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Prefrontal GABA concentration changes in women - Influence of menstrual cycle phase, hormonal contraceptive use, and correlation with premenstrual symptoms

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ABSTRACT

Prefrontal regions are involved in processing emotional stimuli and are a topic of interest in clinical and neurological research. Although sex steroids are potent neuromodulators, the influence of menstrual cycle phase and hormonal contraceptive use is rarely taken into account in neuroimaging studies. Our purpose was to evaluate changes in gamma-aminobutyric acid (GABA) in women, as measured by magnetic resonance spectroscopy (MRS), with phases of the menstrual cycle and use of hormonal contraceptives, and to assess correlations with premenstrual symptoms.

Three MRI sessions per cycle were obtained in the natural cycle group, and two sessions in the hormonal contraceptives group. In addition to an anatomical scan, single voxel MRS in the prefrontal area was performed. After quality control, 10 women with natural cycle and 21 women taking hormonal contraceptives were included for analysis. Peripheral blood samples were obtained to determine endogenous hormone concentrations. Subjects were asked to complete a daily rating of severity of problems questionnaire, to quantify premenstrual symptoms.

In the natural cycle group, we found a significant increase in prefrontal GABA concentration at the time of ovulation. Conversely, in the hormonal contraceptives group, no differences were found between the pill phase and pill-free phase. GABA concentrations did not significantly correlate with endogenous hormone levels, nor with premenstrual symptoms.

Our results indicate that spectroscopically measured GABA concentrations are higher during ovulation in women with a natural menstrual cycle. We suggest that neuroimaging studies should take into account this variability.

1. Introduction

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the human brain, and is therefore of interest in several subfields of clinical and neurological research (Puts and Edden 2012, Mullins et al. 2012). Although in the human brain, it is only present in millimolar concentrations, thanks to advances in magnetic resonance techniques, it has become possible to identify endogenous concentrations of GABA, non-invasively and in-vivo, by using edited MR spectroscopy (MRS). Cortical GABA concentrations are assessed in research for several conditions such as epilepsy (Simister et al. 2003), major depressive disorder (Hasler et al. 2007, Croarkin et al. 2011), bipolar disorder (Kaufman et al. 2009), schizophrenia (Tayoshi et al. 2010), panic disorder (Hasler et al. 2009, Long et al. 2013), attention deficit hyperactivity disorder (ADHD) (Edden et al. 2012), and for brain plasticity in motor neurons (Stagg 2013). Also, local prefrontal GABA modulations have been associated with working memory (Michels et al. 2012).

GABA concentrations have been historically assessed most commonly in the occipital regions, due to practical and technical considerations (absence of air-containing structures, more homogeneous environment, less motion artifacts). However, more recently there is increasing interest in prefrontal GABA concentrations (Hasler et al. 2007 and 2009, Waddell et al. 2011, Long et al. 2013, Stan et al. 2014). This shift of attention is due to the fact that prefrontal regions are commonly associated with emotion-regulation (Yamasaki et al. 2002, Gingnell et al. 2013, Epperson 2013), other higher order cognitive functions (Frith and Dolan 1996), and the prefrontal cortex is of interest in several neuro-psychiatric diseases. Prior to the clinical manifestation of these disorders, there may be associated alterations or imbalances in regional neurotransmitter concentrations (Waddell et al. 2011). Prefrontal and anterior cingulate cortex (ACC) GABA levels may index the degree of negative emotions in depressive episodes (Halser and Northoff 2011).

Early researchers in this field suggested a potential effect of menstrual cycle phase on cortical GABA concentrations, and a possible link to clinical conditions such as premenstrual syndrome (PMS), and/or premenstrual dysphoric disorder (Epperson et al. 2002, Backstrom et al. 2003). Hormonal fluctuations of the menstrual cycle seem to have an effect on cortical GABA levels, or vice-versa. Progesterone has a binding site at the GABA-A receptor complex, modulating neural plasticity and excitability (Kaore et al. 2012). Using transcranial magnetic stimulation (TMS), excitability of the motor cortex was more inhibited during the luteal phase in a control population, as compared to PMS subjects (Smith et al. 2002); these authors pointed to a possible deficiency in GABAergic function in the PMS group. Low GABA plasma levels in the luteal phase of PMS subjects have been observed almost two decades ago (Halbreich et al. 1996). MRS results in the lentiform nucleus, frontal lobe and (non-

significantly) cingulate voxels, show that GABA concentrations decrease in the luteal phase, compared to the follicular phase (Harada et al. 2011).

One other possible factor that is almost never taken into account is the use of hormonal contraceptives (HC). The synthetic progestins contained in HC may also interact with the progesterone binding site of the GABA-A receptor, changing its configuration and behavior (Stell et al. 2003). Also, women can experience PMS-like symptoms during their pill-free week (of inactive phase) (Cullberg 1972, Rapkin et al. 2006, Rapkin and Akopians 2012).

