Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Regional gray matter volume differences and sex-hormone correlations as a function of menstrual cycle phase and hormonal contraceptives use

T. De Bondt[a,*], Y. Jacquemyn[b], W. Van Hecke[c], J. Sijbers[d], S. Sunaert[e], P.M. Parizel[a]

[a]Department of Radiology, University of Antwerp, Antwerp University Hospital, Wilrijkstraat 10, 2650 Antwerp, Belgium
[b]Department of Gynecology and Obstaetrics, University of Antwerp, Antwerp University Hospital, Wilrijkstraat 10, 2650 Antwerp, Belgium
[c]icoMetrix, Zavelstraat 63, 3010 Leuven, Belgium
[d]iMinds-Vision Lab, Department of Physics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium
[e]Department of Radiology, University Hospitals of the Catholic University of Leuven, Herestraat 49, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Accepted 19 July 2013
Available online 26 July 2013

Keywords:
Voxel based morphometry
Menstrual cycle
Hormonal contraception
Hormones

ABSTRACT

During the menstrual cycle, hormone-driven functional and morphological changes occur in the female brain. The influence of hormonal contraceptives on these changes has received only little attention in the medical literature. The purpose of our study is to measure regional gray matter volume changes as a function of the cycle phase and use of hormonal contraceptives, in relation to blood concentrations of sex hormones. We performed a prospective study in 30 healthy young women; 15 women had a natural menstrual cycle and 15 were using monophasic combined hormonal contraceptives. MRI examinations were acquired at 2 specific time-points in the cycle (follicular and luteal phase). MRI studies included a T1-weighted, isotropic, high-resolution 3-D gradient echo acquisition, for the purpose of performing voxel based morphometry. Peripheral venous blood samples were obtained to determine concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and progesterone. We found a highly significant negative correlation of regional gray matter volume in the anterior cingulate cortex with estradiol concentrations. To the best of our knowledge, this result has not been described before, and was only present in the natural cycle group, not in women using hormonal contraceptives. The anterior cingulate cortex is involved in emotion processing and there is literature describing behavioral alternations with changing hormone levels. Our findings provide a structural, morphological basis to support these data. Therefore, we advise neuroscientists to take into account the menstrual cycle phase and use of hormonal

*Corresponding author. Fax: +32 38214532.
E-mail addresses: timo.debondt@ua.ac.be (T. De Bondt), yves.jacquemyn@uza.be (Y. Jacquemyn), wim.vanhecke@icometrix.be (W. Van Hecke), jan.sijbers@ua.ac.be (J. Sijbers), stefan.sunaert@uz.kuleuven.ac.be (S. Sunaert), paul.parizel@ua.ac.be (P.M. Parizel).

http://dx.doi.org/10.1016/j.brainres.2013.07.034

contraceptives, in order to avoid obtaining heterogeneous data sets, leading to a significant loss of accuracy and precision.

1. Introduction

Differences in the human brain between men and women were first reported in the 1960s (Levine, 1966). In the original paper, attention was focused on the hypothalamus, which regulates the secretion of sex hormones. For several decades investigators assumed that sex differences in the human brain were localized and by large negligible. This concept has changed dramatically because, during the last decade, an overwhelming amount of literature has accumulated, describing more extensive sex-related differences, at all levels of the brain (Jazin and Cahill, 2010; Cahill, 2012; McCarthy et al., 2012). Moreover, several investigators have reported differences in the female brain as a function of the menstrual cycle, e.g. in implicit memory (Maki et al., 2002), working memory (Konishi et al., 2008) and emotional memory (Andreano et al., 2008; Andreano and Cahill, 2010; Ferree et al., 2011, 2012 Ertman et al., 2011). These findings should not come as a surprise, because the menstrual cycle is governed by a complex endocrine regulation mechanism, involving fluctuations in the concentration of estradiol and progesterone (Boron and Boulpaep, 2005). These hormones are known to be strong neuromodulators (Naftolin et al., 1988, 2007; Garcia-Segura,1997; Melcangi et al., 2011).

The human menstrual cycle is commonly divided into two phases. The cycle starts with the follicular phase on day 1 of menstrual bleeding, and ends when ovulation takes place, around day 14. This heralds the luteal phase, which ends at the beginning of the next menstrual bleeding, again approximately 14 days later. The most relevant hormones regulating this natural menstrual cycle are the anterior pituitary produced luteinizing hormone (LH) and follicle stimulating hormone (FSH), and the ovarian steroids estradiol and progesterone (Boron and Boulpaep,2005).

Using functional magnetic resonance imaging (fMRI), several research groups published findings of menstrual cycle related changes in brain activation as a response to various stimuli. These studies in healthy subjects include: modulation of the arousal circuitry (Goldstein et al., 2005), response to negative and positive words (Amin et al., 2006), emotional face recognition (Guapo et al., 2009), response to erotic stimuli (Gizewski et al., 2006; Zhu et al., 2010), pain modulation (Rezaii et al., 2012), and the association with several components of empathy (Derntl et al., 2013). Other authors have compared regional gray matter volumes, as a function of the menstrual cycle between patients with premenstrual dysphoric disorder (PMDD) and normal controls (Protopopescu et al., 2008).

Only very recently, approximately 50 years after the introduction of oral combined hormonal contraception (HC), have investigators focused their attention on the effects of HC on the female brain and behavior (Nielsen et al., 2011, 2013; Gingnell et al., 2013). Hormonal concentrations are significantly altered in women using HC, and therefore an effect on the brain and its function is to be expected. This is supported by MRI findings in brain morphometry (Pletzer et al., 2010), function (Andreano and Cahill, 2010) and white matter microstructure (De Bondt et al., 2013). Although differences in gray matter volume were found between the follicular and luteal phase in normally cycling women and HC users (Pletzer et al., 2010), these data have not been previously correlated with hormonal concentrations in the blood.

The goal of our study is to perform a Jacobian-modulated voxel based morphometry (VBM) analysis in healthy young women (with natural cycle and HC) and to correlate gray matter volumes with hormonal levels measured in peripheral venous blood.

