* score of +5 years, this means that this individual shows the typical atrophy pattern of a 75 year old individual (Fig. 1B). Recent work has demonstrated that this method provides reliable and stable estimates, with *BrainAGE* scores in control subjects remaining the same during the follow-up period of up to 4 years, thus indicating only normal age-related atrophy (Franke et al., 2012a; Franke et al., 2013). Additionally, *BrainAGE* scores calculated from two shortly delayed scans on the same MRI scanner, as well as on separate 1.5 T and 3.0 T scanners, produced intraclass correlation coefficients (ICC) of 0.93 and 0.90, respectively (Franke et al., 2012a).

Within this study, the *BrainAGE* framework was applied using preprocessed (as described in the section 'Preprocessing of MRI data and data reduction') GM images. In Franke et al. (2010) it was already shown that performance measures for age estimation showed no differences when test data were collected on a scanner that was not included in training of the age estimation model. Therefore, accounting for possible difference between the scanners used in the training dataset and the test dataset was not necessary. Furthermore, because we use relative changes between the time points these values would be not affected by any differences due to use of different scanners for training and testing.

For training the brain age estimation model as well as for predicting individual brain ages, we used "The Spider" (http://www.kyb.mpg.de/bs/people/spider/main.html), a freely available toolbox running under MATLAB. For an illustration of the most important features (i.e., the importance of voxel locations for regression with age) that were used by the RVR to model normal brain aging and more detailed information please refer to Franke et al. (2010).

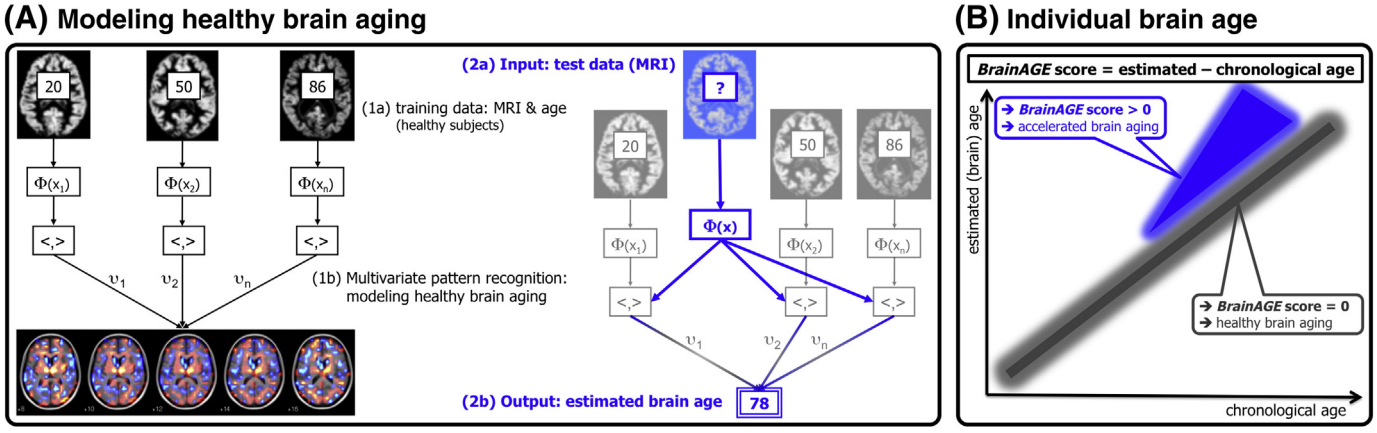

Fig. 1. Depiction of the *BrainAGE* concept. A: The model of healthy brain aging is trained with the chronological age and preprocessed structural MRI data of a training sample (left; with an exemplary illustration of the most important voxel locations that were used by the age regression model). Subsequently, the individual brain ages of previously unseen test subjects are estimated, based on their MRI data (blue; picture modified from Schölkopf and Smola (2002)). B: The difference between the estimated and chronological age results in the *BrainAGE* score. Consequently, positive *BrainAGE* scores indicate accelerated brain aging. (Image reproduced from Franke et al. (2012a), with permission from Hogrefe Publishing, Bern.)