Despite these promising findings, uncertainties remain regarding the validity of these results. The study by Epperson et al. in 2002 is overall well designed, but only considers an occipital voxel, and has a relatively large age-spread, which has been shown to affect GABA concentrations (Gao et al. 2013). Harada et al. in 2011 partially confirmed the cycle-dependent GABA fluctuations, but only in a healthy subgroup without premenstrual symptoms (because these symptoms were not assessed), and their study population contained only 7 women. In order to avoid these possible cycle-dependent effects, some researchers are now advocating scanning only men (Near et al. 2014), which is also a practical confound, and always limits conclusions.

In the scientific literature there is a growing interest in measuring GABA concentrations in prefrontal areas with MRS, and in identifying differences in GABA concentrations in different anatomical regions (Van Der Veen et al. 2013). Therefore, when measuring cortical GABA concentration through edited MRS, it is important to eliminate uncertainties about the influence of menstrual cycle phase, premenstrual symptoms and hormonal contraceptive use. Our aim was to study time-dependent differences of GABA concentrations in the prefrontal region through a longitudinal study in young, healthy women. Although some literature regarding menstrual cycle related GABA changes and possible interactions with hormones and premenstrual symptoms exists, we consider this research as being exploratory, and do not propose a strong a priori hypothesis.

2. Results

2.1. Subjects

Table 1 shows an overview of the performed procedures; several subjects were excluded for further analysis, for a variety of reasons:

(1) Coincidental abnormalities in the brain on the T1 weighted images by a senior radiologist (3 subjects of the HC-group were excluded).

(2) One subject had a markedly abnormal hormonal estradiol value in the follicular phase (more than 6 standard deviations above average), and an unusually long menstrual cycle length (more than 12 standard deviations above average).

(3) Due to suboptimal quality of the edited GABA MR spectra (grade 3 and 4), 38 scans out of 170 were excluded.

Finally, because we to exclude as many inter-subject differences as possible, we only retain data of subjects from which the spectra in *all* sessions have sufficient quality.

What remains, is a total of 76 spectra: 42 (2x21) from the hormonal contraceptives (HC) group, and 33 (3x11) from the natural cycle (NC) group. The mean age of the remaining HC group is (22.5 ± 2.6) years and (24.3 ± 3.6) years for the NC group, which means that there is no significant age difference (T-test p-value = 0.112). The mean cycle length in the remaining NC group is (29.4 ± 2.5) days, ranging from 26.5 to 35 days, which is considered to be normal (Mihm et al. 2011).

2.2. GABA+/Cr

Figure 1 shows the placement of the MRS voxel superimposed on axial, coronal and sagittal slices of the MP-RAGE image, together with the resulting edited spectrum of GABA+ fitted by the Gannet software (see section experimental procedures). The mean GABA fit error of all included spectra was 12.27 % ± 0.36 %. MR spectroscopically measured GABA+/Cr ratios are listed for each group and phase in Table 2 and shown in boxplots in Figure 2. All distributions were found not to differ significantly from a Gaussian, because all Kolmogorov-Smirnov (K-S) tests show a p-value higher than 0.9.

A paired T-test between both phases of the HC group showed no significant difference (p-value = 0.375; T-value = 0.906). A repeated measures ANOVA in the NC group shows a significant effect of phase (p-value = 0.011; F = 5.646). A post-hoc analysis revealed that GABA+/Cr ratios are higher around ovulation as compared to the follicular phase (p-value = 0.020; T-value = 2.764) and as compared to the luteal phase (p-value = 0.046; T-value = 2.272). There were no significant differences between follicular and luteal phases (p-value = 0.530; T-value = -0.650). When comparing HC phases to NC phases, both active pill and inactive, pill-free phases differ significantly from the ovulation phase using an independent sample T-test (p-value = 0.046; T-value = -2.204 and p-value = 0.023; T-value = -2.577 respectively). No significant differences between the HC phases and follicular and luteal phases were found (all p-values > 0.348).

2.3. Hormonal assays

Measured endogenous hormone concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol and progesterone, follow the expected pattern for a natural menstrual cycle and pill-cycle. Results are depicted in Figure 3.

2.4. Premenstrual symptoms

Normalized somatic and psychological premenstrual symptom (sPMS and pPMS) values in both groups were also not found to deviate significantly from a Gaussian distribution (all K-S tests show p-value > 0.327). Mean values and standard deviations are listed in Table 3 and shown in boxplots in Figure 4. Ratings of symptoms were not significantly different between groups, for both sPMS (p-value = 0.914; T-value = -0.109) and pPMS (p-value = 0.578; T-value = -0.563).

2.5. Correlational analysis

Pearson correlation was performed in all separate phases of both groups. We found only one correlation that reached statistical significance: FSH concentrations correlated negatively with pPMS in the pill-free week of the HC group (p-value = 0.024, r = -0.516). Furthermore, no other correlations were found between GABA+/Cr ratios, endogenous hormones and PMS symptoms.

3. Discussion

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the human brain. Recently, GABA research has emerged as an exciting and relatively new field in experimental neuroscience. Some authors have pointed out possible fluctuations of GABA with menstrual cycle phase, although the evidence in the literature is rather scarce (Epperson et al. 2002, Harada et al. 2011). This is why we undertook an experimental study in healthy, young female volunteers in order to assess GABA concentration changes in the prefrontal areas of the brain, and to search for possible correlations with menstrual cycle phase, hormonal contraceptive use and premenstrual symptoms.