2. Results

2.1. Subjects

Our study population consisted of 30 young women (mean age: 21.7 ± ±0.5 years; range: 18–28 years). The study population was subdivided into a group using hormonal contraceptives (HC) ($n=15$; mean age=21.1±0.5 years) and a group with a normal menstrual cycle (NC) ($n=15$; mean age=22.3±0.8 years). There was no significant age difference between both groups (*T*-test, $p=0.177$, $t=1.385$).

Paired *T*-tests indicated no difference in total volume of gray matter (GM) between the follicular and the luteal phase in the NC group ($p=0.922$, $t=0.100$), nor between the inactive (pill-free period) and active pills in the HC group ($p=0.137$, $t=-1.579$).

2.2. Hormonal data

Hormonal data from all subjects were recorded. When performing standard data control, we removed three outliers in the estradiol data and one extreme value of progesterone. Additionally we rejected an extreme value in the NC luteal phase in LH concentration, which is probably due to an excessively long follicular phase, hence we observed a residue of the LH peak initiating ovulation. Boxplots of hormone levels are shown in Fig. 1.

In the luteal/active phase, we found highly significant differences ($p<0.001$) for all hormones between the NC group and the HC group (Mann Whitney *U* for FSH=6; LH=2; estradiol=0; progesterone=1). These differences disappear in the follicular phase (NC group) and during the pill-free period (inactive pill HC group). During this phase of the cycle, we only observe a slight progesterone difference between groups ($p=0.012$; Mann Whitney $U==24$). Thus, hormonal concentrations in the inactive pills and the follicular phase in the NC group, are very similar.

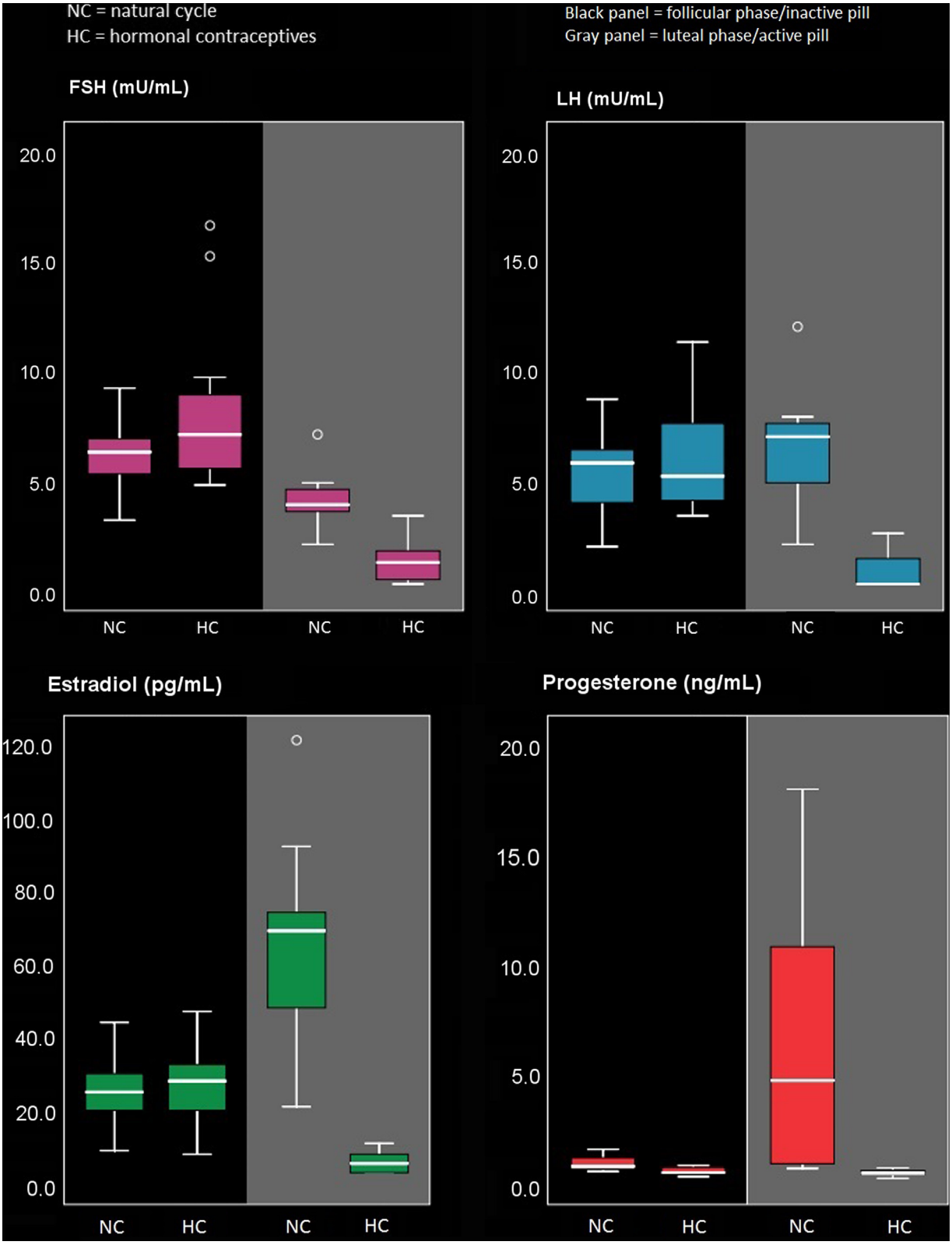

Fig. 1 – Boxplots of all hormonal concentrations in follicular/inactive pill (black panel) and luteal/active pill (gray panel) phase for the natural cycle (NC) and hormonal contraceptives (HC) group.

Comparing the inactive pill phase to the active pill phase, the hormone suppressing effect of the contraceptives is obvious. All hormones have significantly lower concentrations in the active pill phase ($p=0.001$ for LH, FSH and estradiol with Wilcoxon $W=0$ for all; $p=0.030$ for progesterone with Wilcoxon $W=18$) compared to the inactive pill phase.