Statistical analysis

The effect of time point during menstrual cycle (i.e., t1: menses, t2: time of ovulation, t3: midluteal phase, t4: next menses) on hormone levels (i.e., estradiol and progesterone), GM, WM, CSF volumes, and *BrainAGE* was explored using analysis of variance (ANOVA). Determining values at t1 as individual baseline scores, changes in *BrainAGE* at t2, t3, and t4 were analyzed using one-sample *t*-test. To control for random effects during study time, these analyses (except for hormone levels) were also conducted in the paired male subjects, scanned at the same time points during the respective menstrual cycle. Further, receiver operating characteristics (ROC) for the time points during menses based on *BrainAGE* scores, GM, WM, and CSF volumes were computed in the female as well as the male sample, resulting in the area under the ROC curve (AUC), which is also known as C-statistics or c-index. The AUC allows judging the quality of the classification, with 1.0 indicating a perfect discrimination and 0.5 indicating a result obtained by chance only. In order to compare the ability for discrimination, one-tailed *z*-tests are performed to test whether the resulting AUC derived from *BrainAGE* ROC analysis is statistically greater than the AUCs derived from ROC analysis with GM, WM, and CSF volumes. Pearson's pairwise correlation was used to assess the relationship between *BrainAGE* scores and hormone levels (i.e., estradiol, progesterone) during menstrual cycle in women. Calculation of effect size and statistical power was performed utilizing the "AI-Therapy Statistics toolbox" (https://www.ai-therapy.com/psychology-statistics/). All other statistical testing was performed using MATLAB 7.10. (www.mathworks.com).

Results

One woman showed no increase in progesterone at t3, indicating missing ovulation, and was excluded, along with the matched male. So, data from seven females and seven males were included in further analyses.

Estradiol and progesterone levels changed significantly during menstrual cycle in women (Table 1), with estradiol levels significantly increasing at the time of ovulation, then significantly decreasing in the midluteal phase ($p < 0.05$; Fig. 2), and progesterone levels being significantly elevated after ovulation ($p < 0.05$; Fig. 2). Overall GM, WM, and CSF volumes did not differ over the course of the menstrual cycle (i.e., t1: menses, t2: time of ovulation, t3: midluteal phase, t4: next menses), neither in men nor in women (data not shown here). Please note, in contrast to Hagemann et al. (2011) these results rely on overall brain tissue volumes that were averaged across all male/female subjects for means of comprehensive sample characterization, whereas Hagemann

Table 1
Characteristics of the test sample.

		Female		Male	
		Mean (SD)	*F* statistic	Mean (SD)	*F* statistic
No. subjects		7	–	7	–
Age range		21–31	–	23–37	–
Estradiol level (nmol/l)	t1	0.11 (0.05)	**11.6 [p = 0.0001]**	–	–
	t2	0.77 (0.31)		–	
	t3	0.41 (0.22)		–	
	*t4**	*0.22 (0.16)*		–	
Progesterone level (nmol/l)	t1	2.7 (0.6)	**4.04 [p = 0.02]**	–	–
	t2	4.3 (2.7)		–	
	t3	33.0 (33.8)		–	
	*t4**	*5.6 (5.5)*		–	
BrainAGE score (years)	t1	0.41 (0.65)	**3.48 [p = 0.03]**	0.37 (0.65)	0.62 [p = 0.61]
	t2	−0.87 (0.99)		−0.20 (1.27)	
	t3	0.46 (1.13)		−0.17 (1.22)	
	*t4***	*0.57 (0.83)*		*−0.45 (0.98)*	

Notes: t1—menses, t2—ovulation, t3—midluteal phase, and t4—next menses.
Italic type = reduced sample size with * $n = 3$, ** $n = 4$. **Bold** type = significant test results.

et al. (2011) analyzed individual changes in brain tissue volumes in relation to individual baseline brain tissue volumes.

