NeuroImage xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

NeuroImage

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

# Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men

Matthew S. Panizzon [a,*], Richard L. Hauger [a,b], Lindon J. Eaves [c], Chi-Hua Chen [a], Anders M. Dale [d,e], Lisa T. Eyler [a,b], Bruce Fischl [f,g,h], Christine Fennema-Notestine [a,d], Carol E. Franz [a], Michael D. Grant [i], Kristen C. Jacobson [j], Amy J. Jak [a,b], Michael J. Lyons [i], Sally P. Mendoza [k], Michael C. Neale [c], Elizabeth Prom-Wormley [c], Larry J. Seidman [g], Ming T. Tsuang [a,l], Hong Xian [m], William S. Kremen [a,l]

[a] Department of Psychiatry, University of California, San Diego, La Jolla, CA 92093, USA
[b] San Diego Veterans Administration Healthcare System, San Diego, CA 92161, USA
[c] Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA 23298, USA
[d] Department of Radiology, University of California, San Diego, La Jolla, CA 92093, USA
[e] Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093, USA
[f] Department of Radiology, Massachusetts General Hospital, Boston, MA 02114, USA
[g] Harvard Medical School, Boston, MA 02115, USA
[h] Computer Science and AI Lab, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[i] Department of Psychology, Boston University, Boston, MA 02215, USA
[j] Department of Psychiatry, University of Chicago, Chicago, IL 60637, USA
[k] Department of Psychology, University of California, Davis, Davis, CA 95616, USA
[l] Center for Behavioral Genomics, University of California, San Diego, La Jolla, CA 92093, USA
[m] Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA

ARTICLE INFO

*Article history:*
Received 2 March 2011
Revised 18 September 2011
Accepted 19 September 2011
Available online xxxx

*Keywords:*
Heritability
Hippocampal volume
Testosterone
Twin study
Aging

ABSTRACT

The hippocampus expresses a large number of androgen receptors; therefore, in men it is potentially vulnerable to the gradual age-related decline of testosterone levels. In the present study we sought to elucidate the nature of the relationship between testosterone and hippocampal volume in a sample of middle-aged male twins (average age 55.8 years). We found no evidence for a correlation between testosterone level and hippocampal volume, as well as no indication of shared genetic influences. However, a significant moderating effect of testosterone on the genetic and environmental determinants of hippocampal volume was observed. Genetic influences on hippocampal volume increased substantially as a function of increasing testosterone level, while environmental influences either decreased or remained stable. These findings provide evidence for an apparent gene-by-hormone interaction on hippocampal volume. To the best of our knowledge, this is the first study to demonstrate that the heritability of a brain structure in adults may be modified by an endogenous biological factor.



## Introduction

As early as the fourth decade of life, testosterone levels in men begin to decline at a steady rate (Feldman et al., 2002; Ferrini and Barrett-Connor, 1998; Harman et al., 2001; Muller et al., 2003). This decline is especially pronounced for bioavailable or free testosterone, which is not bound to the sex hormone binding globulin and therefore is physiologically active (Feldman et al., 2002; Ferrini and Barrett-Connor, 1998; Harman et al., 2001; Muller et al., 2003). The gradual loss of testosterone leads to functional changes in androgen responsive tissue, those areas of the body in which androgen receptors (AR) are abundant (Morley, 2001; Vermeulen, 2000). In addition to the prostate (Cunha et al., 1987), heart (Marsh et al., 1998), skin (Blauer et al., 1991), and musculoskeletal tissue (Sheffield-Moore and Urban, 2004), the brain is highly responsive to androgens such as testosterone, with the hippocampus being one of the most strongly influenced regions (Beyenburg et al., 2000; Kerr et al., 1995; Simerly et al., 1990). Indeed, the level of AR expression in the hippocampus, as measured by AR mRNA concentration, has been shown to be of the same order of magnitude as expression in the prostate (Beyenburg et al., 2000). Thus, although testosterone is frequently associated with sexually dimorphic physical characteristics, as well as behavioral traits such as aggression, the gradual decrease in the availability of testosterone for hippocampal tissue is likely a key component of the processes underlying brain aging in men (Pike et al., 2006; Veiga et al., 2004).

Animal studies have demonstrated a significant positive association between testosterone and hippocampal volume (Galea et al.,

* Corresponding author at: Department of Psychiatry, University of California, San Diego, 9500 Gilman Drive (MC 0738), La Jolla, CA 92093-0738, USA. Fax: +1 858 822 5856.
*E-mail address:* mspanizz@ucsd.edu (M.S. Panizzon).

1053-8119/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2011.09.044

1999), as well as effects of the hormone on hippocampal neural plasticity (Harley et al., 2000; MacLusky et al., 2006), synaptic density (Leranth et al., 2003; Parducz et al., 2006), and neurogenesis (Galea, 2008; Galea et al., 2006). In humans, testosterone level has been found to correlate positively with hippocampal volume during adolescence (Neufang et al., 2009). On a more global scale, levels of bioavailable testosterone from midlife (roughly age 60) have been shown to predict total cranial volume as well as frontal and parietal lobe volumes 10 to 16 years later (Lessov-Schlaggar et al., 2005). We are aware of no studies in adult humans that have established a direct relationship between testosterone and hippocampal volume; however, higher testosterone levels have been linked to increased regional cerebral blood flow within the human hippocampus (Moffat and Resnick, 2007). While there has been minimal investigation of the relationship between testosterone and the hippocampus in humans, numerous studies have found a positive correlation between testosterone and hippocampally-mediated cognitive processes (e.g., episodic memory and visual–spatial ability) in middle-aged and older adults (Barrett-Connor et al., 1999; Martin et al., 2008; Matousek and Sherwin, 2010; Yaffe et al., 2002).

In addition to associations with the hippocampus and cognition, low testosterone levels have been shown to be predictive of Alzheimer's disease (AD) in men (Hogervorst et al., 2004; Moffat et al., 2004), as well as amnestic mild cognitive impairment (Chu et al., 2008). Evidence indicates that testosterone regulates the accumulation of β-amyloid in the brain, potentially abating the neuropathology underlying AD (Gouras et al., 2000; Rosario et al., 2006). Interactions between testosterone and the apolipoprotein-E (*APOE*) ε4 allele have also been observed for measures of cognitive functioning and hippocampal volume (Raber, 2008; Panizzon et al., 2010), as well as for the risk of AD itself (Hogervorst et al., 2002). Findings such as these have promoted the hypothesis that androgens like testosterone are neuro-protective, sparking interest into hormone replacement as a possible therapeutic intervention (Hammond et al., 2001; Pike et al., 2009; Veiga et al., 2004). It should be noted, however, that the actual risks and benefits of such hormone replacement treatment remain largely undetermined.

The aim of the present study was to elucidate the nature of the relationship between testosterone and hippocampal volume in adult men. Using data from a cohort of middle-aged male twins we examined two potential mechanisms. First, given that multiple animal studies have shown a relationship between testosterone and structural aspects of the hippocampus (Galea et al., 1999, 2006; Leranth et al., 2003; MacLusky et al., 2006), as well as some evidence for a direct relationship in humans (Moffat and Resnick, 2007; Neufang et al., 2009), we examined whether testosterone level and hippocampal volume were significantly correlated with one another, and the extent to which any observed correlation was driven by shared genetic or environmental factors. Second, we tested whether testosterone modifies the degree to which hippocampal volume is influenced by genetic and environmental factors. In any type of tissue, testosterone primarily exerts its influence by binding with the AR (Li and Al-Azzawi, 2009). As a transcriptional activator the AR modulates the expression of numerous downstream genes by regulating the conversion (i.e., transcription) of DNA into RNA (Dalton and Gao, 2010; Li and Al-Azzawi, 2009). Thus, the function of the AR provides a mechanism by which testosterone may alter the degree to which a phenotype like hippocampal volume is influenced by genetic factors. Along these lines, a number of studies have to date shown that testosterone can alter the expression of specific genes within the hippocampus and other brain regions (Chowen-Breed et al., 1989; Kerr et al., 1996; Tirassa et al., 1997; Zhang et al., 1999). We therefore tested whether the heritability of hippocampal volume (i.e., the proportion of variance contributed by genetic factors) would vary as a function of testosterone level. Put another way, we examined whether the level of testosterone alters the balance of latent genetic and environmental factors that contribute to individual differences in hippocampal volume.