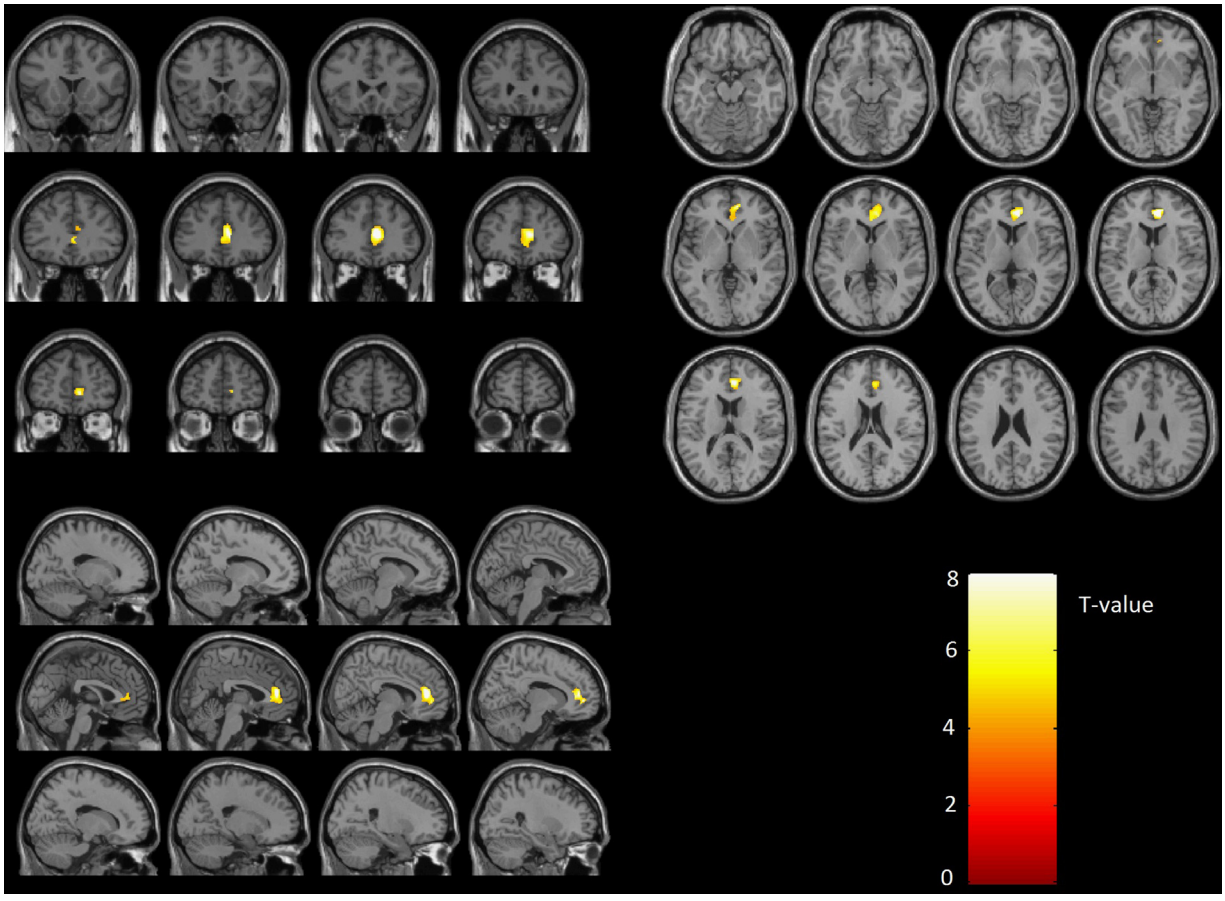

Fig. 2 – Coronal, axial and sagittal slices, showing the relationship between gray matter volume and estradiol concentrations in peripheral blood, in the group of women with a natural cycle. There is a negative correlation in the anterior cingulate gyrus during the luteal phase. An uncorrected threshold of $p<<0.001$ was used.

Table 1 – Mann–Whitney *U*-test and Wilcoxon-test *p*-values of all comparisons of the hormonal data. Key to abbreviations: NC=natural cycle; HC=hormonal contraception; FSH=follicle stimulating hormone; and LH=luteinizing hormone.

P-values		FSH	LH	Estradiol	Progesterone
NC vs. HC	Follicular/inactive pill	0, 167	0, 792	0, 631	0, 012
	Luteal/active pill	<0, 001	<0, 001	<0, 001	<0, 001
Follicular vs. Luteal	NC	0, 010	0, 350	0, 008	0, 010
Inactive vs. active pill	HC	0, 001	0, 001	0, 001	0, 030

In the NC group, our observations are also in agreement with the expectations. Estradiol and progesterone concentrations are significantly higher in the luteal phase ($p=0.008$ and $p=0.010$ with Wilcoxon $W=63$ and 68 respectively). We also found a marked difference in FSH ($p=0.010$; Wilcoxon $W=6$) and no difference in LH ($p=0.350$; Wilcoxon $W=43.5$). This finding reflects that our hormone concentration measurements were made well outside the sharp LH surge preceding ovulation. All *p*-values are listed in Table 1.

2.3. *Voxel based morphometry*

Local gray matter volumetric differences were assessed using Jacobian-modulated voxel based morphometry. All statistical differences are described using a $p<0.001$ uncorrected threshold, in order to keep the comparison in cluster sizes relevant. In addition, the peak level FDR corrected *p*-value per cluster is given. The findings are listed in Tables 2, 3 and 4 in the following subsections.

2.3.1. *Cycle dependent effects*

During the follicular phase, women with a natural menstrual cycle showed an increased local GM volume in the anterior cingulate cortex (ACC), left cingulate gyrus, left insula, left middle temporal gyrus and the right middle frontal gyrus (Table 2). The volume of the right superior temporal gyrus was found to be smaller when compared to the volumes in the luteal phase. Results are tabulated with their MNI coordinates (Table 2).

Table 2 – Clusters of local volume differences in gray matter (GM) in the subject-group with natural cycle, along with MNI coordinates and peak *t*-values. Per cluster, the FDR corrected peak *p* value is shown.