Previous investigators found proof of varying GABA+/Cr concentrations in the occipital cortex (Epperson et al. 2002, Harada et al. 2011). Up to now, it was not clear if these changes also occurred in the prefrontal area, which has emerged as a new focus of research in GABA MRS (Hasler et al. 2007 and 2009, Waddell et al. 2011, Long et al. 2013, Stan et al. 2014). Our results show that, in women with a natural menstrual cycle, there is a slight, but

significant increase in prefrontal GABA+/Cr levels around ovulation, as compared to both follicular and luteal phases, and as compared to the active and inactive pill phases in the HC-group. To the best of our knowledge, this is the first time that a prospective GABA MRS study attempts to include a time-point around ovulation. Also, it is the first time that use of hormonal contraceptives has been explicitly taken into account. It has been shown in other neuroimaging subfields that use of hormonal contraception is a confounding factor that can introduce heterogeneity in the data; examples include voxel based morphometry (Pletzer et al. 2010, De Bondt et al. 2014), diffusion tensor imaging (De Bondt et al. 2013) and functional MRI (Protopopescu et al. 2008, Pletzer et al. 2014, Petersen et al. 2014).

A possible explanation for this increase in cortical GABA levels might be found in a social-cognitive context. GABA deficiency has been associated with major depression (Hasler et al. 2007, Croarkin et al. 2011) and panic disorder (Halser et al. 2009, Long et al. 2013). Conversely this could mean that higher GABA levels around ovulation can induce a more successful social interaction, with an enhanced sensitivity to stimuli with reproductive relevance (Macrae et al. 2002).

Despite the fact that the molecular structure of the synthetic progestins in HC leads to a different interaction with the GABA-A receptor, this does not seem to lead to short term differences of GABA+/Cr when comparing inactive pill-phase with active pill-phase. The inactive pill-phase of the HC group can be considered identical to the early follicular phase of the next cycle if no contraceptive pill would be started. Not restarting the pill after the interval would generally and immediately result in a normal ovulatory cycle in over 75% of women (Seidman et al. 2014). Hence, when comparing the NC follicular phase and the HC inactive pill-phase, we should be able to look at long-term (i.e. not normalized after the washout of synthetic hormones) effects of hormonal contraceptive use. Since we observe no difference between GABA+/Cr ratios in the NC follicular phase and the inactive pill-phase, we have reason to believe that there is also no long term effect of HC use.

We evaluated premenstrual symptoms (PMS), or PMS-like symptoms in the HC-group, in all of our subjects, and we defined 2 normalized parameters for both psychological and somatic symptoms (pPMS and sPMS). Unlike previous studies (Epperson et al. 2002, Smith et al. 2002, Protopopescu et al. 2008, Gignell et al. 2013, Berman et al. 2013, Hoyer et al. 2013), we chose not to split up our subjects based on symptom ratings, but rather to evaluate them as a continuous variable. The reason why we do this, is that the subjective symptom ratings are relatively homogenously distributed, and every splitting based on these parameters could be considered as arbitrary. Instead, we correlated our symptom ratings with GABA+/Cr and hormone concentrations. Based on our results, we could find no significant correlation between measurements of GABA+/Cr in the prefrontal region and pPMS or sPMS.

In the correlational analysis of endogenous hormones with premenstrual symptoms, we found one significant result: apparently pPMS are negatively correlated with FSH in the inactive pill phase. In the literature, there is one study documenting a negative association

between FSH and suicidality scores in a population of women with major depressive disorder (Kim et al. 2013). However, because these authors did not correct for cycle phases and use of HC, and because our study included only healthy subjects, we believe that this finding is a spurious correlation.

In the study of Epperson et al. from 2002, it was shown that occipital GABA concentrations in non-symptomatic women decreased in the mid-luteal and late luteal phase as compared to the follicular phase. In contrast, for women with severe premenstrual symptoms, there was a significant increase of GABA levels from the follicular to both luteal phases (Epperson et al. 2002). Considering these results, one might expect a negative correlation between GABA+/Cr and premenstrual symptom severity. Such effects were not seen in our data. However, results may not be readily comparable between the studies, because of differences in scan timing, and different anatomical localization of the MRS voxel.

One would to some extent expect a correlation of GABA+/Cr ratios with progesterone, considering its binding site to the GABA-A receptor, this is however not observed. Additionally, the significant increase of GABA+/Cr is found around ovulation, a menstrual cycle phase which is mostly associated with an increase in estradiol. Preclinical evidence exists that estradiol can interact with the GABAergic system in the hippocampus during both development and adulthood (Wojtowicz and Mozrzymas 2010). While the hippocampus is not located within our spectroscopy voxel, similar interactions can occur in prefrontal areas. It is plausible that the effect is not determined by absolute concentrations of the endogenous hormones, but is rather the effect of their interaction with each other and possible other factors (Osterlund and Hurd 2001, Brinton et al. 2008).

