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RAPID COMMUNICATION

Hippocampal Structural Changes Across the Menstrual Cycle

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ABSTRACT: Magnetic resonance imaging (MRI) in association with Jacobian-modulated voxel-based morphometry (VBM) was used to test for regional variation in gray matter over the menstrual cycle. T1-weighted anatomical images were acquired using a spoiled gradient recalled acquisition sequence in 21 women. Each subject was scanned twice: once during the postmenstrual late-follicular phase (Days 10–12 after onset of menses), and once during the premenstrual late-luteal phase (1–5 days before the onset of menses). Gray matter was relatively increased in the right anterior hippocampus and relatively decreased in the right dorsal basal ganglia (globus pallidus/putamen) in the postmenstrual phase. Verbal declarative memory was increased in the postmenstrual vs. premenstrual phase. This first report of human brain structural plasticity associated with the endogenous menstrual cycle extends well-established animal findings of hormone-mediated hippocampal plasticity to humans, and has implications for understanding alterations in cognition and behavior across the menstrual cycle. © **2008 Wiley-Liss, Inc.**

KEY WORDS: menstrual cycle; MRI; anatomy; VBM; estrogen; structural

Animal research indicates that sex hormones affect brain structure (McEwen and Alves, 1999), with evidence for sex-hormone mediated hippocampal plasticity, including synaptic remodeling (Woolley and McEwen, 1992; Yankova et al., 2001), gliagenesis, and neurogenesis (Gould et al., 2000). These changes occur rapidly: a 32% decrease in hippocampal synaptic density is detectable 24 h following onset of rat estrus (Woolley and McEwen, 1992). In humans, postmenopausal estrogen supplementation has been associated with greater hippocampal size (Eberling et al., 2003). Here, for the first time, we used MRI in association with voxel-based morphometry (VBM) to assess structural brain changes over the human menstrual cycle.

VBM has been used to test for regional differences in concentration of gray matter (per unit volume in native space) (Ashburner and Friston, 2000). To assess regionally specific changes in the amount (volume) of gray matter, we utilized optimized, Jacobian-modulated VBM in which Jacobian determinants derived from the spatial normalization step are incorporated into analysis (Good et al., 2001).

For this IRB-approved protocol, 21 women (mean age = 29, range = 22–35) were scanned twice: once during the postmenstrual, high-estrogen, late-follicular phase (Days 10–12 after onset of menses), and once during the premenstrual, low-estrogen, late-luteal phase (1–5 days before the onset of menses, when hormone levels are variable, with progesterone levels relatively high and estrogen and progesterone levels both falling). Menstrual phase was determined by ovulation tests and confirmed by onset of menses. About 19/21 women were scanned over one menstrual cycle, with an average of 15 days (standard deviation (SD) = 3.6) between each of these subject's two scans (due to scheduling difficulties, two subjects were scanned over two cycles). So that findings would be generalizable, participants consisted of women with and without premenstrual symptoms: 10 who met DSM-IV criteria for premenstrual dysphoric disorder (PMDD) and 11 with no premenstrual symptoms. Scan order was counterbalanced, with approximately half of subjects in each group scanned first in the luteal phase, and half scanned first in the follicular phase. All subjects were right-handed, native English speakers. Subjects had regular menses, no medical or neurologic problems, and did not take oral contraceptives or any other medications. The structured clinical interview for DSM-IV was administered to ensure that no subjects had psychiatric disorders other than PMDD. Two months of the daily record of severity of problems (developed by Jean Endicott PhD and Wilma Harrison MD) were administered to characterize premenstrual mood symptoms.

Image data were acquired with a GE Sigma 3 Tesla MRI scanner (max gradient strength 40 mT/m, max gradient slew rate 150 mT/m/ms). T1-weighted anatomical images were acquired using a spoiled gradient recalled acquisition sequence (TR/TE = 30/8 ms, flip angle = 45, field of view = 240 mm, 100 transverse

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TABLE 1.