	X (mm)	Y (mm)	Z (mm)	Side	# voxels	t-Value	P FDR
NC follicular > luteal							
Middle frontal gyrus	21	−4.5	49.5	R	160	8.27	0.311
Brodmann area 6	6	1.5	52.5	R/L	143	5.48	0.663
Cingulate gyrus	−7.5	−10.5	39	L	231	7.99	0.311
ACC	12	45	19.5	R	23	5.91	0.659
Middle temporal gyrus	−42	−75	16.5	L	128	7.95	0.311
Insula	−33	13.5	−3	L	30	4.78	0.823
NC follicular < luteal							
Superior temporal gyrus	39	13.5	−31.5	R	79	−5.5	0.963

Table 3 – Clusters of local volume differences in gray matter (GM) in the subject-group of hormonal contraceptives, and comparison with the natural cycle group, along with MNI coordinates and peak *t*-values. Per cluster, the FDR corrected peak *p* value is shown.

	X (mm)	Y (mm)	Z (mm)	Side	# voxels	t-Value	P FDR
HC inactive > active							
Brodmann area 6	−28.5	−9	60	L	31	4.33	0.629
Brodmann area 6	−33	4.5	54	L	18	4.26	0.629
Posterior central gyrus	45	−24	40.5	R	28	5.26	0.629
Caudate (acc)	−4.5	18	−1.5	L	13	4.62	0.629
HC inactive < active							
Anterior cingulate cortex	−1.5	40.5	3	L	9	−4.04	0.926
Insula	−40.5	−24	18	L	8	−3.89	0.926
Inactive HC > follicular NC							
Brodmann area 6	45	0	48	R	260	4.61	0.987
Brodmann area 6	34.5	15	60	R	142	4.29	0.987
Superior frontal gyrus	19.5	48	27	R	29	4.47	0.987
Cingulum	12	27	36	R	52	3.87	0.987
Fusiform gyrus	49.5	−39	−16.5	R	27	4.09	0.987
Fusiform gyrus	−36	−69	−12	L	7	3.79	0.987
Active HC > luteal NC							
Occipital lobe	40.5	−87	−4.5	R	64	4.82	0.69
Brodmann area 6	45	0	48	R	109	4.15	0.881
Active HC < luteal NC							
Fusiform gyrus	−27	−27	−31.5	L	26	−4.73	0.083

Table 4 – Clusters of significant correlation of gray matter volume with estradiol concentrations. An uncorrected threshold of $p < 0.001$ was used. Per cluster, the FDR corrected peak *p* value is shown.

Estradiol	X (mm)	Y (mm)	Z (mm)	Side	# voxels	t-Value	P FDR
NC follicular positive corr.							
Brodmann area 8	31.5	19.5	48	R	37	5.88	0.893
Cingulum	3	−25.5	40.5	R	9	4.27	0.893
Posterior central gyrus	−51	−27	45	L	31	4.63	0.893
Insula	−27	25.5	−6	L	89	5.65	0.893
NC follicular negative corr.							
Fusiform	−42	−43.5	−24	L	10	−4.65	0.826
NC luteal negative corr.							
Anterior cingulate cortex	7.5	39	10.5	R	1155	−14.01	0.033
Superior frontal gyrus	33	49.5	−13.5	R	89	−5.35	0.926
Medial/Superior frontal gyrus	−10.5	60	−10.5	L	147	−6.89	0.866
Mid temporal gyrus	−60	−43.5	−9	L	145	−5.73	0.866
HC active negative corr.							
Fusiform/Culmen	−37.5	−45	−27	L	61	−4.77	0.942
Superior frontal gyrus	−21	52.5	−12	L	80	−5.84	0.942

2.3.2. *Contraceptives effects*

In the HC group, we found short term morphometric differences when comparing the active pill phase (3 weeks) and the inactive pill phase (1 week). More specifically, GM volume in the left mid-frontal gyrus (Brodmann area 6), was found to be larger during administration pause, as was the post central gyrus and caudate nucleus. A few small clusters in the ACC and the insula were larger when taking the contraceptives.

When we compared gray matter volumes between the HC group and the NC group, in both phases, we found larger clusters of differences. In the right mid-frontal gyrus (Brodmann area 6), we identified larger gray matter volumes in the contraceptives group, in both phases, but the difference was greatest during the follicular/inactive pill phase. In the left fusiform gyrus, we found no difference within the HC group, but compared to the NC group, the left fusiform gyrus volume is bigger in the follicular phase and smaller in the luteal phase. There is however a difference in the volume of the left fusiform gyrus between NC and HC groups; and this difference is phase dependent. The right fusiform gyrus was found to be larger in the HC group only in the inactive phase; the same was true for smaller clusters in the cingulum and the superior frontal gyrus. We could not identify clusters of higher regional GM volume in the NC group, compared to the HC group, during the follicular/inactive phase.

2.3.3. *Hormonal correlations*

We correlated local gray matter volumes with hormonal concentrations of estradiol (Table 4) and progesterone (Table 5), for groups and phases seperately. There are highly significant and large clusters in the ACC (peak *t*-value=−14.01; 1155 voxels), the right superior frontal gyrus (peak *t*-value=5.35; 89 voxels), and the left medial/superior frontal gyrus (peak *t*-value=−6.89; 147 voxels). Gray matter volumes in these areas of the brain show a negative correlation with estradiol concentration in the NC group during the luteal phase (Fig. 2). Progesterone correlations with regional GM volumes are almost all positive. The largest clusters are found in the fusiform gyri and neighboring regions during the follicular phase of the NC group (Fig. 3). In the luteal phase, there is only a small cluster in the fusiform gyrus correlating negatively with progesterone concentrations. In the HC group, two large positively correlating clusters appear in the right temporal/parietal lobe. With regard to FSH and LH, no meaningful results were obtained, which is consistent with the information that these hormones are not known to be very strong neuromodulators in gray matter (Peper et al., 2008).

3. Discussion

Although sex steroids are mostly known for their role in procreation and development of genital organs, the brain seems to be an important target as well, affecting brain plasticity and overall development (Garcia-Segura ,1997; Melcangi et al., 2011). In this study, 30 healthy young women were divided into a natural cycle group (NC) and a group on hormonal contraceptives (HC). Regional gray matter volumes were compared using T1 weighted MR images. Correlations with hormone concentrations were assessed, with a focus on estradiol and progesterone.