Measured GABA+/Cr ratios show no significant correlations with the endogenous hormones we measured (LH, FSH, estradiol and progesterone). The development of negative mood symptoms has also been associated with allopregnanolone, a metabolite of progesterone, from which the concentration increases during the luteal phase of the menstrual cycle, together with progesterone (Andreen et al. 2009, Backstrom et al. 2011). Comparing allopregnanolone concentrations between a symptomatic and a control population has shown contradictory results (Rapkin et al. 1997, Wang et al. 1997, Girdler et al. 2001). However there seems to be a relationship between symptom severity and allopregnanolone concentrations (Nyberg et al. 2007). Allopregnanolone acts as a positive modulator of the GABA-A receptor, in a way that is pharmacologically similar to alcohol. It has been suggested that several GABA-A modulators, show a biphasic effect. Low concentrations increase anxiety, and high concentrations have calming properties; the exact mechanism of this effect is not known (Andreen et al. 2009). It seems that some women's GABAergic system is more sensitive to alloprognanolone than others. For an extensive review on the GABA-A receptor mediated effect of allopregnanolone on mood disorders, we refer to the work of Backstrom and coworkers (Backstrom et al. 2014).

Unfortunately, our study has a number of important limitations. One of the limitations is that the MR spectroscopy sequence has only 100 averages, whereas in current literature 200 or 300 averages are recommended in order to improve the SNR of the spectra. We made this choice in order to limit the total scan-time, given the fact that our subjects were volunteers that were asked to return multiple times. In addition we rigorously excluded technically suboptimal spectroscopy data (leaving only 76 out of 176 spectroscopy datasets), and therefore we believe that our results are relevant. A second confound may have been some frequency drift during the MRS sequence due to the cooling of scanner elements. This could widen the difference spectrum, but is not likely to bias the results (Waddell et al. 2007). This drift reduces the editing efficiency of GABA+, although it has been reported that scanner drift remains relatively small when using short (70 ms) TE-values (Harris et al. 2013). Corrections for this are made in the Gannet post-processing software. As mentioned earlier, GABA+ signal is contaminated with some co-edited macromolecular signals, which increase with the above mentioned frequency drift, and unfortunately, this cannot be corrected for in post-processing.

Another confound is that the types of HC used contain both older and newer synthetic progestins. The progestin component in the HC can be subdivided based on their molecular structure, and as a consequence their binding properties. More specifically, the androgenicity of the progestins, the ability to stimulate the development of male characteristics, is significantly different when comparing older second generation HC to newer third and fourth generations of HC. Newer progestins have no androgenic activity and bind purely to the progesterone receptors (Bitzer and Simon 2011). The type of progestin used can also influence the metabolism of the synthetic estrogen component (Jung-Hoffmann and Kuhl 1989). Therefore, the interaction with the GABAergic complex may be different, depending on the type of HC. In our HC subject group, 5 out of 21 used the newer anti-androgenic type. However, comparing these 5 to the 16 other HC users, using a Mann-whitney U-test, revealed no significant differences between both types of HC (p-value = 0.302 during inactive pill-phase and p-value = 0.470 during the active pill-phase). Due to the small sample size of the anti-androgenic HC group, this result needs to be interpreted with care, and is not conclusive.

Some investigators have found an age-related decline of GABA+/Cr ratios in frontal and parietal regions (Gao et al. 2013). In our population of normal volunteers, however, the age-range was very small (18-30 years), and we could not find such a correlation. Finally, an effect of nicotine uptake on the GABAergic system in the occipital cortex has been described (Epperson et al. 2005), for which we did not control.

In conclusion, our results show an increase in GABA+/Cr ratios in the prefrontal area around the time of ovulation in young women with a natural menstrual cycle. Conversely, in women using hormonal contraception we could not find a significant influence of the cycle phase. We suggest that investigators should take into account the slight increase in GABA+/Cr ratio

is measured. Because of this, the easy way out would be only to work with women on HC. However, we do not recommend this; Neuroscience needs to be interested and focused on how the female brain works under natural conditions, and not dodge the subject because of practical concerns. Moreover, GABA+/Cr measurements are, at least not directly, related to hormone levels and premenstrual symptoms.

4. Experimental procedure

4.1. Subjects

A total of 75 healthy young women, with no history of neurological of psychiatric illness, were enrolled in this longitudinal study. The study was approved by the institutional review board, and all subjects signed a written informed consent form. Of our subjects, 38 women had a natural menstrual cycle (NC-group), while 37 women were taking monophasic hormonal contraceptives (HC-group).

In the NC-group, we obtained MR examinations of the brain at three time-points during the menstrual cycle: (1) early follicular phase (days 2 to 7, depending on mean cycle length); (2) periovulatory period (the MR examination was performed after a positive urine-based luteinizing hormone (LH) ovulation-test, Sensitest®, Delfgauw, The Netherlands); (3) during the luteal phase of the cycle, approximately one week after session 2.

In the HC-group, MR examinations were performed at two time-points during the cycle: (1) during the last days of the inactive pill phase (days 5 to 7 of the pill-free week); and (2) approximately 2 weeks in the active pill phase (days 13 to 16 of the pill-intake).