## Methods

### *Participants*

Data were obtained as part of the Vietnam Era Twin Study of Aging (VETSA), a longitudinal study of cognitive and brain aging with baseline in midlife (Kremen et al., 2006). Participants in the VETSA were sampled from the Vietnam Era Twin (VET) Registry, a nationally distributed sample of male–male twin pairs who served in the United States military at some point between 1965 and 1975 (Goldberg et al., 2002). Detailed descriptions of the VET Registry's composition and method of ascertainment have been reported elsewhere (Eisen et al., 1987; Henderson et al., 1990). Although all VETSA participants are military veterans, the vast majority did not experience combat situations during their military careers. In total, 1237 men ages 51 to 60 participated in the primary VETSA project, the average age was 55.4 years (SD = 2.5). Participants were predominantly Caucasian (89.7%), with an average education of 13.8 years (SD = 2.1); 88% described their overall health as good or better. In comparison to U.S. census data, participants in the VETSA are similar in demographic and health characteristics to American men in their age range (Centers for Disease Control and Prevention, 2003). Zygosity for 92% of the sample was determined by analysis of 25 microsatellite markers obtained from blood samples. For the remainder of the sample zygosity was determined through a combination of questionnaire and blood group methods. A comparison of these two approaches in the VETSA sample has demonstrated an agreement rate of 95%. Neuroimaging and endocrine data were collected as part of two related studies: neuroimaging began in 2003 (N = 474); and endocrine data collection began in 2005 (N = 783).

As part of the primary VETSA project, participants traveled to either the University of California San Diego (UCSD) or Boston University for a daylong series of physical, psychosocial, and neurocognitive assessments. Informed consent was obtained from all participants prior to data collection. To be eligible for the primary VETSA project both members of a twin pair had to agree to participate and be between the ages of 51 and 59 at the time of recruitment. Similar eligibility criteria were imposed for the VETSA MRI study, with the exception that standard MRI exclusion criterion were imposed in order to ensure participant safety (e.g., the absence of metal in the body). No exclusion criteria were used for the endocrine study other than the participant's willingness to participate. In total, both neuroimaging and testosterone data were available for 382 VETSA participants: 89 monozygotic (MZ) pairs, 68 dizygotic (DZ) pairs, and 68 unpaired twins. This subset did not significantly differ from the rest of the sample with respect to age (average age = 55.9 years, SD = 2.6), years of education (average education = 13.8 years, SD = 2.1), ethnicity (86.7% Caucasian), or self-reported health status (90% described as good or better).

### *Procedures*

#### *Testosterone collection and assay*

Testosterone levels were obtained through saliva collection on two days at home during a participant's typical week, as well as on the assessment day. The at-home samples were collected approximately two weeks prior to the assessment day. Saliva was collected at waking, 30 min after waking, 10:00 a.m., 3:00 p.m., and bedtime on all days. Participants were mailed a saliva collection kit which included individualized instructions, labeled 4.5 ml Cryotube vials, Trident original sugarless gum, straws, tissues, a daily log, pen, reminder watch, and a storage container with an electronic track cap for determining compliance with the protocol. Samples were sent via overnight mail to the University of California at Davis for assay.

Prior to assay, saliva samples were centrifuged at 3000 rpm for 20 min to separate the aqueous component from mucins and other suspended particles. Salivary concentrations of free testosterone

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044

were determined in duplicate using commercial radioimmunoassay kits (Beckman Coulter Inc., formerly Diagnostics Systems Laboratories, Webster, TX). Assay procedures were identical to those previously described by Granger and colleagues (Granger et al., 1999). Intra-assay and inter-assay coefficients of variation were 3.141 pg/ml and 4.878 pg/ml, respectively. The least detectable dose for the assay was 1.3697 pg/ml. All samples from each participant were assayed together; and data from one to three individuals were included in each assay batch. Assays were always performed without knowledge of the zygosity of the twin pairs. Values greater than three standard deviations above the mean waking testosterone level, the highest value of the day, were set to missing in order to eliminate outlying data points. Data from participants who reported taking testosterone supplements or other medications known to alter testosterone levels were also set to missing. All analyses utilized the average testosterone value for all time points across the three collection days. This value was square-root transformed in order to normalize the distribution, and adjusted for the effects of age prior to formal structural equation modeling. Hereafter, all references to testosterone results refer to these transformed, age-adjusted values.

*MRI acquisition and processing*

The acquisition parameters and post-processing details for the VETSA MRI study have been described in detail elsewhere (Kremen et al., 2010). Briefly, neuroimaging was performed within 24 h of the assessment day at either the UCSD Medical Center or the Massachusetts General Hospital (MGH) in Boston. Images were acquired on Siemens 1.5 T scanners. Scanning sequences were specifically designed for use across different scanners and vendors. Sagittal T1-weighted MPRAGE sequences were employed with a TI = 1000 ms, TE = 3.31 ms, TR = 2730 ms, flip angle = 7°, slice thickness = 1.33 mm, voxel size 1.3 × 1.0 × 1.3 mm. Raw DICOM MRI scans from both sites were transferred to MGH for post-processing and quality control.

Hippocampal volumes were obtained using segmentation methods based on the publically available FreeSurfer software package (Fischl et al., 2002). The semi-automated, fully 3D whole brain segmentation procedure uses a probabilistic atlas and applies a Bayesian classification rule to assign a neuroanatomical label to each voxel. Adhering to the parcellation system described by Makris and colleagues (Makris et al., 1999), the FreeSurfer definition of the hippocampus includes the dentate gyrus, the CA fields, the subiculum/parasubiculum, and the fimbria. Segmentation of the hippocampus, the region of interest for the present study, required a visual inspection of the automated output to ensure no technical failure of the application or mislabeling. For the purposes of the VETSA MRI study, a project-specific atlas was created from 20 unrelated, randomly selected VETSA participants. Automated volumetric measurements based on this atlas were within the 99% confidence interval with respect to the "gold standard" manual measurements (Kremen et al., 2010). Direct comparisons of FreeSurfer to manually derived hippocampal volume measurements in other samples have also demonstrated high degrees of agreement between the approaches (Fischl et al., 2002; Morey et al., 2009). A measure of total intracranial volume (ICV) was also estimated based on a scaling factor related to the transformation of the full brain mask into atlas space (Buckner et al., 2004). Estimated ICV was used to adjust the hippocampal volume measures for individual differences in head size (Kremen et al., 2010). In addition to controlling for ICV, measures of left and right hippocampal volume were statistically adjusted for the effects of participant age and scanning site. All future references to hippocampal volume results refer to volumes adjusted for ICV, age, and scanning site.

*Statistical analyses*

The standard univariate twin model decomposes the variance of any phenotype into the proportion attributed to additive genetic (A) influences, common or shared environmental (C) influences (i.e., environmental factors that make both members of a twin pair similar to one another), and unique environmental (E) influences (i.e., environmental factors that make members of a twin pair different from one another, including measurement error) (Eaves et al., 1978; Neale and Cardon, 1992). The resulting model is commonly referred to as the "ACE" model. Analyses for the present study extend this model so as to determine the degree of covariance between the A, C, and E contributions to testosterone level and hippocampal volume, as well as determine the degree to which the A, C, and E contributions to hippocampal volume vary as a function of testosterone (Purcell, 2002). Models were fit to the raw data using the maximum-likelihood based structural equation modeling software Mx (Neale et al., 2004).

*Multivariate twin analysis*

Multivariate twin analysis can be used, in part, to calculate genetic and environmental correlations between phenotypes of interest. Conceptually, these correlations represent the degree to which genetic and environmental influences of one phenotype are predictive of the influences for another phenotype (Carey, 1988). In order to determine if testosterone level and hippocampal volume correlated significantly, and if they possess significant genetic and environmental correlations, we fit a trivariate (i.e., testosterone, left hippocampal volume, and right hippocampal volume) correlated factors model. A simplified bivariate version of the relationships derived from this model is depicted in Fig. 1. In addition to estimating the genetic and environmental contributions to each phenotype, the correlated factors model decomposes the total covariance between phenotypes into genetic and environmental components. As such, the sum of the standardized genetic and environmental covariance estimates is equal to the phenotypic correlation. The genetic and environmental covariance estimates can then be used to calculate genetic and environment correlations. In statistical terms, the genetic correlation between two phenotypes is equal to their genetic covariance, divided by the square root of the product of their separate genetic variances (Neale and Cardon, 1992). Shared environmental and unique environmental correlations are calculated in a similar fashion using the corresponding variance and covariance estimates. Unlike other structural equation models that utilize twin data, the correlated factors model tests no specific hypothesis regarding the data; rather, the model simply estimates the genetic and environmental contributions to the variance of each phenotype and the covariances between them, while imposing no other structure on the data. Thus, the model is analogous to a simple correlation analysis that would be conducted in standard, non-twin studies.

