Mood and the pill

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Chapter 7

The role of the mineralocorticoid receptor haplotype and oral contraceptives use in resting state EEG theta/beta ratio

Under review

Abstract

Objective

Resting-state electroencephalography (rs EEG) theta/beta ratio (TBR) is a biomarker of cognitive control over the affective system. Theta power may be associated with prefrontal cortex (PFC)-mediated goal-directed behavior and beta power with arousal. Female hormones modulate cortical-subcortical neural circuits. We examined whether users of oral contraceptives (OC) and naturally cycling (NC) women differ in TBR. Since the mineralocorticoid receptor (MR) haplotype moderates the influence of the female hormonal status on emotional information processing, we also investigated whether any effect of OC-use on rs EEG TBR was moderated by MR-haplotype.

Method

Frontal and parietal rs EEG recordings were acquired in 44 OC-users and 44 NC women in a counterbalanced mixed within-subject design. At both sessions estradiol and progesterone concentrations were assessed.

Results

OC-users and NC women did not differ in rs EEG TBR ($p > .58$). TBR was different between MR-haplotypes ($p = .022$). MR-haplotype 2 homozygotes had lower TBR scores than MR-haplotype 2 heterozygotes ($p = .006$).

Conclusion

Genetic variation associated with enhanced MR expression may modulate regulation of arousal in females irrespective of oral contraceptive use or menstrual cycle phase. Lower parietal TBR in MR-haplotype 2 homozygotes may reflect improved PFC-mediated effortful control and resilience to stressful events.

Introduction

Emotion regulation involves the control of emotional responses to external stimuli or internal mental representations by regulating their occurrence, intensity, and expression (Ochnsner & Gross, 2005). Neural systems involved in emotion regulation comprise cortical and subcortical regions. A cortical regulatory network involving the prefrontal cortex (PFC) and the anterior cingulate cortex (ACC), exerts a top-down influence on an emotional appraisal system residing in subcortical structures, including the amygdala (Ochsner et al., 2012).

The connectivity and plasticity of the limbic-cortical circuits is regulated by sex- and stress hormones (Arnsten & Rubia, 2012; Sacher et al., 2013). Estrogen and progesterone receptors, which mediate these central actions of the sex steroids, are expressed amongst others in the PFC, the amygdala, and the hippocampus (McEwen et al, 2012). Accordingly, estrogen and progesterone enhance cortical-subcortical communication, which may contribute to improved emotion regulation [for a review, see Peper et al., 2011; van Wingen et al., 2011). For instance, estrogen contributed to higher resting-state connectivity between the amygdala and PFC regions (Ottowitz et al., 2008; Engman et al., 2016) and progesterone increased functional connectivity between the amygdala and dorsal ACC in response to emotional faces (van Wingen et al., 2008). OC, however, contain synthetic versions of estrogen and progesterone that suppress endogenous estrogen and progesterone levels (Fleischman et al., 2010). So, OC-use may influence neural circuits underlying emotion regulation and has also been associated with modified emotion recognition (Hamstra et al., 2014, 2015, 2016, 2017a, 2017b, Osorio et al., 2018). A prospective resting-state fMRI study showed that OC-use contributed to a negative connectivity between the amygdala and the dorsolateral PFC (Lisoksky et al., 2016). In addition, both menstrual cycle phase and OC-use have been related to variation in the default mode and executive control networks (Petersen et al., 2014). Literature on how resting-state networks are affected by the female hormonal status is still limited, however (Engman et al., 2018).

Resting-state (rs) theta/beta ratio (TBR) is an electroencephalographic (EEG) marker suggested to represent cortical regulation over subcortical affective systems (Schutter & van Honk, 2005; Knyazev, 2007) and has repeatedly been found to be associated with executive control over threat-related responses in healthy individuals, cross-sectionally and with a one-week predictive interval (Putman et al., 2010; Angelidis et al., 2016). TBR predicted interpersonal differences in (mal)adaptive emotional information processing after exposure to stimuli with different threat levels (Putman et al., 2014; Angelidis et al., 2018; van Son et al., 2018ab) and results from dividing the slow-wave theta power (4-7 Hz) by the fast-wave beta power (13-30 Hz). Theta may stem from emotion-oriented and evolutionary old subcortical structures such as the amygdala and has been associated with goal directed behavior and motivational sensitivity (Knyazev, 2007;

Siegel & Sapru, 201; Massar et al., 2014; Cavanagh & Shackman, 2015). Beta may originate from more complex cognitively-oriented cortical structures (Knyazev & Slobodskaya, 2003) and is associated with higher-order cognitive processes (Singer, 1993; Hofman et al., 2013). Hence, cortical control over subcortical systems may be reduced if theta dominates over beta, resulting in an increased TBR (Schutter & van Honk, 2005; Putman et al., 2014), which in turn may impair down-regulation of negative emotions like anxiety (Tortella-Feliu et al., 2014) and relates to inhibited attentional control (Angelidis et al., 2016). Theta and beta power correlated negatively with progesterone levels (Becker et al, 1982) and differed between menstrual cycle phases (Creutzfeld et al., 1976; Solis-Ortiz et al., 1994). Accordingly, OC-use may influence TBR: an effect which, as far as we know, has not been investigated yet.