Regions Showing Differential Gray Matter Volume by VBM in the Postmenstrual Versus Premenstrual Phases of the Cycle

	Volume (mm^3)	*x*	*y*	*z*	*Z*-score	*P* value
Postmenstrual > premenstrual						
L occipital (lingual gyrus, BA18)	1,464	−6	−80	−16	3.62	0.0001
R hippocampus/parahippocampus	560	20	−8	−22	3.33	0.0004
L middle frontal gyrus	*480*	*−42*	*16*	*44*	*3.42*	*0.0003*
Premenstrual > postmenstrual						
L superior parietal lobule (BA7)	1,016	−22	−54	66	−3.88	<0.0001
R dorsal basal ganglia (globus pallidus/putamen)	1,880	18	−4	4	−3.74	<0.0001
R medial frontal gyrus/anterior cingulate	1,608	16	−4	54	−3.38	0.0004
R thalamus (pulvinar)	*392*	*26*	*−30*	*8*	*−3.05*	*0.001*

Voxel-wise *P*-value <0.005, cluster volume >0.5 cm^3; regions with spatial extents slightly below threshold are listed in italics.

slices with thickness = contiguous 1.5 mm, number of averages = 1, matrix = 256 × 256, voxel resolution = 0.9375 × 0.9375 × 1.5 mm^3).

Image processing within a customized statistical parametric mapping consisted of: manual AC-PC reorientation of images; correction for tissue intensity inhomogeneities; coregistration of each subject's follicular image to her luteal image; stereotactic transformation into standard Montreal Neurological Institute (MNI average 152 T1 brain) space based on the mean image of each subject's coregistered and deskulled follicular and luteal images; segmentation into gray matter, white matter, and CSF component images. Visual inspection revealed uniformly high quality transformations, and no subjects were excluded from analysis. Optimized VBM was performed according to established methods (Ashburner and Friston, 2000) using Jacobian modulation (Good et al., 2001), with removal of nonbrain tissues (brain extraction) for improved spatial normalization quality (Fein et al., 2006). The resulting inhomogeneity-corrected, coregistered, deskulled, normalized, and Jacobian-modulated gray matter component images were smoothed using a 12-mm FWHM isotropic Gaussian kernel and voxel-wise transformed with a logit function.

Menstrual cycle phase effects (postmenstrual vs. premenstrual) were assessed by paired *t* tests of voxel-wise transformed gray matter values between cycle phases, with intracranial gray matter volume (global mean voxel value of the Jacobian-modulated normalized gray matter component images) and group membership (asymptomatic or PMDD) entered as covariates of no interest in a repeated-measures ANCOVA model. Regionally-specific differences in gray matter between cycle phases were considered statistically significant if the peak voxel-wise *P*-value was less than 0.001 for the *t*-statistic and associated cluster had a spatial extent of at least 0.5 cm^3. Based on prior studies (Woolley and McEwen, 1992; Korol, 2004b), two a priori regions of interest (ROIs) were defined: anterior hippocampus (AAL hippocampal mask with $y > -30$ mm) and dorsal basal ganglia (AAL masks of caudate, putamen and pallidum with $z > 0$ mm) (Tzourio-Mazoyer et al., 2002). Within these ROIs, results were considered significant if they survived correction for multiple comparisons across the ROI at $P < 0.05$, according to the Gaussian Random Field Theory.

Verbal memory was assessed at the time of each scan session using two 360 item computerized recognition tasks, each of which consisted of 240 words the subject had seen in the scanner (during an fMRI task) and 120 distractor words. Verbal memory performance was evaluated by the bias-free discrimination index d' (estimated by the *z*-score of the false alarm rate minus the *z*-score of the hit rate) based on Signal Detection Theory. The Wilcoxon signed ranks test was used to compare postmenstrual and premenstrual discrimination indices. Using a multiple regression model, with the group factor and whole gray matter volume as covariates of no interest, postmenstrual minus premenstrual change in d' was correlated with contrast images (postmenstrual minus premenstrual gray matter changes) to assess correlation between memory and ROI gray matter changes.