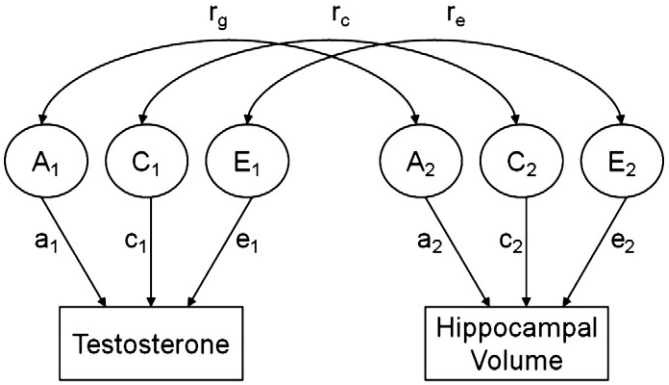


**Fig. 1.** Bivariate correlated factors model. A = Additive genetic influences; C = Shared or common environmental influences; E = Nonshared or unique environmental influences; $r_g$ = Genetic correlation; $r_c$ = Shared environment correlation; $r_e$ = Unique environment correlation. Lower case a, c, and e represent the parameter estimates for the corresponding variance components.

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044

*Moderation of variance components*

To determine if the heritability of hippocampal volume varies as a function of testosterone, we utilized a model that allowed for the inclusion of an additional measured variable in the previously mentioned univariate ACE model that would act as a moderator of the genetic and environmental influences. The model is depicted in Fig. 2. As described by Purcell (Purcell, 2002), the model specifies a linear increase or decrease in each of the variance components and the mean of a phenotype as a function of the continuous moderator variable. Each moderating effect ($\beta$) is independent of the other, thereby allowing multiple variations of the model to be fit (e.g., moderation of the genetic variance only, moderation of the unique environment variance only). The independence of the effects also allows for a moderating influence to be present on the variance components of a phenotype while the mean remains stable. The presence of a significant moderator effect on the mean simply indicates that testosterone, the moderating variable, is phenotypically correlated with hippocampal volume. A moderator effect on either the genetic or environmental variance components would indicate that the relative contributions of these factors to the variance of hippocampal volume are not constant across different levels of testosterone. Because the proportions of genetic and environmental variances determine heritability, a moderator effect on the variance components may result in differential heritability of hippocampal volume at different levels of testosterone.

In total, six models were fit to the data in order to test for the presence of a moderating effect of testosterone. We began by first fitting a model with no moderating effects. This provided a comparison model against which the fit of all other models would be judged. Moderating effects were then systematically added to the mean and each of the variance components. Evaluation of relative model fit was performed using the likelihood-ratio-test (LRT), which is calculated as the difference in the negative 2 log-likelihood (−2LL) of one model relative to another (i.e., a model with one or more moderating effects relative to the model with none). Under certain regulatory conditions, the LRT is distributed as a chi-square with degrees of freedom equivalent to the difference in the number of parameters between the competing models (Steiger et al., 1985). Significant LRT values ($p<.05$) indicate that the addition of moderating effects significantly improves the fit of the model. In addition, we used the Akaike Information Criterion (AIC), calculated as the −2LL minus twice the degrees of freedom, as a secondary indicator of model parsimony (Akaike, 1987). Lower AIC values represent a better balance on the part of the model between goodness-of-fit and the number of parameters.

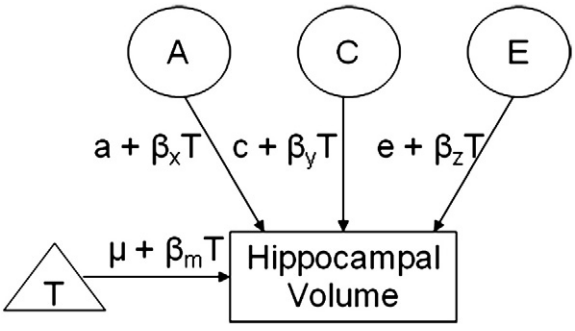


**Fig. 2.** Univariate ACE model with moderation effects. A = Additive genetic influences; C = Shared or common environmental influences; E = Nonshared or unique environmental influences; T = Testosterone level; $\beta_x$ = Moderating effect on the genetic variance; $\beta_y$ = Moderating effect on the shared environment variance; $\beta_z$ = Moderating effect on the unique environment variance; $\beta_m$ = Moderating effect on the mean. Lower case a, c, and e represent the parameter estimates for the corresponding variance components.

## Results

Prior to statistical adjustment, the average testosterone level was 98.4 pg/ml (SD = 29.3), and a small but significant correlation was observed between the hormone level and age, $r=-.13$ ($p=.01$). The unadjusted, average left and right hippocampal volumes were 3974.7 $mm^3$ (SD = 393.7) and 4198.0 $mm^3$ (SD = 426.1), respectively. These volumes are consistent with those reported for similarly aged samples that were collected utilizing nearly identical imaging and post-processing methods (Walhovd et al., 2011). The difference in the average volumes for the two structures was highly significant ($p<.0001$) and was consistent with the established pattern of hippocampal asymmetry (Watson et al., 1992; Weis et al., 1989). After accounting for ICV, small correlations were observed between both hippocampal volumes and the age of the participants (Left Hippocampus: $r=-.10$, $p=.02$; Right Hippocampus: $r=-.11$, $p=.02$). There were no significant effects observed for scanning site on either measure of hippocampal volume after adjusting for ICV.

*Trivariate correlated factors model*

Estimates of the genetic, environmental and phenotypic correlations (as derived from the trivariate correlated factors model) are presented in Table 1. The average testosterone level was found to have a significant heritability ($a^2$) of .34 (i.e., 34% of the variance could be attributed to additive genetic influences), common environmental influences ($c^2$) of .20, and unique environmental influences ($e^2$) of .46. The observed common environmental influences on testosterone were likely due to the effects of assay batch, which introduced additional within-pair covariance for twins that were processed together. Left and right hippocampal volumes were both strongly heritable, .62 and .66 respectively, and both had minimal common environmental influences, .02 and .00 respectively. Consistent with previous findings from our group, the genetic correlation ($r_g$) between the left and right hippocampal volumes was .99 (95% CI: .94 to 1.0), indicating that the same genes influence the volume of both structures (Eyler et al., 2011).

At the phenotypic level the correlations between testosterone and both hippocampal volumes were extremely small and not statistically significant (Left Hippocampus: $r_p=.00$, Right Hippocampus: $r_p=-.02$). Genetic correlations with testosterone were .13 for the left hippocampus and .18 for the right; neither was significant based on the 95% confidence intervals. Although the correlation between the common environmental influences ($r_c$) appeared to be large in magnitude (Left Hippocampus $r_c=-.74$; Right Hippocampus $r_c=-.99$), these values were not significant due the minimal impact of the common environment on either hippocampal volume. The unique environmental correlation ($r_e$) between testosterone and left hippocampal volume was small and non-significant, −.03; however, a significant relationship was observed with right hippocampal volume ($r_e=-.21$). Overall, the results indicated the presence of minimal genetic or environmental covariance between testosterone level and hippocampal volume.

*Moderation of variance components*

The model fitting results for the moderating effects of testosterone on the left and right hippocampal volumes are presented in Table 2. Unlike the previous analysis, separate models were fit for the left and right hippocampal volumes. We began by fitting models with only additive genetic and unique environment variance components, referred to as an AE model, and no moderating effects for each hippocampal volume (Models 1a and 1b). This provided us with baseline models to which moderating effects could systematically be added in order to see if doing so resulted in significant improvements in model fit. Given the minimal effects of the common environment on hippocampal volume observed in the previous analysis, we elected to set this parameter to zero in all subsequent models. The 20%

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044

**Table 1**
Results of the trivariate correlated factors model.

| | Standardized variance components | | | Correlations with testosterone | | | |
|---|---|---|---|---|---|---|---|
| | $a^2$ | $c^2$ | $e^2$ | $r_p$ | $r_g$ | $r_c$ | $r_e$ |
| Testosterone level (pg/ml) | .34 (.04;.61) | .20 (.00;.43) | .46 (.37;.58) | – | – | – | – |
| Left hippocampal volume ($mm^3$) | .62 (.41;.72) | .02 (.00;.21) | .36 (.28;.47) | .00 (−.11;.11) | .13 (−.34;.90) | −.74 (−1.0;1.0) | −.03 (−.21;.16) |
| Right hippocampal volume ($mm^3$) | .66 (.50;.74) | .00 (.00;.14) | .34 (.26;.44) | −.02 (−.13;.09) | .18 (−.23;.91) | −.99 (−1.0;1.0) | −.21 (−.38;−.01) |

$a^2$ = Additive Genetic Variance Component (Heritability); $c^2$ = Common Environment Variance Component; $e^2$ = Unique Environment Variance Component; $r_p$ = Phenotypic Correlation; $r_g$ = Genetic Correlation; $r_c$ = Common Environment Correlation; $r_e$ = Unique Environment Correlation. 95% confidence intervals are presented in the parentheses. Measures of hippocampal volume are adjusted for the effects of ICV, age, and scanning site. Testosterone level is adjusted for age, and square-root transformed to normalize the distribution.

contribution of the common environment to testosterone was not impacted by this step because only the observed level of the hormone was represented in the model and not its individual variance components.