Female hormones also act upon the mineralocorticoid receptor (MR) (Handa & Weiser, 2014). MRs and glucocorticoid receptors (GRs) are crucial for the regulation of the hypothalamic-pituitary-adrenal (HPA) axis by mediating the actions of cortisol (Joels et al., 2008). MR synthesis in the brain is suppressed by estrogen (Carey et al., 1995). Progesterone is a competitive antagonist of the MR, because it binds to the MR with almost the same affinity as cortisol and aldosterone (Carey et al., 1995; Quinkler et al, 2002; Handa & Weiser, 2014). The MR is abundantly expressed in the hippocampus and amygdala and also in the PFC (Joels et al., 2008; De Kloet et al, 2005). Pharmacological and genetic studies have shown that the MR is involved in the appraisal of a stressful event, the regulation of the initial psychological stress reactions like vigilance, selective attention, selection of an appropriate coping style and encoding of emotional memory (De Loet et al, 2005; Cornelisse et al., 2011; Henckens et al., 2012).

Genetic variation in the MR was found to be associated with behavior, autonomic function and neuroendocrine responses to stress (DeRijk et al., 2006). The most widely studied MR haplotype is based on two common functional single nucleotide polymorphisms (SNP's): the rs5522 (A/G) is located in codon 180 of exon 2 and causes an amino acid change from isoleucine (ATT) to valine (GTT), and rs2070951 (G/C) located in the MR promoter region two nucleotides before the translation start site (DeRijk et al., 2006; van Leeuwen et al, 2010, 2011). Three common MR-haplotypes were identified: haplotype 1 (GA) frequency ~ 49 %, haplotype 2 (CA) frequency ~ 42%, and haplotype 3 (CG) frequency ~ 9% and a very rare haplotype 4 (GG) (DeRijk et al., 2006). *In vitro* in a cell line MR-haplotype 2 showed the highest transcriptional, translational and transactivational activity of the MR-gene variants (DeRijk et al., 2006, 2007; van Leeuwen et al., 2010, 2011; Kumsta et al., 2018). Female carriers of MR-haplotype 2 reported higher dispositional optimism, less rumination and fewer thoughts of hopelessness, and were also less vulnerable to depression than MR-haplotype 1/3 carriers and following childhood maltreatment (Klok et al., 2011; Vinkers et al., 2015; Hamstra

et al., 2017a). MR promotes the stress-induced shift from cognitive towards 'habitual' learning (Schwabe et al., 2010, 2013), particularly in MR-haplotype 2 carriers (Wirz et al., 2017).

We have previously reported that these MR-haplotypes moderate the influence of the menstrual cycle phase, OC- use, estrogen and progesterone on emotional information processing, as assessed with both behavioral measures and self-reports (Hamstra et al., 2015, 2016, 2017a, 2017b). Psychophysiological studies, however, show effects related to cortical arousal and may reveal subtle changes in information processing that may remain unnoticed in self-reported arousal or behavioral studies. In sum, the aim of the present study was to examine rs EEG TBR in healthy OC-users and naturally cycling women. We also investigated whether any effect of OC-use and levels on estrogen of progesterone was moderated by MR-haplotype. The same counterbalanced within-subject design was applied as in our earlier behavioral study (see Hamstra et al., 2017a).

Materials and methods

Participants

Data was collected from May 2016 till April 2017 at Leiden University. Participants were healthy, Dutch-speaking, right-handed female students of Northwestern European origin, aged between 18 and 35 years. Participants were recruited at the local university campus and through social media. All participants provided written informed consent and received €50 or course credits for participation. The study was approved by the Psychology Research Ethics Committee at Leiden University (approval number: CEP16-0318/139).