In women, *BrainAGE* scores significantly differed during menstrual cycle ($F = 3.48$, $p < 0.05$; Table 1), with *BrainAGE* scores decreasing by −1.27 years ($SD = 1.23$; $p < 0.05$) from t1 to t2 (i.e., ovulation; Fig. 3). Calculation of the effect size for ovulation on *BrainAGE* resulted in $d = 1.52$, while statistical power was 0.91. Changes in individual *BrainAGE* scores from t1 to t3 as well as t4 resulted in 0.05 years ($SD = 1.56$; $p = 0.93$) and 0.10 years ($SD = 0.56$; $p = 0.74$), respectively. In men, *BrainAGE* scores did not differ across scanning time points ($F = 0.62$, $p = 0.61$; Table 1), with mean changes in *BrainAGE* score of −0.57 ($SD = 1.60$) years at t2 ($p = 0.38$), −0.54 ($SD = 1.49$) years at t3 ($p = 0.37$), and 0.40 ($SD = 0.97$) years at t4 ($p = 0.47$).

To explore the quality of classification using *BrainAGE* as compared to overall GM, WM, and CSF volumes, ROC analysis was conducted. Since change in individual *BrainAGE* was significant for t1–t2 in women, data were classified as being acquired at t1 vs. t2. In the female sample, the ROC analyses resulted in accuracy rates of 86%, 57%, 43%, and 64%, as well as AUCs (or c-index) of 0.88, 0.55, 0.51, and 0.55 for *BrainAGE* scores, GM, WM, and CSF volumes, respectively (Fig. 4). Performing one-tailed *z*-tests showed that AUC derived from ROC analysis based on *BrainAGE* is statistically greater than AUCs derived from ROC analysis with GM, WM, and CSF volumes ($p < 0.05$). Thus, classification between time of menses vs. time of ovulation is much more precise when based on *BrainAGE* as compared to GM, WM, and CSF volumes.

As stated above, estradiol levels are elevated at t2, whereas progesterone levels are elevated at t3. Lower *BrainAGE* scores were significantly correlated to higher estradiol levels ($r = -0.42$, $p < 0.05$; Fig. 5), whereas progesterone levels did not correlate with individual *BrainAGE* scores ($r = 0.08$, $p = 0.71$).

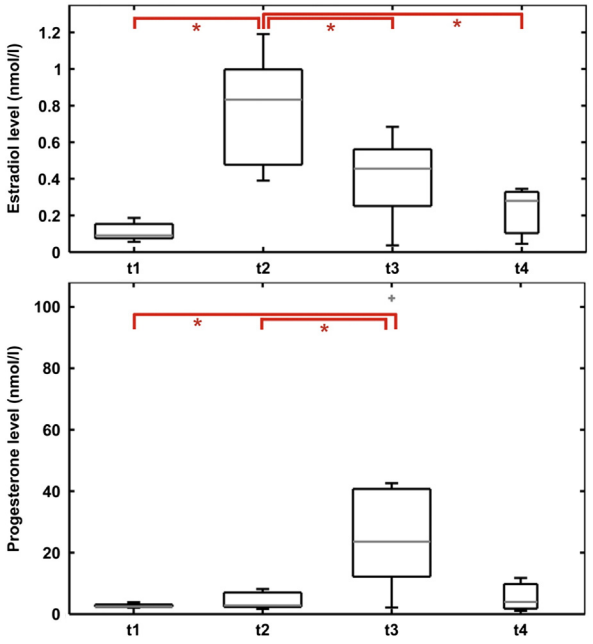

Fig. 2. Quantitative hormonal changes during the menstrual cycle for estradiol and progesterone in the female sample. In estradiol (top chart), there is a significant increase at the time of ovulation and a significant decrease in the midluteal phase ($p < 0.05$, asterisk). Progesterone levels (bottom chart) are significantly elevated after ovulation ($p < 0.05$, asterisk). t1: menses, t2: ovulation; t3 midluteal phase; and t4: next menses. The data are displayed as boxplots, containing the values between the 25th and 75th percentiles of the samples, including the median (gray lines). Lines extending above and below each box symbolize data within 1.5 times the interquartile range (outliers are displayed with a +). The width of the boxes depends on the sample size. Note: reduced sample size at t4.