There was no significant moderating effect of testosterone on the mean of the right or the left hippocampal volumes, Models 2a and 2b. This was consistent with the near zero phenotypic correlations that were observed between testosterone and both hippocampal volumes in the correlated factors model. For the right hippocampus, there was no significant moderating effect of testosterone on the additive genetic variance alone, Model 3a ($p = .248$), and no significant moderating effect of testosterone on the unique environment variance alone, Model 4a ($p = .390$). However, a highly significant change in model fit ($p = .006$) was observed when moderating effects were added to both the additive genetic and unique environment variance components but not the mean, Model 5a. The inclusion of a moderating effect on the mean as well as the two variance components, Model 6a, also resulted in a significant change in model fit ($p = .012$); however, examination of the LRT values for both models indicated that relative to Model 5a the increase in LRT for Model 6a was small and did not reach statistical significance (LRT = 0.88, ΔDF = 1, $p = .350$). In addition, the smaller AIC value for Model 5a indicates that this scenario of moderating effects (i.e., on both variance components but not on the means) achieved a better balance between overall fit and parsimony. As shown in Fig. 3a, the moderating effects from Model 5a resulted in a steady increase in the genetic variance of hippocampal volume as a function of increasing testosterone levels, whereas an inverse relationship was observed for the unique environment variance. As a result, the heritability of hippocampal volume increased from .27 at the lowest testosterone level, to .96 when the hormone level was at its highest point (see Fig. 3b).

**Table 2**
Model fitting results for the moderating effects of testosterone on hippocampal volume.

| Model | | −2LL | DF | LRT | ΔDF | p | AIC |
|---|---|---|---|---|---|---|---|
| *Right hippocampus* | | | | | | | |
| 1a. | AE model with no moderating effects | 4532.552 | 311 | – | – | – | 3910.552 |
| 2a. | AE model + moderating effects on the means | 4531.492 | 310 | 1.06 | 1 | .303 | 3911.492 |
| 3a. | AE model + moderating effects on the genetic variance | 4531.215 | 310 | 1.34 | 1 | .248 | 3911.215 |
| 4a. | AE model + moderating effects on the unique environment variance | 4531.812 | 310 | 0.74 | 1 | .390 | 3911.812 |
| **5a.** | **AE model + moderating effects on the genetic and unique environment variances** | **4522.473** | **309** | **10.08** | **2** | **.006** | **3904.473** |
| 6a. | AE model + moderating effects on the means and all variance components | 4521.589 | 308 | 10.96 | 3 | .012 | 3905.589 |
| *Left hippocampus* | | | | | | | |
| 1b. | AE model with no moderating effects | 4509.678 | 311 | – | – | – | 3887.678 |
| 2b. | AE model + moderating effects on the means | 4509.574 | 310 | 0.10 | 1 | .747 | 3889.574 |
| **3b.** | **AE model + moderating effects on the genetic variance** | **4504.863** | **310** | **4.82** | **1** | **.028** | **3884.863** |
| 4b. | AE model + moderating effects on the unique environment variance | 4508.784 | 310 | 0.89 | 1 | .344 | 3888.784 |
| 5b. | AE model + moderating effects on the genetic and unique environment variances | 4504.535 | 309 | 5.14 | 2 | .076 | 3886.535 |
| 6b. | AE model + moderating effects on the means and all variance components | 4504.268 | 308 | 5.41 | 3 | .144 | 3888.268 |

* Models 2 through 6 are tested against the fit of model 1. −2LL = Negative 2 log-likelihood; DF = Degrees of freedom; LRT = Likelihood-ratio chi-square test; ΔDF = Change in degrees of freedom; AIC = Akaike information criterion. Best fitting models appear in **bold** font.

For the left hippocampus, the only significant change in model fit was obtained when a moderating effect was added to the additive genetic variance, Model 3b ($p = .028$). The inclusion of moderating effects on both the genetic and unique environment variance components, Model 5b, resulted only in a trend level reduction in fit ($p = .067$). Thus, a model equivalent to the one fitted to the right hippocampus proved to be a poor representation of the data. The moderating effect from Model 3b resulted in a steady increase in the genetic variance of left hippocampal volume as a function of increases in testosterone level, whereas the unique environment variance remained stable. At the lowest testosterone levels the heritability of left hippocampal volume was .47, while at the higher testosterone levels the heritability increased to .72 (see Supplemental Fig. 1).

### *Secondary analyses*

In order to ensure that testosterone had no effect on the common environmental determinants of hippocampal volume, analyses were repeated with the common environment parameter freely estimated. The best-fitting model for the right hippocampus was again obtained when moderating effects were added to additive genetic and unique environment variance components, while the common environment and the mean remained unaffected. For the left hippocampus, the best-fitting model was obtained when moderating effects were only allowed to influence the additive genetic variance component. In both cases the relative increase in heritability as function of testosterone was identical to what was observed in the previous analyses (Right Hippocampus: .27 to .96; Left Hippocampus: .47 to .72).

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044

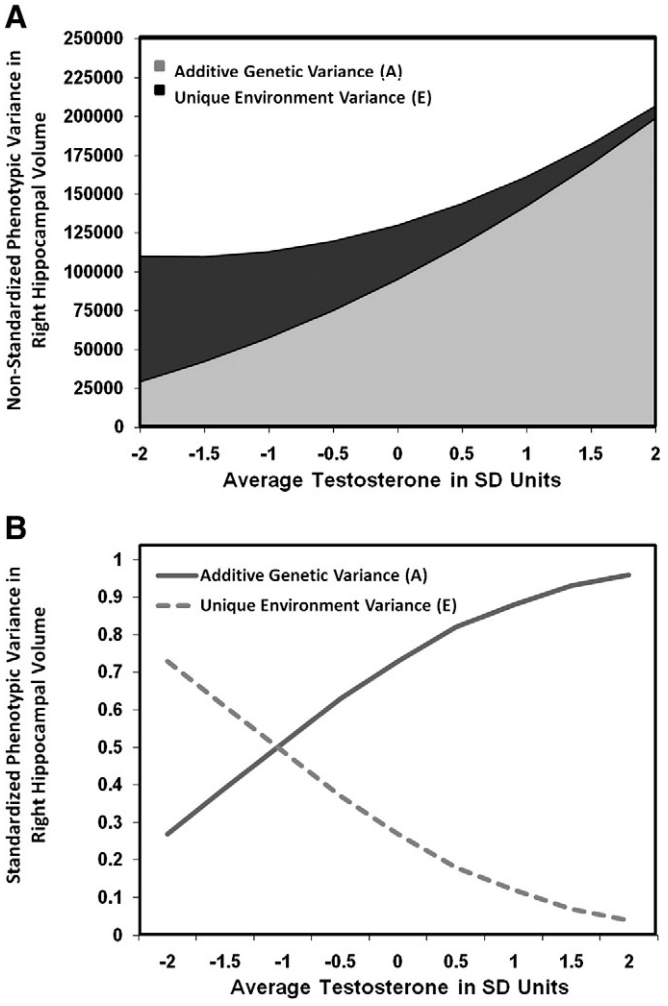


**Fig. 3.** Best fitting model for the moderating effects of testosterone on right hippocampal volume. (A) Effect of testosterone on the non-standardized variance components of hippocampal volume. (B) Effect of testosterone on the standardized variance components of hippocampal volume. The additive genetic variance in this case indicates the heritability of hippocampal volume at the given testosterone level. In both figures the testosterone level has been standardized to a mean of zero and a standard deviation of 1.0.