Inclusion and exclusion criteria

In this study we recruited a new sample of 106 women, of whom 60 were using oral contraceptives (OC) and 46 had a natural menstrual cycle (NC). OC-users used second generation monophasic OC containing Ethinylestradiol (EE; 0.03 mg)/ Levonorgestrel (LNG; 0.15 mg) and applied a pill-free week. Only NC women with a regular menstrual cycle between 25 and 35 days were included. All participants were in their current hormonal status for three months or longer. Participants were excluded in case of pregnancy, lactation, and use of abortifacients or morning-after pill, current physical or psychological illness. Additionally, participants were excluded when screened positive for psychiatric present and past by the MINI International Neuropsychiatric Interview (van Vliet & de Beurs, 2007) or premenstrual syndrome [as determined by the Menstrual Distress Questionnaire (MDQ; Moos, 1968). Lastly, participants were excluded in case of excessive alcohol use (> 14 units per week), smoking, regular soft- or hard-drug use, and use of prescribed medication.

Procedure

Design

This study had a within-subject counterbalanced design. OC-users and NC women were tested in a counterbalanced entry-order. OC-users were tested during active OC-use (day 8–14), and during the pill-free week (day 4–7). NC women were tested during the early follicular phase (day 2–6), when both estrogen and progesterone levels are low, and in the mid-luteal phase (3–10 days prior to onset of the new cycle), when progesterone levels are at its maximum and E2 reaches a second peak (Jones et al., 2012). Information on the onset of the next cycle – day 1 of the new pill-strip for OC-users, start of menses for NC participants – was used to confirm whether participants had been tested at the right moment.

Clinical characteristics

Vulnerability for depression was assessed with the revised version of the Leiden Index of Depression Sensitivity (LEIDS-R; (van der Does, 2002). Explicit affect was assessed with the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988). Implicit affect was assessed with the Implicit Positive and Negative Affect Test (IPANAT; Quirin et al., 2009). Personality traits were assessed with the NEO-Five Factor Inventory (NEO-FFI; McCrae & Costa, 1987).

Biological measures

Hormonal assessment

Estradiol (E2) and progesterone (P4) were assessed in saliva, collected at three time points during the experiment with at least a 30 min interval (see 2.5). In order to control for pregnancy, the estriol level in saliva was assessed as well. Participants were not allowed to eat or chew gum 30 minutes prior to participation nor to drink coffee before participation. After rinsing their mouth with water, participants directly expectorated 1ml of saliva into a sterile tube (SaliCap Sets; Innovation Beyond Limits, Hamburg, Germany). Samples were immediately stored and kept frozen at −20 °C until the day of assaying. The three samples were pooled and analyzed with highly sensitive luminescence assays of IBL at Ganzimmun Diagnostics AG. Reference values of free estradiol (E2) in saliva were: follicular phase 0.2–10.4 pg/ml; ovulation 5.8–21.2 pg/ml; luteal phase 0.8–10.8 pg/ml. For free progesterone (P4) in saliva: follicular phase 28–82 pg/ml; luteal phase 127–445 pg/ml; post-menopause and OC: 18–51 pg/ml.

Mineralocorticoid (MR)-haplotype

Analysis of the rs2070951 and rs5522 polymorphisms.

To determine the rs2070951 and rs5522 polymorphisms, PCR fragments were sequenced using the forward primers (5'-GTTCCYTAGATTCCAGCTCAG-3') respectively (5'-AGAGGAGTTCCCTGGGTGAT-3') and dye terminator chemistry (BigDye v3.1, Applied Biosystems). Sequence reactions were run on an ABI-3730 automated sequencer and sequence data were analyzed using SeqScape software (Applied Biosystems).

DNA isolation.

Buccal swabs/saliva from individuals were collected in lysisbuffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/ml proteinase K and 0.5% w/v SDS). Genomic DNA was isolated from the samples using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany).

PCR amplification.

The rs2070951 and rs5522 regions were amplified by PCR using the following primers: a forward primer (5′-GCTGGAAACAGAGCACCTTG-3′) and a reverse primer (5′-GCAAGCCACCCACTTCACTA-3′). Typical PCR reactions contained between 10–100 ng genomic DNA template, 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies) in a total volume of 30µl using the following cycling conditions: initial denaturation step of 4 min at 95C, followed by 40 cycles of 30 s 94°C, 30 s 50°C, 120 s 72°C and a final extension step of 10 min 72°C. After the first PCR 1ul of the amplification product was used directly in a second PCR amplification with nested primers. The following primers were used: a forward primer (5′ GGAGGSCTGGAAATTGAGGA-3′) and a reverse primer (5′-CGACAAGCTGTAGTCAATACTC-3′). The PCR reactions contained 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies) in a total volume of 30µl using the following cycling conditions: initial denaturation step of 4 min at 95°C, followed by 40 cycles of 30 sec 94°C, 30 sec 50°C, 120 sec 72°C and a final extension step of 10 min 72°C. According to the observed frequency in the population (DeRijk et al., 2006, 2008), MR-haplotype 1 (GA) is composed by MR-2 (G) and MR-I180 V (A), MR-haplotype 2 (CA) by MR-2 (C) and MR-I180 V (A), MR-haplotype 3 (CG) by MR-2 (C) and MR-I180 V (G) and the in vivo seldom observed MR-haplotype 4 (GG) by MR-2 (G) and MR-I180 V (G).