Our results indicate a strong negative correlation between the anterior cingulate cortex (ACC) volume and the concentration of estradiol during the high hormone luteal phase of the NC women. Moreover we found that the ACC is slightly larger during the follicular phase, as compared to the luteal

Table 5 – Clusters of significant correlation of gray matter volume with progesterone concentrations. An uncorrected threshold of $p<0.001$ was used. Per cluster, the FDR corrected peak p value is shown.

Progesterone	X (mm)	Y (mm)	Z (mm)	Side	# voxels	t-Value	P FDR
NC follicular positive corr.							
Fusiform	−39	−27	−27	L	462	8.74	0.469
Lingual gyrus/parahip. gyr.	−28.5	−48	−1.5	L	126	6.35	0.469
Fusiform/culmen	40.5	−36	−31.5	R	104	7.11	0.469
Occipital lobe/fusiform	34.5	−85.5	−19.5	R	130	6.15	0.483
Precentral gyrus	63	−4.5	31.5	R	29	7.84	0.469
NC luteal positive corr.							
Brodmann area 8	−21	30	43.5	L	185	5.95	0.612
Superior motor area	9	−7.5	57	R	37	4.55	0.888
NC luteal negative corr.							
Fusiform	−42	−55.5	−19.5	L	35	−4.48	0.805
HC inactive positive corr.							
Temporal/parietal lobe	61.5	−46.5	18	R	556	6.37	0.652
Temporal/parietal lobe	49.5	−18	1.5	R	650	5.44	0.652
HC inactive negative corr.							
Post central gyrus	40.5	−24	39	R	63	−5.18	0.48
HC active positive corr.							
Fusiform	30	−49.5	−4.5	R	12	4.4	0.506
HC active negative corr.							
Post central gyrus	43.5	−30	37.5	R	257	−5.89	0.297
Lingual gyrus	−9	−90	−16.5	L	209	−6.54	0.297
Cingulum	12	−16.5	45	R	227	−4.82	0.693
Inferior temporal gyrus	60	−30	−19.5	R	132	−5	0.666

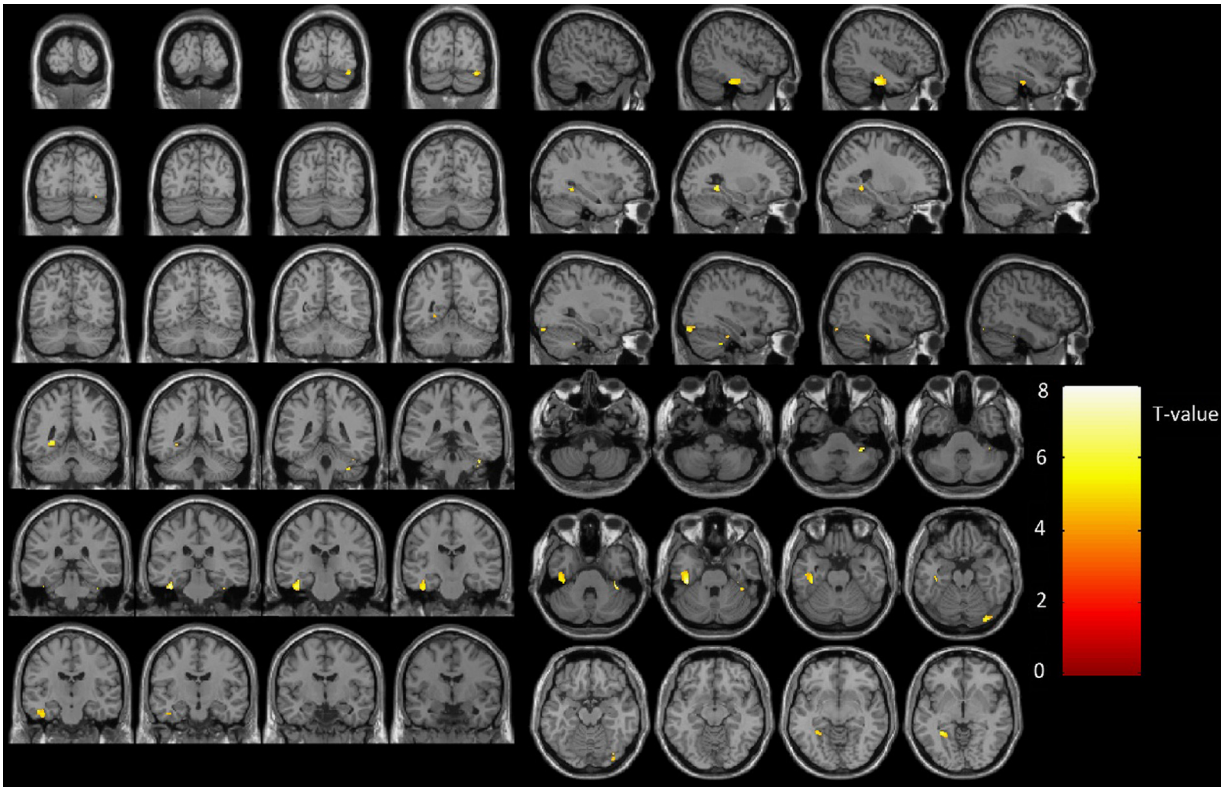

Fig. 3 – Coronal, sagittal and axial slices, showing the relationship between gray matter volume and progesterone concentrations in peripheral blood, in the group of women with a natural cycle. There is a positive correlation located in the fusiform gyrus areas during follicular phase. An uncorrected threshold of $p<<0.001$ was used.

phase. To the best of our knowledge, regional gray matter volumes were never before correlated with hormone levels, as a function of menstrual cycle phase. A study of Peper et al., performed a VBM study on developing pubertal girls, without taking cycle phases into account (Peper et al., 2009). These authors describe that a higher estradiol level was associated with lower gray matter density in prefrontal, parietal and middle-temporal areas. The correlational findings of prefrontal gray matter with estradiol concentrations tend to agree with our population.