Additional analyses were also performed in order to determine whether the observed effects of testosterone were unique to hippocampal volume, or were indicative of a more global brain process. Thus, we tested for effects of testosterone on a measure of total brain volume. There was no significant effect of testosterone on the mean of total brain volume, indicating the absence of a significant phenotypic correlation between them. Moreover, there were no significant effects of testosterone on the genetic and environmental variance components of total brain volume. Altogether, a model in which moderating effects of testosterone were allowed on all parameters resulted in minimal change in fit relative to a model with no moderating effects ($p = .741$), once again indicating non-significant effects of testosterone. Equivalent results were obtained for a measure of total brain volume adjusted for ICV.

## Discussion

### *No correlation between testosterone and hippocampal volume*

In the present study, there was no evidence for either a phenotypic or genetic correlation between testosterone level and hippocampal volume. In other words, hippocampal volumes did *not* increase or decrease as a function of testosterone levels, and there was no evidence of shared genetic variance between the hormone and the brain structure. A small but significant unique environmental correlation was observed between testosterone level and right hippocampal volume; however, the magnitude of this correlation indicated a minimal degree of shared variance. These results allowed us to conclude that testosterone level and hippocampal volume were phenotypically and genetically independent of one another.

### *Testosterone moderates genetic and environmental variance components*

Despite the absence of significant phenotypic or genetic covariance, we found that testosterone moderated the heritability of hippocampal volume, such that individual differences in the brain structure were more genetically driven when testosterone levels were higher. The effect was most pronounced for the right hippocampus where the heritability ranged from .27 when testosterone levels were two standard deviations below the mean, to .96 when the testosterone levels were two standard deviations above the mean. For the left hippocampus, which was on average smaller than the right and had a smaller overall variance, the heritability ranged from .47 to .72. Once again, testosterone level did not modify the volume of the hippocampus; rather, the results indicate that regardless of whether the hippocampus is large or small, the determinants of its size differ as a function of testosterone level. Genetic influences played a greater role in determining hippocampal volume when testosterone levels were high, whereas unique environmental influences played a greater role when testosterone levels were low. To the best of our knowledge, this is the first study to show that the heritability of a brain structure in adults can be modified by an endogenous biological factor.

The dynamic nature of heritability estimates is a well established concept (Jinks and Fulker, 1970), and recent studies of phenotypes such as general cognitive ability (Turkheimer et al., 2003; Vinkhuyzen et al., 2011), reading ability (Kremen et al., 2005), personality (Krueger et al., 2008), and physical health (Johnson and Krueger, 2005) have repeatedly demonstrated this fact. While multiple twin and family studies have shown that individual differences in brain structures (e.g., cortical thickness and subcortical volumes) are under significant genetic influence (Glahn et al., 2007; Peper et al., 2007; Schmitt et al., 2007), we are aware of only two studies that have reported changes in the heritability of brain imaging phenotypes as a result of some quantifiable factor. In a pediatric sample of twins and siblings, Lenroot and colleagues reported increases in the heritability of cortical thickness from early childhood to late adolescence (Lenroot et al., 2009). More recently, Chiang and colleagues found similar effects of age and socioeconomic status on measures of white matter integrity in a slightly older cohort of twins and siblings ages 12 to 29 (Chiang et al., 2011). These findings, as well as those from the present study, suggest that changes in the balance of genetic and environmental influences on brain structures may occur as a function of age as well as in response to factors either in the external environmental or within the individual. The identification of these potential moderating factors may prove critical to future studies that attempt to elucidate the specific genetic determinants of neuroimaging phenotypes.

At the present time, the precise mechanism through which testosterone influences the heritability of hippocampal volume is unclear. It may be the case that the level of testosterone changes the degree to which the genes that influence hippocampal volume are expressed. Thus, while a fixed number of genes might influence hippocampal volume, the magnitude of their impact could depend upon the exposure of neurons to certain levels of hormones such as testosterone. Animal studies have established that testosterone, as well as hormones such as estrogen and dehydroepiandrosterone, can modify gene expression within the hippocampus and other brain regions

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044

(Aenlle et al., 2009; Chowen-Breed et al., 1989; Kerr et al., 1996; Mo et al., 2009; Tirassa et al., 1997; Zhang et al., 1999). Such a mechanism would certainly be consistent with the known function of the AR, wherein as the concentration of testosterone increases, activation of ARs within the hippocampus will be greater (Dalton and Gao, 2010; Li and Al-Azzawi, 2009). Stronger AR activation in turn, will cause greater expression of AR-regulated genes that may alter hippocampal structure and function. Conversely, these processes will be considerably less or will not occur at all when testosterone levels are low. Alternatively, it may be the case that these results reflect the switching on or off of genes as the level of testosterone changes. In other words, the genes that influence hippocampal volume when testosterone is low may be different from the genes that influence it when the hormone levels are high. An enhanced version of the model used in the present study may be able to ultimately address this question (Neale and Keller, 2007); however, that model is currently still under development.

Although it has been well established that testosterone levels in men decline with increasing age (Feldman et al., 2002; Ferrini and Barrett-Connor, 1998; Harman et al., 2001; Muller et al., 2003), the cross-sectional design of the present study does not allow us to determine whether the observed effects of testosterone are long standing, or the result of age-related changes in the hormone level. Nevertheless, we believe that the nature of the effect, specifically the modulation of genetic influences in the absence of mean level changes, is suggestive of an underlying aging-related process. In an animal model of cognitive aging, Blalock and colleagues showed that changes in gene expression preceded observable age-related changes in the phenotypes they examined (Blalock et al., 2003). Thus, it may be the case that changes in the genetic influences of a phenotype are one of the earliest indicators of cognitive and brain aging. The results of the present study, as well as recent findings from our group in which we found heritability differences but not mean level differences in cognitive domains as a function of hypertension status (Vasilopoulos et al., in press), would appear to be consistent with this hypothesis. Ultimately, longitudinal investigations, and to some extent cross-sectional studies spanning from early adulthood to old age, that utilize advances in genome-wide association and gene expression methods will be needed in order to address this issue.

It is possible that variation in AR sensitivity and signaling might, in part, account for the results of the present study. Functional polymorphisms of the AR gene are determined by a variable number of CAG repeats in exon 1, which subsequently code for a polyglutamine sequence in the receptor protein (Zitzmann and Nieschlag, 2003). The length of this polyglutamine sequence inversely relates to the transcriptional activity of the receptor (Chamberlain et al., 1994), and when pathologically elongated, results in severe androgen insensitivity (i.e., Kennedy's disease) (La Spada et al., 1991). Longer CAG repeat sequences have been negatively associated with cognitive performance in older men (Yaffe et al., 2003), and also appear to slow the decline in testosterone levels with increasing age (Krithivas et al., 1999). Perhaps most relevant to the present findings is the mediating role of the CAG repeat length on the relationships between testosterone and depressive symptoms in middle-aged men (Seidman et al., 2001), amygdala reactivity in men (Manuck et al., 2010), and white matter development in male adolescents (Perrin et al., 2008). The interactions observed in these studies suggest that the modifying influence of testosterone on the heritability of hippocampal volume might vary in accordance with the number of CAG repeats in the AR gene.

### *Limitations*

This study is not without its limitations; however, we believe that each of these represents an additional avenue for further investigation. For example, it remains to be seen whether testosterone exerts similar influences on other brain regions. In addition to the hippocampus, ARs are also highly expressed in the amygdala and areas of the prefrontal cortex; therefore these regions may demonstrate similar relationships with testosterone, although transcriptional regulation can differ due to cellular background (Beyenburg et al., 2000; Finley and Kritzer, 1999; Roselli et al., 2001; Simerly et al., 1990). The role of other hormones in this relationship is also uncertain. The hippocampus has many different types of steroid hormone receptors (Tohgi et al., 1995), of which the glucocorticoid receptors are particularly notable (McEwen, 2002; McEwen et al., 1968). Like testosterone, cortisol has been found to influence hippocampal volume as well as performance on hippocampal-mediated cognitive tasks (Lupien et al., 1994; Lupien et al., 1998). Moreover, the two hormones are known to interact with one another, such that testosterone may suppress cortisol by inhibiting the function of the hypothalamic-pituitary-adrenal axis (Viau, 2002). A more comprehensive model of neuroendocrine action in the hippocampus, therefore, will ultimately have to include the combined actions of testosterone, cortisol, and possibly other hormones. Finally, it remains to be seen if and how these results will generalize to women. Testosterone levels in women, although markedly lower than in men, also decrease with age (Longcope et al., 1986), and higher levels have been associated with better cognitive functioning in later life (Barrett-Connor and Goodman-Gruen, 1999). The effect of testosterone in women, however, is likely secondary to the role of estrogen. Paralleling much of the work done with testosterone, estrogen has been associated with cognitive functioning (Genazzani et al., 2007), structural aspects of the hippocampus (Chen et al., 2009), and the risk of developing AD (Pike et al., 2009). Animal studies have also shown that estrogen can influence hippocampal gene expression (Aenlle et al., 2009). Given the similarity of effects of both hormones on cognition and brain structure, as well as the prominence of both types of receptors within the hippocampus, examination of whether estrogen modifies the heritability of hippocampal volume in women would appear warranted.