EEG measures

Recording and software

EEG was recorded from 15 Ag/AgCl scalp electrodes (F_3, F_Z, F_4, C_Z, CP_1, CP_Z, CP_2, P_3, P_1, P_Z, P_2, P_4, PO_3, PO_4, O_Z) placed in accordance with the International 10/20 System. Scalp electrodes were referenced online to CMS/DRL positions and mastoid electrodes were used as offline reference channels. Vertical electro-oculogram (EOG) was recorded from electrodes placed at the supraorbital and the infraorbital ridge of the right eye. Horizontal EOG was recorded from the outer canthi of the left and the right eye. EEG signal was pre-amplified at the electrode to improve the signal-to-noise ratio and amplified with a gain of 16x by a BioSemi ActiveTwo system (BioSemi B.V., Amsterdam).

Resting-state paradigm

RsEEG was recorded for eight minutes, in alternating one-minute trials of eyes open/eyes closed. During eyes open trials, participants were instructed to look at a fixation cross on the computer screen (see also Angelidis et al., 2016).

Procedure

Screening procedure

Interested participants completed an online questionnaire screening for in- and exclusion criteria. Subsequently participants were screened with the MINI by phone interview. Lastly, the average cycle duration of the NC participants was registered. This cycle onset information was used to confirm whether participants had been tested at the right moment.

First session

Participants were seated in a room adjacent to the experimenters' room. All participants read the study's information letter and provided written informed consent and were interviewed for inclusion and exclusion criteria. Consecutively mucus and saliva were collected, and the PANAS, LEIDS-R, NEO-FFI, and IPANAT were administered. After the self-report measures, EEG electrodes were applied by two experimenters. Following another saliva sample, participants underwent an eight-minute resting-state EEG recording. The first session ended after the collection of a third saliva sample.

Second session

Procedures of the second session were identical, except for the absence of mucus collection and assessment of the NEO-FFI and LEIDS-R. Furthermore, at the end of the session, the MDQ was administered and participants were debriefed on the study objectives. Participation ended after confirmation of the start of the new menstrual cycle or pill-strip.

EEG processing

Data processing was performed with Brain Vision Analyzer V2.0.4 (Brain Products GmbH, Germany). Data were sampled at 500 Hz. The data were high-pass filtered at 0.1-Hz, low-pass filtered at 100-Hz low-pass filter, and a 50-Hz notch filter was applied (as in Putman et al., 2014). Ocular correction was done automatically (Gratton & Coles, 1983). Segments containing remaining muscle movements, amplitudes above 200 μV [as in Putman et al., 2014 (25)], or other artifacts were rejected automatically. A fast Fourier transformation with a resolution of 0.25 Hz and a 10% Hamming window length was used to estimate the spectral power density (μV2/Hz) for the frontal (F3, Fz, F4) and parietal (P3, Pz, P4) electrodes in the theta (4-7 Hz) and beta (13-30 Hz) bands.

Following Putman et al. (2010, 2016), all eyes-open/eyes-closed segments were collapsed for the analysis. Frontal power density averages were obtained from frontal electrodes at F3, Fz, and F4 positions. Parietal power density averages were obtained from parietal electrodes at P3, Pz, and P4 positions. Frontal and parietal averages were calculated over both the theta and the beta power density. Non-normally distributed theta and beta power densities from frontal and parietal positions were log-normalized. Frontal and parietal TBR were calculated by dividing the log-normalized theta power density by the log-normalized beta power density (Angelidis et al., 2016).

Statistical analyses

Background variables

OC and NC groups, and MR-haplotype subgroups within the OC and NC groups, were characterized in terms of their average age, cognitive vulnerability to depression (LEIDS-R), personality traits (NEO-FFI), and explicit and implicit affect (PANAS; IPANAT). The distribution of MR-haplotypes over naturally cycling and OC groups was analyzed by chi-square tests.