Discussion

The scope of this study was the quantification of the effects of hormonal changes during the course of the menstrual cycle on individual *BrainAGE* estimations using a novel MRI-based biomarker derived from the recently presented *BrainAGE* framework. The *BrainAGE* approach was applied to seven women, who got three to four MRI scans during their menstrual cycle. The results provide evidence that hormonal changes across the course of the menstrual cycle have significant effects on individual brain structure. Thus, this study supports the results presented in Hagemann et al. (2011), which gave evidence of short-term hormone-dependent structural brain changes during the course of the menstrual cycle in humans. In detail, Hagemann et al. (2011) showed significant increases in individual GM volumes at the time of ovulation, possibly due to fast modulation of synaptic plasticity by estrogen (Baroncini et al., 2010; Mukai et al., 2010). The results presented here show a corresponding pattern, with individual *BrainAGE* scores based on GM images significantly decreasing at time of ovulation.

Furthermore, classifying individual brain structure as being scanned at either time of menses or time of ovulation was more precise using *BrainAGE* as compared to overall GM, WM, and CSF volumes. If *BrainAGE* would simply reproduce overall GM, WM or CSF volume, AUCs as well as classification accuracies based on *BrainAGE* or brain tissue volumes would be similar. Consequently, the information content of *BrainAGE*, which aggregates the wide-spread, multidimensional GM pattern across the whole brain, appears to be much higher as compared to overall brain tissue volumes.

Even more interesting, higher levels in estradiol levels were significantly related to lower *BrainAGE* scores. Previous *BrainAGE* studies in elderly subjects showed increasing *BrainAGE* scores being related to measures of clinical disease severity in patients with Alzheimer's disease and prospective decline in cognitive functioning (Franke et al., 2012a; Gaser et al., 2013). Research on sex hormones suggested a neuroprotective role for estrogen on age-related brain atrophy and cognition, whereas increased brain atrophy, faster decline and greater deterioration of cognition, as well as higher risk for AD were observed in postmenopausal women and in women receiving antiestrogens (Eberling et al., 2003; Eberling et al., 2004; Erickson et al., 2005; Sherwin, 2006; Shuster et al., 2010; Witte et al., 2010; Li et al., 2014; Li and Singh, 2014). Furthermore, estrogen was found to protect premenopausal females from the adverse effects of obesity and metabolic complications of inflammation, such as cardiovascular disease (Shi et al., 2009; Morselli et al., 2014). However, the prevalence of metabolic disorders is significantly increased in postmenopausal women (Ford, 2005), suggesting an important role of estrogen in modulating the effects of those lifestyle-related diseases. Amongst others, the metabolic syndrome and obesity are strongly associated with lower brain volume (Enzinger et al., 2005; Debette et al., 2010), advanced brain aging (Franke et al., 2014) as well as increased risk for dementia in late-life (Chen et al., 2009; Fitzpatrick et al., 2009). Within this study, the *BrainAGE* approach demonstrated its ability to depict even short-term effects of brain volume variations probably related to estrogen levels. Therefore, future work may incorporate the *BrainAGE* approach to further explore the modulating effects of hormones in (lifestyle-related) diseases and dementia.

At a first glance, the actual sample size in this study of $n = 7$ for each group seems to be quite low. However, a longitudinal design with repeated measures is much more powerful than a simple cross-sectional design with only one measure per subject, because the sample size necessary for obtaining the same statistical power decreases with increasing number of repeated measures (Lui and Cumberland, 1992). In practice, while achieving the same statistical power the sample size for typical values in variance decreases to 50–79% for three repeated measures in a longitudinal design as compared to a single time point measure in a cross-sectional design (Vickers, 2003). With an effect size of $d = 1.5$ and achieved statistical power of 0.9, the effect of ovulation on *BrainAGE* in women proves to be profound.