A step-wise regression model was used to test whether the Group factor (PMDD or control) played any significant role in menstrual cycle difference shown in VBM analysis and summarized in Table 1. It was found that the Group factor does not explain any significant variation in the data for all but the last coordinate in Table 1, for which the Group factor only explained an extremely minor portion of the overall variation in the data (0.7%). In all cases, the menstrual cycle factor explained the major proportion of the variation in the data (63.2, 90.5, 96.3, 92.9, and 90.8%) for the five coordinates reported in Table 1, respectively.

In addition to employing optimized, Jacobian-modulated VBM to assess volumetric effects, we have further quantified the volume changes in the anterior hippocampal and basal ganglia ROI as follows: pre- and postmenstrual mean gray matter volumes of these ROIs (defined stereotactically using standard anatomical masks) were calculated as regional mean voxel values of the Jacobian-modulated normalized gray matter component images within the corresponding ROI masks. Right anterior hippocampal gray matter increased on average from 3,653 mm^3 (SD = 377 mm^3) premenstrually to 3,694 mm^3 (SD = 370 mm^3) postmenstrually, while right dorsal basal ganglia gray matter decreased on average from 7,117 mm^3 (SD = 748

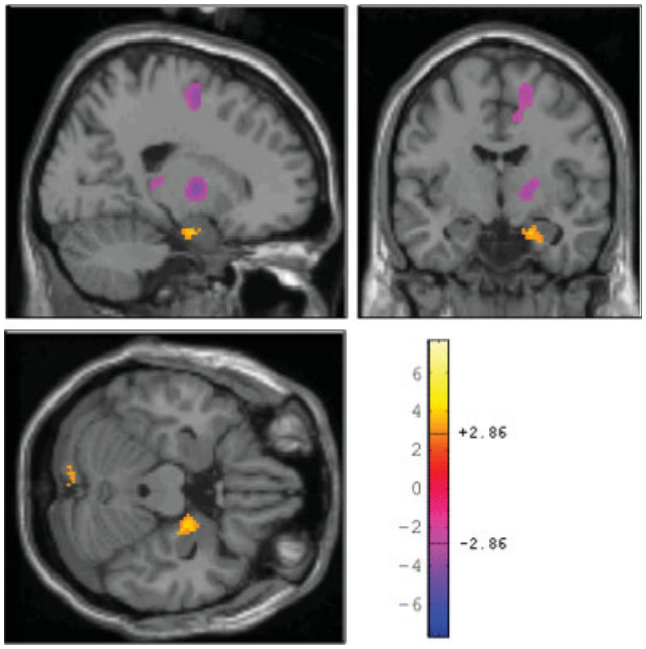

FIGURE 1. Three-section view *t*-map rendering ($x = 20$, $y = -8$, $z = -22$) showing increased anterior hippocampal and decreased basal ganglia gray matter in the postmenstrual vs. premenstrual phase ($n = 21$; $P < 0.005$) (Coordinates presented are in MNI space).

mm^3) premenstrually to 6,941 mm^3 (SD = 711 mm^3) postmenstrually.

This report of human structural brain plasticity across the menstrual cycle, obtained using an unbiased, standard technique, demonstrates specific increases in right hippocampal/parahippocampal gray matter and specific decreases in right basal ganglia gray matter in the postmenstrual phase (Fig. 1), in accord with a large body of literature describing similar effects in animals (Woolley and McEwen, 1992; Korol, 2004b). Verbal memory was increased in the postmenstrual vs. premenstrual phase (postmenstrual $d' = 1.234 \pm 0.112$, premenstrual $d' = 0.968 \pm 0.096$; $P < 0.026$). There was a strong trend towards a positive correlation between premenstrual to postmenstrual improvement in verbal memory performance and premenstrual to postmenstrual gray matter increase in right anterior hippocampus (peak at (28, −6, −28), $z = 2.48$, voxel-wise $P = 0.006$).

Additional regions showing gray matter increases or decreases in the postmenstrual phase as compared with the premenstrual phase are presented in Table 1. These regions did not represent a priori hypothesis or survive whole-brain correction.