4.2. MRI protocol

All MR examinations were obtained with a 32-channel phased array head coil on a 3T scanner (Magnetom Trio Tim, Siemens, Erlangen, Germany). High resolution anatomical T1-weighted images were acquired with a magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence in the axial plane (176 slices, 1 mm isotropic resolution, field-of-view: 256 mm x 192 mm; repetition time TR: 1910 ms; echo time TE: 3.37 ms; flip angle: 15°). For MR spectroscopy, we used a work-in-progress (WIP) sequence, which is based on a previously described J-difference method (Mescher et al. 1998). Edited MRS separates the small GABA peak from superimposed larger peaks, mainly of creatine (Cr). Two subspectra were acquired: one with a frequency selective pulse to GABA spins at 1.9 ppm (“on resonance”), and one placed at 7.5 ppm, symmetrically about the water resonance (“off resonance”). The “on resonance” modifies the J-coupled spins at 3 ppm, while the “off

resonance" pulse does not. The difference between the spectra contains a GABA signal at 3 ppm, while eliminating the overlying Cr peak. In this procedure, there is a co-editing of macromolecules at 3 ppm due to a coupling at 1.7 ppm, which is partly inverted by the editing pulses, therefore we will refer to the GABA signal as GABA+ (Harris et al. 2013). A single voxel of 2 cm x 2 cm x 2 cm was placed in the prefrontal area of the dominant hemisphere based on the MP-RAGE scan. The edited MRS sequence used a repetition time (TR) of 1.5 s and an echo time (TE) of 70 ms, and the number of averages (on-off pairs, acquired interleaved) was 100. Prior to launching the MRS sequence, we always performed a second order manual shimming procedure.

4.3. Hormonal assays

After each MR examination, we obtained peripheral blood samples in order to measure endogenous concentrations of the following hormones: luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol and progesterone. The samples were analyzed using standard assays in the institutional laboratory. FSH and LH were assessed using a Siemens Dimension Vista® 1500 Intelligent Lab System. Progesterone and estradiol concentrations were measured in a Roche MODULAR Analytics E170. Results were used for correlational analyses, and as a confirmation for the cycle phases.

4.4. Auto-assessment of premenstrual symptoms

Premenstrual symptoms are defined by the cyclical appearance of several mental and physical symptoms in naturally cycling women, mostly during the luteal phase. In women taking HC, similar complaints may occur during the pill-free week, or inactive pill phase of the HC cycle (Cullberg 1972). We asked each participating subject to fill out a daily record of severity of problems (DRSP), during 2 natural cycles or 2 pill-cycles (Endicott et al. 2006). This questionnaire records 11 different complaints, 7 of which concern mental or psychological issues, and 4 of which assess physical issues. Women were asked to attribute scores ranging from 1 (not present) to 6 (very severe) (Endicott et al. 2006).

For the NC-group, premenstrual symptoms were defined as the difference between the average daily ratings during the last 5 to 7 days of the cycle (dependent of mean cycle length) and during 9 mid-cycle days (day 8 to 16). In the HC-group, inactive pill-week symptoms were defined as the difference between average ratings during 6 days in the pill-free week and average ratings during 10 days in the active pill-phase (days 7 to 17 of pill-intake). Normalized somatic and psychological PMS-factors (sPMS and pPMS) were defined by averaging the 3 (out of 4) and 4 (out of 7) worst complaints respectively.

4.5. Data analysis

GABA+ spectra were analyzed using the MATLAB-based software package Gannet 2.0, which, unlike other software packages, is specifically targeted for GABA edited MRS. Gannet 2.0 takes raw time domain data input and processes it to a frequency domain edited spectrum; afterwards, a non-linear least-squares fitting procedure is performed (Edden et al. 2013). GABA+ concentrations are referenced to total Cr concentrations. Parameters of this analysis were extracted from the MATLAB structure and further analysis was performed with IBM SPSS 21 Statistical Software package, together with PMS and hormonal data. All spectra were visually rated by 2 independent observers; scores from 1 (good quality) to 4 (bad quality) were attributed. Kappa analysis revealed a moderate agreement between the ratings (kappa = 0.495). Spectra rated 3 or 4 by both observers were excluded from analysis. Spectra where there was doubt, were discussed for inclusion afterwards. Differences were considered to be statistically significant for p-values lower than 0.05.