Menstrual cycle related changes in regional gray matter volume were first investigated with MRI in 2008 (Protopopescu et al., 2008); these authors performed VBM on T1-weighted anatomical images. They found, inter alia, smaller GM volumes in the anterior cingulate gyrus during the late follicular phase, as compared to the late luteal phase. They obtained follicular phase data at day 10 to 12 after onset of the menses, and luteal phase was measured 1–5 days before the next menses. These time-points do not coincide with our measurements, which might explain the differences between our results and those previously published (Protopopescu et al., 2008). We surmise that this difference constitutes evidence for the constant modulation of the brain, at least partly, through variations in estradiol and progesterone levels.

The ACC is commonly associated with emotional processing and empathy (Yamasaki et al., 2002; Mak et al., 2009). Literature data indicate that women are more interested in social signs, interactions and show more empathy (Singer et al., 2004) during the follicular phase. Women appear to focus more attention on emotional expressions, in order to have a more successful social interaction, because it enhances mating chances. During the luteal phase, this is no longer of primary concern (Macrae et al., 2002). Findings overall suggest, that women during the follicular phase tend to be more "efficient" in processing emotional events, than during the high hormonal luteal phase. This implies that, during the follicular phase, women tend to handle emotional challenges with greater equanimity and with less impact on their social environment.

Women show behavioral alternations during different hormonal phases, and this has been well documented in previous studies using fMRI (Maki et al., 2002; Wrase et al., 2003; Goldstein et al., 2005, 2010; Gizewski et al., 2006; Derntl et al., 2008, 2013; Rupp et al., 2009; Guapo et al. 2009; Zhu et al., 2010; Ossewaarde et al., 2010; Andreano and Cahill, 2010; Rezaii et al., 2012, Gingnell et al., 2013) or other non-imaging techniques (Konishi et al., 2008; Ferree et al., 2011, 2012; Nielsen et al., 2011, 2013). Although our study lacks behavioral data, from the fMRI literature, we know that the ACC is associated with emotional memory processing (Yamasaki et al., 2002; Mak et al., 2009). It has been shown that the performance of the ACC appears to be dependent on the menstrual cycle phase, better in low estradiol situations than in high estradiol situations (Guapo et al., 2009; Andreano and Cahill, 2010). Because regional brain volume is related to the relative importance of a brain structure, we expect a negative correlation of estradiol concentration with regional GM volume in the ACC. We think that the strong

negative correlation between the concentration of estradiol with the ACC volume is relevant to explain the reaction to emotional events. Recent literature data provide supporting evidence for the link between gray matter changes and differences in behavior (Mak et al., 2009; Lee et al., 2011; Riva et al., 2013).

A secondary finding describes differences in several fusiform gyrus areas. This is an interesting part of the brain because it has been linked to the processing of faces (Kanwisher et al., 1997). Because most of the fMRI studies on emotion-processing use pictures of faces (IAPS) to extract results, this is a relevant structure to consider.

When considering only the NC group, no cycle-dependent volume changes in the fusiform gyrus are detected (Table 2). Progesterone concentrations in the follicular and luteal phase showed clusters of correlation, positive and negative respectively, with regional gray matter volume (Table 5). We believe that the different correlation results with progesterone levels during the luteal and follicular phases can possibly be explained by the relatively low spread of progesterone concentrations in the follicular phase. As a result, correlations with progesterone in the follicular phase are less reliable, especially given the relatively small sample size of our study. Future work might give a better understanding of the role of the fusiform gyrus.

The possible effect of hormonal contraceptives use was also taken into account by the VBM study of Pletzer et al. (2010). These authors compared 2 phases of a natural menstrual cycle with women on HC. These authors found a larger fusiform gyrus bilaterally, in the active pills, when compared to the luteal phase of the natural cycle group. Our active pill group however shows a smaller left fusiform gyrus (P-FDR=0.083), when compared to the NC group. Because of these different results, we think that the mechanism of local volume changes in the fusiform gyrus is not trivial, because additionally none of our correlations spatially coincide exactly with the local fusiform gyrus volume changes. Despite of these contradictory results, it is reasonable to assume that certain structural differences can occur. Several fMRI studies show significant differences as a function of cycle phase (Derntl et al., 2008; Guapo et al., 2009).

It is an interesting finding that our data show gray matter differences to a far lesser extent than the Pletzer study, especially in the prefrontal cortex. The performance of VBM is crucially dependent on registration performance (Ashburner, 2007), and we at least partly ascribe this difference to the superior non-linear registration algorithm that we used (DARTEL). Additionally, before starting DARTEL, we used the "New Segment" tool, which offers a more robust initial affine transformation. A comparative study between earlier SPM non-linear registrations (among others) and DARTEL shows the superiority of DARTEL and therefore we have high confidence in our data processing pipeline (Klein et al., 2009).

Finally, the study of Pletzer et al., did not take into account the administration pause ("stop week/inactive pills") of the contraceptives users. We note that, although smaller, there are significant short term differences within the HC group between inactive pill phase and active pill phase, in the left Brodmann 6 area and the right posterior central gyrus, along with some smaller clusters.

Confounding factors of our study might be the relatively small size and age range of the population. As a consequence of the small sample size, high statistical power is not achievable with our dataset. In addition we believe that data in the ovulatory phase of the NC group might be of significant interest due to the contrast between estradiol and progesterone concentrations at that time-point. In present study two phases of the natural cycle are considered, when both concentrations are either low or high. A study shows a gray matter volume peak and CSF loss at the time of ovulation (Hagemann et al., 2011), interesting observations can probably be made here. Overall, correlations with progesterone and estradiol do not always spatially coincide with the volumetric differences described. The endogenous release of progesterone in the luteal phase of the NC group is always accompanied by release of estrogen. Release of other hormones in a natural situation may alter the effect or responsiveness of progesterone or estradiol on the brain (Andreano and Cahill, 2010). Therefore, the use of exogenous progesterone (and/or estrogen), differs from the natural situation. Because the artificially introduced hormones through the contraceptives may have a slightly different effect than endogenously produced hormones, the correlations with hormones in the HC group and the NC group need not mean the same thing. Finally, this study did not include behavioral experiments, interpretation of the results is based on literature data.