Taking a closer look into regional volume differences across the menstrual cycle, increased GM volumes in hippocampal and parahippocampal structures during the postmenstrual, high-estrogen, follicular phase in

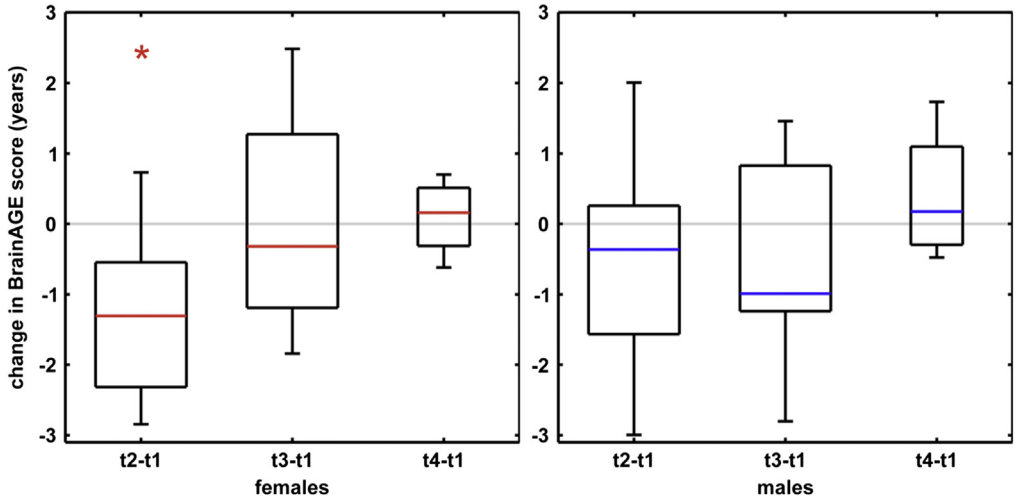

Fig. 3. Change in *BrainAGE* scores during the menstrual cycle in women and at corresponding time points in men, respectively. In women (left chart), *BrainAGE* scores significantly decreased by −1.27 years (SD = 1.23) at time of ovulation ($p < 0.05$, asterisk), whereas no differences could be found in men (right chart). The data are displayed as boxplots, containing the values between the 25th and 75th percentiles of the samples, including the median (red/blue lines). Lines extending above and below each box symbolize data within 1.5 times the interquartile range. The width of the boxes depends on the sample size. Note: reduced sample size at t4.

humans were recently reported (Protopopescu et al., 2008; Pletzer et al., 2010). On the other hand, with normal aging the hippocampus undergoes structural and biochemical changes, decreasing in (late) adulthood (Driscoll et al., 2003; Jernigan and Gamst, 2005; Knoops et al., 2012). Additionally, increased volume loss in hippocampal and parahippocampal structures was also observed in several disease states, like depression (Sheline et al., 1996; Sheline et al., 1999; Bremner et al., 2000; Sheline et al., 2003; Colla et al., 2007; Balu and Lucki, 2009), schizophrenia (Adriano et al., 2012), diabetes mellitus (Gispen and Biessels, 2000; Gold et al., 2007), or Alzheimer's disease (Henneman et al., 2009; Schuff et al., 2009). Applying the *BrainAGE* approach to various populations, its ability to recognize subtle changes in individual brain structure in depressive subjects (Franke et al., 2013), schizophrenia (Koutsouleris et al., 2014), diabetes mellitus (Franke et al., 2013), and even health parameters (Franke et al., 2014), as well as its superiority over state-of-the-art biomarkers in predicting AD (Gaser et al., 2013) or differentiating neighboring age groups in healthy children and adolescents (Franke et al., 2012b) was recently demonstrated. Additionally, with an intraclass correlation coefficient (ICC) of 0.93 calculated from two shortly delayed scans the *BrainAGE* method already proved its ability to provide reliable estimates (Franke et al., 2012a). Therefore, this study strongly suggests that the *BrainAGE* framework reliably indicates even temporary neuroanatomical changes as for example occurring during the course of the menstrual cycle. Consequently, in future the *BrainAGE* method could be applied to monitor subtle neuroanatomical changes in longitudinal intervention and treatment studies, e.g. exploring the effects of daily activity, protective nutrients, or medication on individual brain structure.