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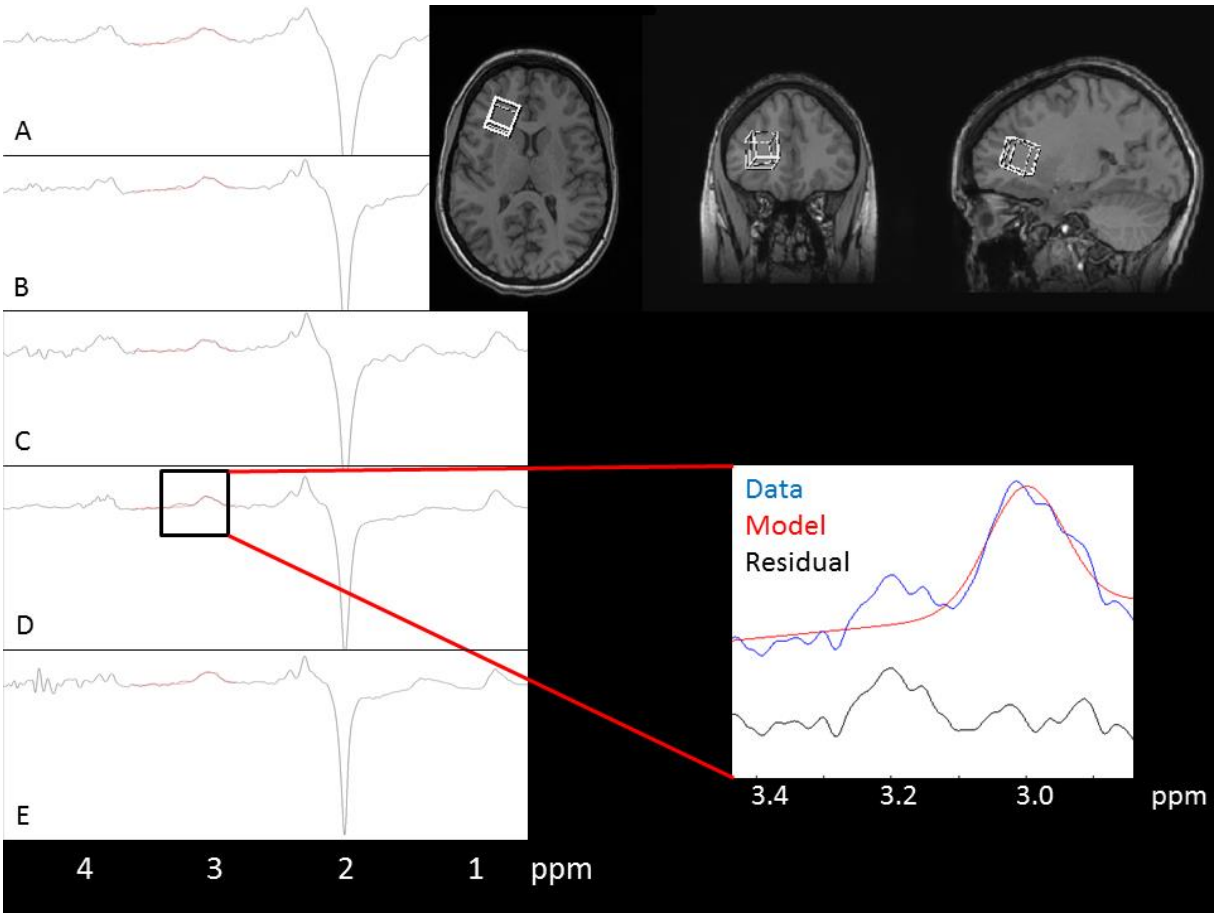
6. Figure captions

- Figure 1: Axial, coronal and sagittal MP-RAGE sections showing the position of the MRS voxel (2x2x2 cm^3). The left panels show edited spectra with the GABA+ peak at 3 ppm for a subject in the NC follicular phase (A), ovulation (B), luteal phase (C), HC inactive pill-phase (D) and active pill-phase (E). One fit of the GABA+ peak is zoomed in: the blue line shows the data, with superimposed (red line) the fitted model by Gannet 2.0; the residual is shown at the bottom (black line).

- Figure 2: Boxplots of the GABA+/Cr ratios in different phases of both naturally cycling women (NC) in red and hormonal contraceptive users (HC) in orange.

- Figure 3: Boxplots of all measured hormones in the different natural cycle phases (left panels) and hormonal contraception phases (right panels).

- Figure 4: Boxplots of the normalized psychological (pPMS) and somatic (sPMS) ratings in both natural cycle (NC) and hormonal contraception (NC) groups. NC-group symptoms are defined as the difference between the average daily ratings during the last 5 to 7 days of the cycle (dependent of mean cycle length) and during 9 mid-cycle days (day 8 to 16). HC-group symptoms are defined as the difference between average ratings during 6 days in the pill-free week and average ratings during 10 days in the active pill-phase (days 7 to 17 of pill-intake). Normalized psychological and somatic PMS-factors were defined by averaging the 3 (out of 4) and 4 (out of 7) worst complaints respectively.