We conclude that sex hormones have an effect on brain structures and influence short term plasticity, a topic that has been ignored for much too long. Hence, we suggest that for all neuroimaging studies in women, the phase of the menstrual cycle and the use of hormonal contraceptives should be taken into account. Further research is needed to elucidate the mechanism and sex dependency of neurologic and psychiatric illness (May, 2011; Lentini et al., 2012). When investigators do not take into account the menstrual cycle phase and use of hormonal contraceptives, this introduces heterogeneity within the test-population, and may lead to significant loss of precision and accuracy of findings.

4. Experimental procedures

4.1. *Subjects*

Thirty healthy young women, with no history of neurological or psychiatric illness, were enrolled in the study. Participants ranged in age between 18 and 28 years, with a mean of 21.7±0.5 years. All participants signed a written informed consent form. Our study was approved by the institutional review board. Participants consisted of 15 women using combined monophasic contraception (different formulations were allowed, but subjects using multiphasic or progestogen only pills were excluded), and 15 women with a natural cycle (i.e. no use of hormonal contraception). MR examinations of the brain were performed during the follicular and luteal phase of the menstrual cycle, and during inactive and active phase of subjects on HC. The order of MR-sessions was counterbalanced across subjects. The naturally cycling women underwent MRI on the 3rd (follicular phase) and the

21st day (luteal phase) of their menstrual cycle. The women on HC underwent MRI on the last day of the inactive pill week and the 14th day after starting the next active pill phase. For practical organizational reasons, a variation of 1 day was accepted in both groups. Consequently, a total of 60 MR data sets were acquired. In addition, after each MR examination, we obtained blood samples, in order to measure total venous plasma concentrations of sex hormones (LH, FSH, progesterone and estradiol). Due to non-normality of some of the hormonal data, we performed non-parametric testing (Mann–Whitney *U* test and Wilcoxon rank test), differences were considered significant when $p < < 0.05$.

4.2. *Hormonal assays*

Peripheral venous blood samples were analyzed using standard assays. Follicle stimulating hormone and luteinizing hormone were assessed using a Siemens Dimension Vista® 1500 Intelligent Lab System. Progesterone and estradiol concentrations were measured in a Roche MODULAR Analytics E170.

4.3. *MR data acquisition*

All MR examinations were performed on a 3T scanner (Magnetom Trio Tim, Siemens AG, Siemens Medical Solutions, Erlangen, Germany). A 32-channel head coil was used to obtain images with an isotropic resolution of 1 mm^3. For volumetric measurements, we acquired a high resolution anatomical T1-weighted MR dataset, using a magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence with 176 slices and a field of view of 256 mm × 192 mm. Repetition time TR and echo time TE were 1910 ms and 3.37 ms, respectively; the flip angle was 15°.

4.4. *Data analysis*

For Jacobian-modulated VBM preprocessing we used the SPM8 software (Ashburner and Friston, 2000). Images are bias-corrected for the smoothly varying intensity inhomogeneity caused by magnetic field imperfections, using a linear combination of low frequency Discrete Cosine Transformation basis functions as the intensity inhomogeneity field model. The New Segment tool was used to segment images into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). This feature uses unified tissue segmentation, warping of tissue probability maps to match the image (Ashburner and Friston, 2005). Intensities are modeled by a Gaussian Mixture Model. Unlike previous investigators (Protopopescu et al., 2008; Pletzer et al., 2010), we made use of a newly described technique "Diffeomorphic Anatomical Registration Through Exponentiated Lie-Algebra" (DARTEL) to account for more detailed shape variability in the population (Ashburner, 2007). The DARTEL registration involves alternating between computing a template, based on the average tissue probability maps of all subjects, and warping the tissue maps of all subjects into increasingly good alignment with the template. Images were then transformed to standard Montreal Neurological Institute (MNI) space, followed by a smoothing procedure with a Gaussian Kernel of 8 mm. Previous authors have used a larger (12 mm) smoothing kernel (Protopopescu et al., 2008; Pletzer et al., 2010); however since we used the DARTEL algorithm, which delivers a significantly better registration (Klein et al., 2009), we preferred to use a smaller smoothing kernel. This results in a better resolution of localized differences. *T*-test or paired *T*-test were used appropriately.

Acknowledgment

Jan Sijbers greatfully acknowledges support from the inter-university attraction pole (IUAP) Belgium.

REFERENCES

Amin, Z., et al., (2006). Effects of estrogen variation on neural correlates of emotional response inhibition. NeuroImage 32, 457–464.
Andreano, J.M., Arjomandi, H., Cahill, L., 2008. Menstrual cycle modulation of the relationship between cortisol and long-term memory. Psychoneuroendocrinology 33, 874–882.
Andreano, J.M., Cahill, L., 2010. Menstrual cycle modulation of medial temporal activity evoked by negative emotion. NeuroImage 53, 1286–1293.
Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry—the methods. NeuroImage 11, 805–821.
Ashburner, J., Friston, K.J., 2005. Unified segmentation. NeuroImage 26, 839–851.
Ashburner, J., 2007. A fast diffeomorphic image registration algorithm. NeuroImage 38, 95–113.
Boron, W., Boulpaep, E., 2005. Medical Physiology. Elsevier, Philadelphia.
Cahill, L., 2012. A half-truth is a whole lie: on the necessity of investigating sex influences on the brain. Endocrinology 153, 2541–2543.
Derntl, B., et al., (2008). Emotion recognition accuracy in healthy young females is associated with cycle phase. Horm. Behav. 53, 90–95.
Derntl, B., et al., (2013). Association of menstrual cycle phase with the core components of empathy. Horm. Behav. 63, 97–104.
De Bondt, T., (2013). Does the use of hormonal contraceptives cause microstructural changes in cerebral white matter? Preliminary results of a DTI and tractography study. Eur. Radiol. 23, 57–64.
Ertman, N., Andreano, J.M., Cahill, L., 2011. Progesterone at encoding predicts subsequent emotional memory. Learn. Mem. 18, 759–763.
Ferree, N.K., Kamat, R., Cahill, L., 2011. Influences of menstrual cycle position and sex hormone levels on spontaneous intrusive recollections following emotional stimuli. Conscious. Cognit. 20, 1154–1162.
Ferree, N.K., Wheeler, M., Cahill, L., 2012. The influence of emergency contraception on post-traumatic stress symptoms following sexual assault. J. Forensic Nurs. 8, 122–130.
Garcia-Segura, L.M., 1997. Estrogen-induced hypothalamic synaptic plasticity. Aging (Milano) 9, 59.
Gingnell, M., (2013). Oral contraceptive use changes brain activity and mood in women with previous negative affect on the pill–a double-blinded, placebo-controlled randomized trial of a levonorgestrel-containing combined oral contraceptive. Psychoneuroendocrinology 38, 1133–1144.
Gizewski, E.R., et al., (2006). There are differences in cerebral activation between females in distinct menstrual phases during viewing of erotic stimuli: a fMRI study. Exp. Brain Res. 174, 101–108.