### *Conclusion*

The present study demonstrates that in middle-aged men testosterone influences the degree to which hippocampal volume is determined by genetic and environmental influences. To our knowledge, these results are the first to show that in addition to age and aspects of the individual's environment, an endogenous factor can alter the magnitude of genetic influences on a brain structure. Given the gradual decline in testosterone levels that is observed with increasing age, the present results suggest that the level of this hormone is likely a valuable biomarker for brain aging in men.

Supplementary materials related to this article can be found online at doi:10.1016/j.neuroimage.2011.09.044.

## Disclosure statement

Dr. Anders M. Dale is a founder and holds equity in CorTechs Laboratories, Inc., and also serves on the Scientific Advisory Board. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies. All other authors state that there are no actual or potential conflicts of interest.

## Acknowledgments

The VETSA project is supported by National Institutes of Health/National Institute on Aging (NIH/NIA) Grants R01 AG18386, RO1 AG18384, RO1 AG22381, and RO1 AG22982. The U.S. Department of Veterans Affairs has provided support for the development and maintenance of the Vietnam Era Twin Registry. Numerous organizations have provided invaluable assistance, including VA Cooperative Studies Program; Department of Defense; National Personnel Records Center, National Archives and Records Administration; the Internal Revenue Service; National Institutes of Health; National Opinion Research

Center; National Research Council, National Academy of Sciences; the Institute for Survey Research, Temple University; Schulman, Ronca, and Bucuvalas, Inc. Most importantly, we gratefully acknowledge the cooperation and participation of the members of the Vietnam Era Twin Registry and their families. Without their contribution this research would not have been possible.

## References

Aenlle, K.K., Kumar, A., Cui, L., Jackson, T.C., Foster, T.C., 2009. Estrogen effects on cognition and hippocampal transcription in middle-aged mice. Neurobiol. Aging 30, 932–945.

Akaike, H., 1987. Factor analysis and AIC. Psychometrika 52, 317–332.

Barrett-Connor, E., Goodman-Gruen, D., 1999. Cognitive function and endogenous sex hormones in older women. Journal of the American Geriatric Society 47, 1289–1293.

Barrett-Connor, E., Goodman-Gruen, D., Patay, B., 1999. Endogenous sex hormones and cognitive function in older men. J. Clin. Endocrinol. Metab. 84, 3681–3685.

Beyenburg, S., Watzka, M., Clusmann, H., Blumcke, I., Bidlingmaier, F., Elger, C.E., Stoffel-Wagner, B., 2000. Androgen receptor mRNA expression in the human hippocampus. Neurosci. Lett. 294, 25–28.

Blalock, E.M., Chen, K.C., Sharrow, K., Herman, J.P., Porter, N.M., Foster, T.C., Landfield, P.W., 2003. Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J. Neurosci. 23, 3807–3819.

Blauer, M., Vaalasti, A., Pauli, S.L., Ylikomi, T., Joensuu, T., Tuohimaa, P., 1991. Location of androgen receptor in human skin. J. Invest. Dermatol. 97, 264–268.

Buckner, R.L., Head, D., Parker, J., Fotenos, A.F., Marcus, D., Morris, J.C., Snyder, A.Z., 2004. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. Neuroimage 23, 724–738.

Carey, G., 1988. Inference about genetic correlations. Behav. Genet. 18, 329–338.

Centers for Disease Control and Prevention, 2003. Health data for all ages. National Center for Health Statistics.

Chamberlain, N.L., Driver, E.D., Miesfeld, R.L., 1994. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res. 22, 3181–3186.

Chen, J.R., Yan, Y.T., Wang, T.J., Chen, L.J., Wang, Y.J., Tseng, G.F., 2009. Gonadal hormones modulate the dendritic spine densities of primary cortical pyramidal neurons in adult female rat. Cereb. Cortex 19, 2719–2727.

Chiang, M.C., McMahon, K.L., de Zubicaray, G.I., Martin, N.G., Hickie, I., Toga, A.W., Wright, M.J., Thompson, P.M., 2011. Genetics of white matter development: A DTI study of 705 twins and their siblings aged 12 to 29. Neuroimage 54, 2308–2317.

Chowen-Breed, J.A., Steiner, R.A., Clifton, D.K., 1989. Sexual dimorphism and testosterone-dependent regulation of somatostatin gene expression in the periventricular nucleus of the rat brain. Endocrinology 125, 357–362.

Chu, L.W., Tam, S., Lee, P.W., Wong, R.L., Yik, P.Y., Tsui, W., Song, Y., Cheung, B.M., Morley, J.E., Lam, K.S., 2008. Bioavailable testosterone is associated with a reduced risk of amnestic mild cognitive impairment in older men. Clin. Endocrinol. 68, 589–598.

Cunha, G.R., Donjacour, A.A., Cooke, P.S., Mee, S., Bigsby, R.M., Higgins, S.J., Sugimura, Y., 1987. The endocrinology and developmental biology of the prostate. Endocrinology Reviews 8, 338–362.

Dalton, J.T., Gao, W., 2010. Androgen receptor. In: Bunce, C.M., Campbell, M.J. (Eds.), Nuclear Receptors, Proteins and Cell Regulation. Springer Science and Business Media, pp. 143–182.

Eaves, L.J., Last, K.A., Young, P.A., Martin, N.G., 1978. Model-fitting approaches to the analysis of human behavior. Heredity 41, 249–320.

Eisen, S.A., True, W.R., Goldberg, J., Henderson, W., Robinette, C.D., 1987. The Vietnam Era Twin (VET) registry: method of construction. Acta Genet. Med. Gemellol. 36, 61–66.

Eyler, L.T., Prom-Wormley, E., Fennema-Notestine, C., Panizzon, M.S., Neale, M.C., Jernigan, T.L., Fischl, B., Franz, C.E., Lyons, M.J., Stevens, A., Pacheco, J., Perry, M.E., Schmitt, J.E., Spitzer, N.C., Seidman, L., Thermenos, H.W., Tsuang, M., Dale, A., Kremen, W.S., 2011. Genetic patterns of correlation among subcortical volumes in humans: results from a magnetic resonance imaging twin study. Hum. Brain Mapp. 32, 641–653.

Feldman, H.A., Longcope, C., Derby, C.A., Johannes, C.B., Araujo, A.B., Coviello, A.D., Bremner, W.J., McKinlay, J.B., 2002. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J. Clin. Endocrinol. Metab. 87, 589–598.

Ferrini, R.L., Barrett-Connor, E., 1998. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am. J. Epidemiol. 147, 750–754.

Finley, S.K., Kritzer, M.F., 1999. Immunoreactivity for intracellular androgen receptors in identified subpopulations of neurons, astrocytes and oligodendrocytes in primate prefrontal cortex. J. Neurobiol. 40, 446–457.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.

Galea, L.A., 2008. Gonadal hormone modulation of neurogenesis in the dentate gyrus of adult male and female rodents. Brain Res. Rev. 57, 332–341.

Galea, L.A., Perrot-Sinal, T.S., Kavaliers, M., Ossenkopp, K.P., 1999. Relations of hippocampal volume and dentate gyrus width to gonadal hormone levels in male and female meadow voles. Brain Res. 821, 383–391.

Galea, L.A., Spritzer, M.D., Barker, J.M., Pawluski, J.L., 2006. Gonadal hormone modulation of hippocampal neurogenesis in the adult. Hippocampus 16, 225–232.

Genazzani, A.R., Pluchino, N., Luisi, S., Luisi, M., 2007. Estrogen, cognition and female ageing. Hum. Reprod. Update 13, 175–187.