Moreover, since the menstrual cycle obviously changes brain volume (Protopopescu et al., 2008; Pletzer et al., 2010; Hagemann et al., 2011), this study also encourages future neuroimaging research to account for sex as well as hormonal status or stage of the menstrual cycle. This extends the results of previous studies suggesting distinct gender-specific patterns of health parameters being associated with brain aging (Franke et al., 2014) as well as the prevention, detection, treatment, and outcome of illnesses affecting men and women differently, including differences in basic aspects of their normal function and their experience of the same illness (Pinn, 2003; Grossi et al., 2005; Azad et al., 2007).

In conclusion, *BrainAGE* scores decrease during the course of the menstrual cycle in young women and inversely correlate to estradiol levels, qualifying the *BrainAGE* approach to further explore hormonal influences on brain size and structure. Additionally, in order to identify

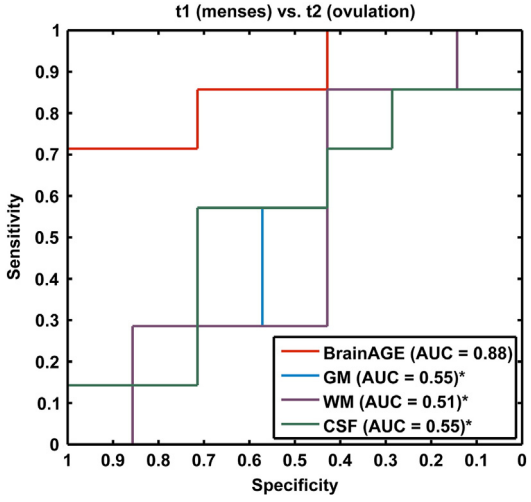

Fig. 4. ROC curves in women for t1 (menses) vs. t2 (ovulation). ROC curves for individual classification as t1 (menses) vs. t2 (ovulation) based on *BrainAGE* scores, GM, WM, and CSF volumes in the female sample, resulting in the area under the ROC curve (AUC). The AUC shows the quality of the classification, with 1.0 indicating a perfect discrimination and 0.5 indicating a result obtained by chance only. AUCs based on GM, WM, and CSF volumes were significantly lower than AUC based on *BrainAGE* scores (* $p < 0.05$).

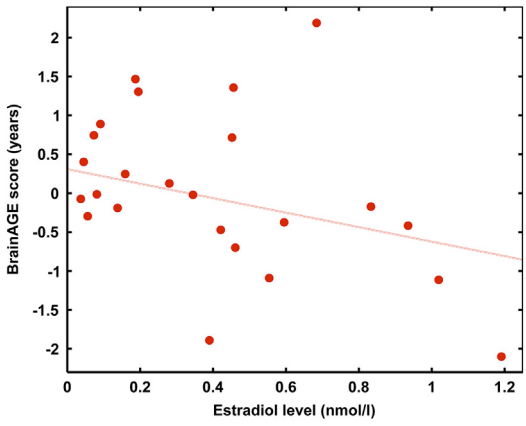

Fig. 5. Correlation between *BrainAGE* scores and estradiol levels in females. Higher estradiol levels were significantly associated with lower *BrainAGE* scores ($r = -0.42$, $p < 0.05$).

subtle neuroanatomical alterations, the *BrainAGE* approach demonstrated its potential to work very sensitive as well as to be easy to apply since *BrainAGE* scores are calculated from structural MRI, using fully automated processing techniques. The implications of this study may lead to a clinical tool that identifies subtle, yet clinically significant, changes in brain structure, thus facilitating as well as monitoring early treatment or preventative interventions, such as hormone replacement therapies.

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