Goldstein, J.M., et al., (2005). Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. J. Neurosci. 25, 9309–9316.
Goldstein, J.M., et al., (2010). Sex differences in stress response circuitry activation dependent on female hormonal cycle. J. Neurosci. 30, 431–438.
Guapo, V.G., et al., (2009). Effects of sex hormonal levels and phases of the menstrual cycle in the processing of emotional faces. Psychoneuroendocrinology 34, 1087–1094.
Hagemann, G., et al., (2011). Changes in brain size during the menstrual cycle. PLoS One 6, e14655.
Jazin, E., Cahill, L., 2010. Sex differences in molecular neuroscience: from fruit flies to humans. Nat. Rev. Neurosci. 11, 9–17.
Kanwisher, N., McDermott, J., Chun, M.M., 1997. The fusiform face area: a module in human extrastriate cortex specialized for face perception. J. Neurosci. 17, 4302–4311.
Klein, A., et al., (2009). Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. NeuroImage 46, 786–802.
Konishi, K., et al., (2008). Effects of the menstrual cycle on working memory: comparison of postmenstrual and premenstrual phases. Ind. Health 46, 253–260.
Lee, H.Y., et al., (2011). Demonstration of decreased gray matter concentration in the midbrain encompassing the dorsal raphe nucleus and the limbic subcortical regions in major depressive disorder: an optimized voxel-based morphometry study. J. Affective Disord. 133, 128–136.
Lentini, E., et al., 2012. Sex Differences in the Human Brain and the Impact of Sex Chromosomes and Sex Hormones. Cereb Cortex.
Levine, S., 1966. Sex differences in the brain. Sci. Am. 214, 84–90.
Macrae, C.N., et al., (2002). Person perception across the menstrual cycle: hormonal influences on social-cognitive functioning. Psychol. Sci. 13, 532–536.
Mak, A.K., et al., (2009). Gray matter reduction associated with emotion regulation in female outpatients with major depressive disorder: a voxel-based morphometry study. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 1184–1190.
Maki, P.M., Rich, J.B., Rosenbaum, R.S., 2002. Implicit memory varies across the menstrual cycle: estrogen effects in young women. Neuropsychologia 40, 518–529.
May, A., 2011. Experience-dependent structural plasticity in the adult human brain. Trends Cognit. Sci. 15, 475–482.
McCarthy, M.M., et al., (2012). Sex differences in the brain: the not so inconvenient truth. J. Neurosci. 32, 2241–2247.
Melcangi, R.C., Panzica, G., Garcia-Segura, L.M., 2011. Neuroactive steroids: focus on human brain. Neuroscience 191, 1–5.
Naftolin, F., et al., (1988). The cellular effects of estrogens on neuroendocrine tissues. J. Steroid Biochem. 30, 195–207.
Naftolin, F., et al., (2007). Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge. Reprod. Sci. 14, 101–116.
Nielsen, S.E., et al., (2011). Hormonal contraception usage is associated with altered memory for an emotional story. Neurobiol. Learn. Mem. 96, 378–384.
Nielsen, S.E., et al., (2013). Hormonal contraception use alters stress responses and emotional memory. Biol. Psychol. 92, 257–266.
Ossewaarde, L., et al., (2010). Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle. Psychoneuroendocrinology 35, 47–55.
Peper, J.S., et al., (2008). Cerebral white matter in early puberty is associated with luteinizing hormone concentrations. Psychoneuroendocrinology 33, 909–915.
Peper, J.S., et al., (2009). Sex steroids and brain structure in pubertal boys and girls. Psychoneuroendocrinology 34, 332–342.
Pletzer, B., et al., (2010). Menstrual cycle and hormonal contraceptive use modulate human brain structure. Brain Res. 1348, 55–62.
Protopopescu, X., et al., (2008). Hippocampal structural changes across the menstrual cycle. Hippocampus 18, 985–988.
Rezaii, T., et al., (2012). The influence of menstrual phases on pain modulation in healthy women. J. Pain 13, 646–655.
Riva, D., et al., 2013. Gray Matter Reduction in the Vermis and CRUS-II is Associated with Social and Interaction Deficits in Low-Functioning Children with Autistic Spectrum Disorders: A VBM-DARTEL Study. Cerebellum.
Rupp, H.A., et al., (2009). Neural activation in the orbitofrontal cortex in response to male faces increases during the follicular phase. Horm. Behav. 56, 66–72.
Singer, T., et al., (2004). Empathy for pain involves the affective but not sensory components of pain. Science 303, 1157–1162.
Wrase, J., et al., (2003). Gender differences in the processing of standardized emotional visual stimuli in humans: a functional magnetic resonance imaging study. Neurosci. Lett. 348, 41–45.
Yamasaki, H., LaBar, K.S., McCarthy, G., 2002. Dissociable prefrontal brain systems for attention and emotion. Proc. Natl. Acad. Sci. U S A 99, 11447–11451.
Zhu, X., et al., (2010). Brain activation evoked by erotic films varies with different menstrual phases: an fMRI study. Behav. Brain Res. 206, 279–285.