Glahn, D.C., Thompson, P.M., Blangero, J., 2007. Neuroimaging endophenotypes: strategies for finding genes influencing brain structure and function. Hum. Brain Mapp. 28, 488–501.

Goldberg, J., Curran, B., Vitek, M.E., Henderson, W.G., Boyko, E.J., 2002. The Vietnam Era Twin Registry. Twin Res. Hum. Genet. 5, 476–481.

Gouras, G.K., Xu, H., Gross, R.S., Greenfield, J.P., Hai, B., Wang, R., Greengard, P., 2000. Testosterone reduces neuronal secretion of Alzheimer's beta-amyloid peptides. Proceeds Nat. Acad. Sci. 97, 1202–1205.

Granger, D.A., Schwartz, E.B., Booth, A., Arentz, M., 1999. Salivary testosterone determination in studies of child health and development. Horm. Behav. 35, 18–27.

Hammond, J., Le, Q., Goodyer, C., Gelfand, M., Trifiro, M., LeBlanc, A., 2001. Testosterone-mediated neuroprotection through the androgen receptor in human primary neurons. J. Neurochem. 77, 1319–1326.

Harley, C.W., Malsbury, C.W., Squires, A., Brown, R.A., 2000. Testosterone decreases CA1 plasticity in vivo in gonadectomized male rats. Hippocampus 10, 693–697.

Harman, S.M., Metter, E.J., Tobin, J.D., Pearson, J., Blackman, M.R., 2001. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore longitudinal study of aging. J. Clin. Endocrinol. Metab. 86, 724–731.

Henderson, W.G., Eisen, S.E., Goldberg, J., True, W.R., Barnes, J.E., Vitek, M., 1990. The Vietnam Era Twin Registry: A resource for medical research. Public Health Rep. 105, 368–373.

Hogervorst, E., Lehmann, D.J., Warden, D.R., McBroom, J., Smith, A.D., 2002. Apolipoprotein E epsilon4 and testosterone interact in the risk of Alzheimer's disease in men. Int. J. Geriatr. Psychiatry 17, 938–940.

Hogervorst, E., Bandelow, S., Combrinck, M., Smith, A.D., 2004. Low free testosterone is an independent risk factor for Alzheimer's disease. Exp. Gerontol. 39, 1633–1639.

Jinks, J.L., Fulker, D.W., 1970. Comparison of the biometrical genetical, MAVA, and classical approaches to the analysis of human behavior. Psychol. Bull. 73, 311–349.

Johnson, W., Krueger, R.F., 2005. Genetic effects on physical health: lower at higher income levels. Behav. Genet. 35, 579–590.

Kerr, J.E., Allore, R.J., Beck, S.G., Handa, R.J., 1995. Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. Endocrinology 136, 3213–3221.

Kerr, J.E., Beck, S.G., Handa, R.J., 1996. Androgens selectively modulate C-fos messenger RNA induction in the rat hippocampus following novelty. Neuroscience 74, 757–766.

Kremen, W.S., Jacobson, K.C., Xian, H., Eisen, S.A., Waterman, B., Toomey, R., Neale, M.C., Tsuang, M.T., Lyons, M.J., 2005. Heritability of word recognition in middle-aged men varies as a function of parental education. Behav. Genet. 35, 417–433.

Kremen, W.S., Thompson-Brenner, H., Leung, Y.J., Grant, M.D., Franz, C.E., Eisen, S.A., Jacobson, K.C., Boake, C., Lyons, M.J., 2006. Genes, environment, and time: the Vietnam Era Twin Study of Aging (VETSA). Twin Res. Hum. Genet. 9, 1009–1022.

Kremen, W.S., Prom-Wormley, E., Panizzon, M.S., Eyler, L.T., Fischl, B., Neale, M.C., Franz, C.E., Lyons, M.J., Pacheco, J., Perry, M.E., Stevens, A., Schmitt, J.E., Grant, M.D., Seidman, L.J., Thermenos, H.W., Tsuang, M.T., Eisen, S.A., Dale, A.M., Fennema-Notestine, C., 2010. Genetic and environmental influences on the size of specific brain regions in midlife: the VETSA MRI study. Neuroimage 49, 1213–1223.

Krithivas, K., Yurgalevitch, S.M., Mohr, B.A., Wilcox, C.J., Batter, S.J., Brown, M., Longcope, C., McKinlay, J.B., Kantoff, P.W., 1999. Evidence that the CAG repeat in the androgen receptor gene is associated with the age-related decline in serum androgen levels in men. J. Endocrinol. 162, 137–142.

Krueger, R.F., South, S., Johnson, W., Iacono, W., 2008. The heritability of personality is not always 50%: gene-environment interactions and correlations between personality and parenting. J. Pers. 76, 1485–1522.

La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E., Fischbeck, K.H., 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352, 77–79.

Lenroot, R.K., Schmitt, J.E., Ordaz, S.J., Wallace, G.L., Neale, M.C., Lerch, J.P., Kendler, K.S., Evans, A.C., Giedd, J.N., 2009. Differences in genetic and environmental influences on the human cerebral cortex associated with development during childhood and adolescence. Hum. Brain Mapp. 30, 163–174.

Leranth, C., Petnehazy, O., MacLusky, N.J., 2003. Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. J. Neurosci. 23, 1588–1592.

Lessov-Schlaggar, C.N., Reed, T., Swan, G.E., Krasnow, R.E., DeCarli, C., Marcus, R., Holloway, L., Wolf, P.A., Carmelli, D., 2005. Association of sex steroid hormones with brain morphology and cognition in healthy elderly men. Neurology 65, 1591–1596.

Li, J., Al-Azzawi, F., 2009. Mechanism of androgen receptor action. Maturitas 63, 142–148.

Longcope, C., Franz, C., Morello, C., Baker, R., Johnston, C.C., 1986. Steroid and gonadotropin levels in women during the peri-menopausal years. Maturitas 8, 189–196.

Lupien, S., Lecours, A.R., Lussier, I., Schwartz, G., Nair, N.P.V., Meaney, M.J., 1994. Basal cortisol levels and cognitive deficits in human aging. J. Neurosci. 14, 2893–2903.

Lupien, S.J., Leon, M.D., Santi, S.D., Convit, A., Tarshish, C., Nair, N.P.V., Thakur, M., McEwen, B.S., Hauger, R.L., Meaney, M.J., 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat. Neurosci. 1, 69–73.

MacLusky, N.J., Hajszan, T., Prange-Kiel, J., Leranth, C., 2006. Androgen modulation of hippocampal synaptic plasticity. Neuroscience 138, 957–965.

Makris, N., Meyer, J.W., Bates, J.F., Yeterian, E.H., Kennedy, D.N., Caviness, V.S., 1999. MRI-Based topographic parcellation of human cerebral white matter and nuclei