These results support models of estrogen-dependent cyclical alterations in hippocampal synaptic density and function proposed to account for neuronal and cognitive differences seen across the menstrual cycle (Desmond and Levy, 1997; Korol, 2004a). Cognitive differences have been seen across the human menstrual cycle, with some women performing better on spatial and abstract reasoning tasks during menstruation (when estrogen levels are low) and better on verbal, articulatory and fine motor tasks midcycle (when estrogen levels are high) (Hampson, 1990). It is important to note that much of the animal literature using spatial tasks refers to hippocampus dependent spatial tasks such as place or allocentric strategies which are reliant upon cognitive mapping, while the tasks used in the Hampson (1990) study may involve egocentric or response strategies (in which route is determined based on decisions at stimuli/landmarks rather than via abstract spatial memory), and depend more upon parietal cortex and/or basal ganglia. One model has proposed two distinct optimal ranges of estradiol in the hippocampus: high-concentrations maintaining high hippocampal synapse density for declarative memory encoding (including verbal tasks in humans) and low-concentrations maintaining lower hippocampal synapse density for optimization of other learning strategies (Desmond and Levy, 1997). However, the majority of the literature currently supports high hippocampal synapse density (as maintained by high estrogen concentrations) as optimal for hippocampal dependent cognitive mapping learning strategies including spatial (place or allocentric) tasks (Korol, 2004a; Bohbot et al., 2007) and verbal declarative memory tasks (Hampson, 1990). A neuroprotective role of estrogens on hippocampal volume in aging is supported by a recent study demonstrating that women using estrogen replacement therapy (ET) had large hippocampal volumes compared to men and women who never used ET or used it in the past (Lord et al., 2008). Progesterone, less studied, may also have significant effects on cognitive performance and brain structure.

Although estrogen-mediated structural plasticity is not as well-documented in the basal ganglia as in the hippocampus, estrogens have been shown to increase striatal dopamine release, to influence striatal serotonergic and dopaminergic innervation density, and to promote striatal medium size spiny neuronal maturation in vivo (Korol, 2004b). The apparent opposite effect of high estrogen levels on hippocampal and basal ganglia gray matter may relate to the finding in rats that high estrogen promotes use of a hippocampally-mediated spatial (place or allocentric) learning strategy, while low levels promote use of a nonhippocampal, possibly striatally-mediated navigational (response or egocentric) strategy (Korol, 2004b). In humans, MRI studies have shown that navigational ability correlates with level of activity in the basal ganglia (putamen) (Epstein et al., 2005), and more specifically, that navigation using a response strategy is associated both with greater activity (Iaria et al., 2003) and gray matter (Bohbot et al., 2007) in the basal ganglia (caudate), though it should be noted that menstrual cycle effects were not assessed in any of these studies. The Bohbot (2007) study further showed that while caudate gray matter correlates with the use of a response strategy, hippocampal gray matter correlates with the use of a spatial strategy, with an inverse correlation between hippocampal and caudate gray matter, suggesting a competitive interaction between these two brain areas. In accord with this finding, a correlation analysis with transformed gray matter values at the peak coordinate in right anterior hippocampus (derived from the postmenstrual vs. premenstrual contrast) as the main regressor in a repeated-measures ANCOVA model revealed a significant negative corre-

lation in the basal ganglia (peak (12,4,14), $z = -3.78$, voxelwise $P < 0.001$, corrected P-value $= 0.029$), with no cycle-dependent changes in this negative correlation.

Menstrual cycle-related structural changes are likely due to alterations in dendritic morphology and glia, though differences in the time course of maturation or migration of newly-generated dentate or subventricular proliferative zone cells could play a role (Gould and Gross, 2002). Another possible mechanism is shifting fluid balance, considered to occur systemically in the premenstrual period (Vellacott and O'Brien, 1987). However, MRI findings of a mean premenstrual cranial CSF increase (when compared to midcycle) of 11.5 ml indicated that the CSF changes observed reflected a relative premenstrual reduction of brain volume and did not support cerebral swelling, in general agreement with present findings (Grant et al., 1988).

Our results illustrate the high degree of structural plasticity of the normal human brain even over short intervals, and call attention to the importance of careful evaluation and consideration of menstrual status when interpreting neuroimaging findings.

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