A
B
C
D
E
4
3
2
1
ppm
Data
Model
Residual
3.4
3.2
3.0
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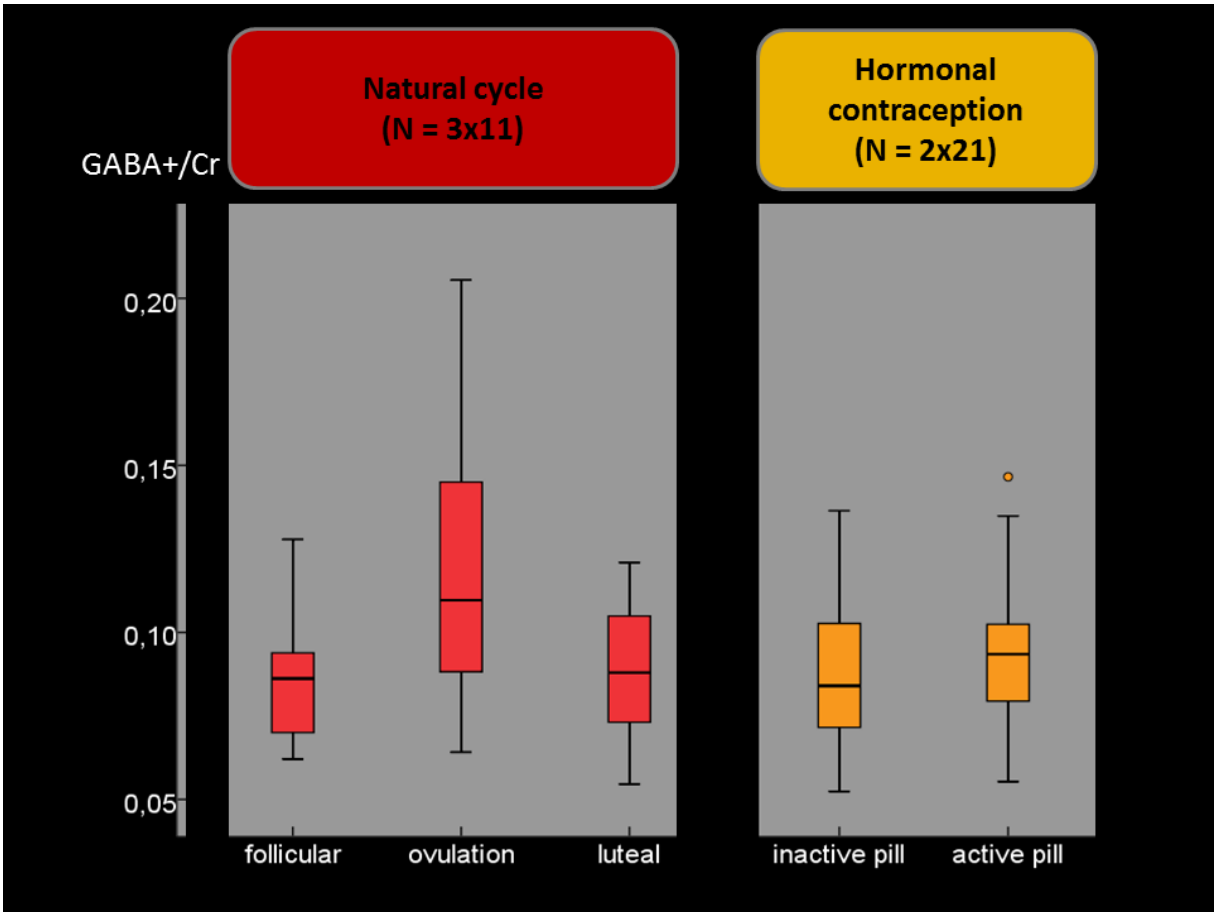
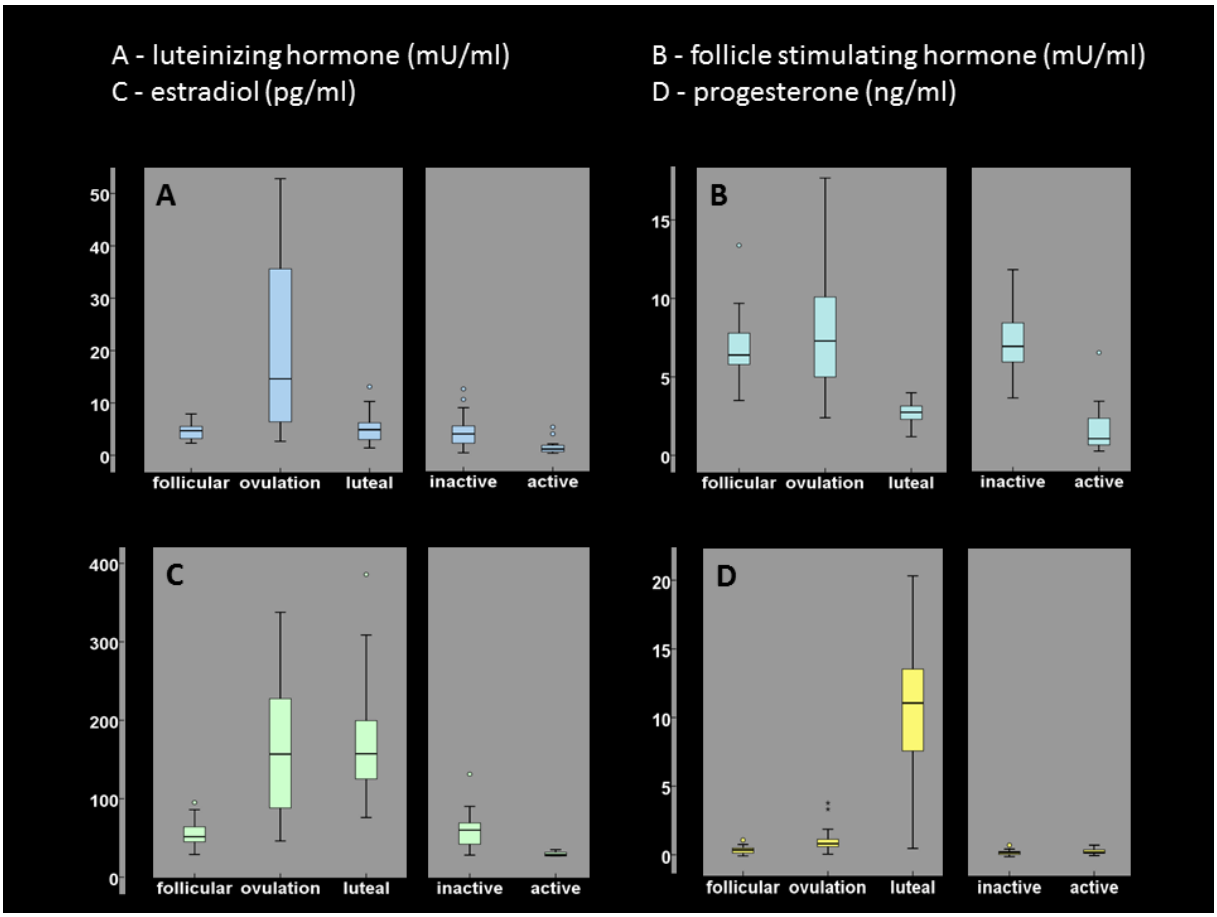
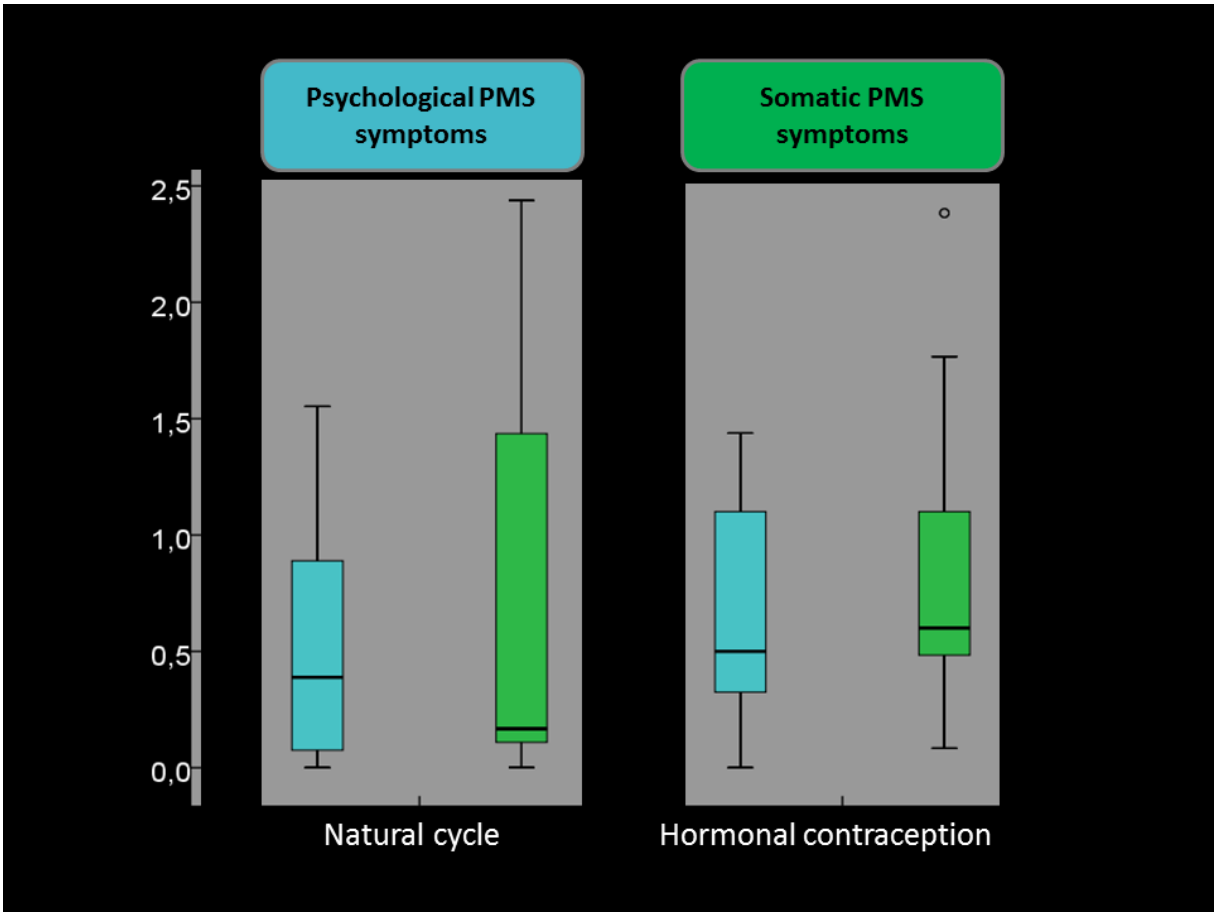

GABA+/Cr
Natural cycle
(N = 3x11)
Hormonal
contraception
(N = 2x21)
0,20
0,15
0,10
0,05
follicular
ovulation
luteal
inactive pill
active pill


A - luteinizing hormone (mU/ml)
B - follicle stimulating hormone (mU/ml)
C - estradiol (pg/ml)
D - progesterone (ng/ml)
A
B
C
D
follicular
ovulation
luteal
inactive
active


Psychological PMS symptoms
Somatic PMS symptoms
Natural cycle
Hormonal contraception