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044

II. Rationale and applications with systematics of cerebral connectivity. Neuroimage 9, 18–45.
Manuck, S.B., Marsland, A.L., Flory, J.D., Gorka, A., Ferrell, R.E., Hariri, A.R., 2010. Salivary testosterone and a trinucleotide (CAG) length polymorphism in the androgen receptor gene predict amygdala reactivity in men. Psychoneuroendocrinology 35, 94–104.
Marsh, J.D., Lehmann, M.H., Ritchie, R.H., Gwathmey, J.K., Green, G.E., Schiebinger, R.J., 1998. Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 98, 256–261.
Martin, D.M., Wittert, G., Burns, N.R., McPherson, J., 2008. Endogenous testosterone levels, mental rotation performance, and constituent abilities in middle-to-older aged men. Horm. Behav. 53, 431–441.
Matousek, R.H., Sherwin, B.B., 2010. Sex steroid hormones and cognitive functioning in healthy, older men. Horm. Behav. 57, 352–359.
McEwen, B.S., 2002. Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. Neurobiol. Aging 23, 921–939.
McEwen, B.S., Weis, J.M., Schwartz, L.S., 1968. Selective retention of corticosterone by limbic structure in rat brain. Nature 220, 911–912.
Mo, Q., Lu, S., Garippa, C., Brownstein, M.J., Simon, N.G., 2009. Genome-wide analysis of DHEA- and DHT-induced gene expression in mouse hypothalamus and hippocampus. J. Steroid Biochem. Mol. Biol. 114, 135–143.
Moffat, S.D., Resnick, S.M., 2007. Long-term measures of free testosterone predict regional cerebral blood flow patterns in elderly men. Neurobiol. Aging 28, 914–920.
Moffat, S.D., Zonderman, A.B., Metter, E.J., Kawas, C., Blackman, M.R., Harman, S.M., Resnick, S.M., 2004. Free testosterone and risk for Alzheimer disease in older men. Neurology 62, 188–193.
Morey, R.A., Petty, C.M., Xu, Y., Hayes, J.P., Wagner II, H.R., Lewis, D.V., LaBar, K.S., Styner, M., McCarthy, G., 2009. A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. Neuroimage 45, 855–866.
Morley, J.E., 2001. Androgens and aging. Maturitas 38, 61–71.
Muller, M., den Tonkelaar, I., Thijssen, J.H., Grobbee, D.E., van der Schouw, Y.T., 2003. Endogenous sex hormones in men aged 40–80 years. Eur. J. Endocrinol. 149, 583–589.
Neale, M.C., Cardon, L.R., 1992. Methodology for Genetic Studies of Twins and Families. Kluwer Academic Publishers, Dordrecht, The Netherlands.
Neale, M.C., Keller, M.C., 2007. An extended model for GxE interactions in twin data. 12th International Society on Twin Studies Congress, Ghent, Belgium.
Neale, M.C., Boker, S.M., Xie, G., Maes, H.H., 2004. Mx: Statistical Modeling, 6th ed. Department of Psychiatry, Medical College of Virginia, Richmond, VA.
Neufang, S., Specht, K., Hausmann, M., Gunturkun, O., Herpertz-Dahlmann, B., Fink, G.R., Konrad, K., 2009. Sex differences and the impact of steroid hormones on the developing human brain. Cereb. Cortex 19, 464–473.
Panizzon, M.S., Hauger, R., Dale, A.M., Eaves, L.J., Eyler, L.T., Fischl, B., Fennema-Notestine, C., Franz, C.E., Grant, M.D., Jak, A.J., Jacobson, K., Lyons, M.J., Mendoza, S.P., Neale, M.C., Prom-Wormley, E., Seidman, L., Tsuang, M.T., Xian, H., Kremen, W.S., 2010. Testosterone modifies the effect of APOE genotype on hippocampal volume in middle-aged men. Neurology 75, 874–880.
Parducz, A., Hajszan, T., Maclusky, N.J., Hoyk, Z., Csakvari, E., Kurunczi, A., Prange-Kiel, J., Leranth, C., 2006. Synaptic remodeling induced by gonadal hormones: neuronal plasticity as a mediator of neuroendocrine and behavioral responses to steroids. Neuroscience 138, 977–985.
Peper, J.S., Brouwer, R.M., Boomsma, D.I., Kahn, R.S., Hulshoff Pol, H.E., 2007. Genetic influences on human brain structure: a review of brain imaging studies in twins. Hum. Brain Mapp. 28, 464–473.
Perrin, J.S., Herve, P.Y., Leonard, G., Perron, M., Pike, G.B., Pitiot, A., Richer, L., Veillette, S., Pausova, Z., Paus, T., 2008. Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. J. Neurosci. 28, 9519–9524.
Pike, C.J., Rosario, E.R., Nguyen, T.V., 2006. Androgens, aging, and Alzheimer's disease. Endocrine 29, 233–241.
Pike, C.J., Carroll, J.C., Rosario, E.R., Barron, A.M., 2009. Protective actions of sex steroid hormones in Alzheimer's disease. Front. Neuroendocrinol. 30, 239–258.
Purcell, S., 2002. Variance component models for gene-environment interaction in twin analyses. Twin Res. 5, 554–571.
Raber, J., 2008. AR, apoE, and cognitive function. Horm. Behav. 53, 706–715.
Rosario, E.R., Carroll, J.C., Oddo, S., LaFerla, F.M., Pike, C.J., 2006. Androgens regulate the development of neuropathology in a triple transgenic mouse model of Alzheimer's disease. J. Neurosci. 26, 13384–13389.
Roselli, C.E., Klosterman, S., Resko, J.A., 2001. Anatomic relationships between aromatase and androgen receptor mRNA expression in the hypothalamus and amygdala of adult male cynomolgus monkeys. Journal of Comparative Neurobiology 439, 208–223.
Schmitt, J.E., Eyler, L.T., Giedd, J.N., Kremen, W.S., Kendler, K.S., Neale, M.C., 2007. Review of twin and family studies on neuroanatomic phenotypes and typical neurodevelopment. Twin Res. Hum. Genet. 10, 683–694.
Seidman, S.N., Araujo, A.B., Roose, S.P., McKinlay, J.B., 2001. Testosterone level, androgen receptor polymorphism, and depressive symptoms in middle-aged men. Biol. Psychiatry 50, 371–376.
Sheffield-Moore, M., Urban, R.J., 2004. An overview of the endocrinology of skeletal muscle. Trends Endocrinol. Metab. 15, 110–115.
Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J. Comp. Neurol. 294, 76–95.
Steiger, J.H., Shapiro, A., Browne, M.W., 1985. On the multivariate asymptotic-distribution of sequential chi-square statistics. Psychometrika 50, 253–264.
Tirassa, P., Thiblin, I., Agren, G., Vigneti, E., Aloe, L., Stenfors, C., 1997. High-dose anabolic androgenic steroids modulate concentrations of nerve growth factor and expression of its low affinity receptor (p75-NGFr) in male rat brain. J. Neurosci. Res. 47, 198–207.
Tohgi, H., Utsugisawa, K., Yamagata, M., Yoshimura, M., 1995. Effects of age on messenger RNA expression of glucocorticoid, thyroid hormone, androgen, and estrogen receptors in postmortem human hippocampus. Brain Res. 700, 245–253.
Turkheimer, E., Haley, A., Waldron, M., D'Onofrio, B., Gottesman, I.I., 2003. Socieconomic status modifies heritability of IQ in young children. Psychol. Sci. 14, 623–628.
Vasilopoulos, T., Kremen, W.S., Kim, K., Panizzon, M.S., Stein, P.K., Xian, H., Grant, M.D., Lyons, M.J., Toomey, R., Eaves, L.J., Franz, C.E., Jacobson, K.C., in press. Untreated Hypertension Decreases Heritability of Cognition in Late Middle Age. Behavior Genetics.
Veiga, S., Melcangi, R.C., Doncarlos, L.L., Garcia-Segura, L.M., Azcoitia, I., 2004. Sex hormones and brain aging. Exp. Gerontol. 39, 1623–1631.
Vermeulen, A., 2000. Andropause. Maturitas 34, 5–15.
Viau, V., 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. J. Neuroendocrinol. 14, 506–513.
Vinkhuyzen, A.A., van der Sluis, S., Posthuma, D., 2011. Life events moderate variation in cognitive ability (g) in adults. Mol. Psychiatry 16, 4–6.
Walhovd, K.B., Westlye, L.T., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Agartz, I., Salat, D.H., Greve, D.N., Fischl, B., Dale, A.M., Fjell, A.M., 2011. Consistent neuroanatomical age-related volume differences across multiple samples. Neurobiol. Aging 32, 916–932.
Watson, C., Andermann, F., Gloor, P., Jones-Gotman, M., Peters, T., Evans, A., Olivier, A., Melanson, D., Leroux, G., 1992. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. Neurology 42, 1743–1750.
Weis, S., Haug, H., Holoubek, B., Orun, H., 1989. The cerebral dominances: quantitative morphology of the human cerebral cortex. Int. J. Neurosci. 47, 165–168.
Yaffe, K., Lui, L.Y., Zmuda, J., Cauley, J., 2002. Sex hormones and cognitive function in older men. J. Am. Geriatr. Soc. 50, 707–712.
Yaffe, K., Edwards, E.R., Lui, L.Y., Zmuda, J.M., Ferrell, R.E., Cauley, J.A., 2003. Androgen receptor CAG repeat polymorphism is associated with cognitive function in older men. Biol. Psychiatry 54, 943–946.
Zhang, L., Ma, W., Barker, J.L., Rubinow, D.R., 1999. Sex differences in expression of serotonin receptors (subtypes 1A and 2A) in rat brain: a possible role of testosterone. Neuroscience 94, 251–259.
Zitzmann, M., Nieschlag, E., 2003. The CAG repeat polymorphism within the androgen receptor gene and maleness. Int. J. Androl. 26, 76–83.

Please cite this article as: Panizzon, M.S., et al., Genetic influences on hippocampal volume differ as a function of testosterone level in middle-aged men, NeuroImage (2011), doi:10.1016/j.neuroimage.2011.09.044