Analyses of variance (ANOVA)

Outcomes on frontal and parietal TBR, theta, and beta power were analyzed with repeated measures (rm) ANOVAS, with session (1, 2) as a within-subject factor. Between-subject factors were hormonal status (OC, NC) and MR-haplotype (amount MR-haplotype 2 alleles: 0, 1, 2). Counterbalancing-order (AB, BA) was added as a covariate to all (rm) ANOVAS. Significant session effects were reported but not further interpreted (Hamstra et al., 2017a). Univariate outliers on EEG outcomes ($Z \leq -3$ or ≥ 3), influential cases (Cook's distance ≥ 1) and bivariate outliers (Mahalanobis distance significant at $p > .001$) were excluded from analyses (Tabachnik & Fidell, 2013). Effects on MR-haplotype ($p < .05$) were investigated with contrasts. Partial eta squared (η_p^2) and power are reported as estimates of effect size. Effects were tested on a two-tailed alpha of .05.

Moderated regression analyses

We applied the same approach as in our previous study (Hamstra et al., 2017a). We performed moderated regression analyses to explore the correlations between sex steroids (estradiol, progesterone), MR-haplotype on the data from the first and second sessions separately. Dependent variables were frontal and parietal EEG outcomes. Main predictor variables in the regression models were mean-centered sex steroid values (estrogen/ progesterone), MR-haplotype, and corresponding interaction-terms. For reasons of multicollinearity, estrogen (E2) and progesterone (P4) were investigated in separate analyses. Progesterone outcomes were log-transformed. Assumptions of multicollinearity were satisfied when Tolerance was >.01 and VIF < 10. Cases were identified as multivariate outliers and excluded from analyses if Mahalanobis distance was significant at $p > .001$, and if Cook's distance ≥ 1. Regression estimates were deemed statistically different at $p < .05$. Semi-partial correlations (*sr*) were reported as an indication of the unique contribution of the variable to the model (Aiken & West, 1991).

Results

Hormonal measures

Hormonal analyses from the first session failed of one NC MR-haplotype 2 heterozygous participant. Six participants exceeded laboratory reference values and sample mean by 3 *SD*s on progesterone (P4) or estrogen (E2). These participants were included in the remaining analyses, since their estriol levels did not indicate pregnancy. The outcomes were within the expected laboratory pattern (see table 1).

Table 1. Means (SE) hormonal levels per phase.

	E2 pg/ml	P4 pg/ml
Early follicular phase (EF)	3.0 (.3)	80.4 (11.2)
Inactive OC-use (IU)	2.1 (.2)	61.3 (7.2)
Mid-luteal phase (ML)	3.8 (.2)	201.8 (19.9)
Active OC-use (AU)	2.0 (.3)	56.6 (10.0)

Participant characteristics

A total of 455 women showed interest in the study and were screened for eligibility, of whom 107 signed informed consent. After inclusion, 12 participants were excluded due use of medication on prescription (n = 3), valerian (n = 1), soft drugs (n = 1), the abortion pill (n = 1), change of hormonal status after inclusion (n = 2), irregular menstrual cycle > 35 days (n = 1) and scheduling difficulties (n = 3). After participation, seven participants had to be excluded because they had not been tested in the intended cycle phase. Two participants (one OC and one NC participant) were excluded because genotyping failed. No participant was screened positive for premenstrual dysphoric disorder (Moos, 1968). The final sample consisted of 86 participants.

Table 2 displays general characteristics of the sample. Background information (NEO-FFI; PANAS; IPANAT; LEIDS-R) of the first session was lost from one participant due to technical problems. Participant's ages ranged from 18 to 26. Scores on cognitive vulnerability to depression (LEIDS-R) were within a non-depressed range (Solis et al., 2017). There were no statistically significant differences between groups in age, personality traits (NEO-FFI), explicit affect (PANAS), LEIDS-R scores and implicit affect (IPANAT).

Table 2. Participant general background information (n = 85)

	Hormonal status		**NC**			**OC**		
	NC	**OC**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**
N (% total sample)	43 (50)	43 (50)	16 (18.6)	20 (22.3)	7 (8.1)	17 (19.8)	19 (22.1)	7 (8.1)
Age	21.79 (0.29)	22.05 (0.28)	22.06 (0.54)	21.60 (0.44)	21.71 (0.52)	22.00 (0.50)	22.05 (0.40)	22.14(0.59)
NEO-FFI								
Agreeableness	33.95 (0.60)	34.51 (0.61)	34.69 (0.90)	33.95 (0.94)	32.29 (1.55)	33.65 (0.83)	34.89 (0.91)	35.57 (2.00)
Conscientiousness	40.40 (0.48)	40.77 (0.48)	41.19 (0.87)	40.05 (0.60)	39.57 (1.27)	40.18 (0.71)	40.74 (0.79)	42.29 (0.87)
Extraversion	43.31 (0.95)	44.33 (0.69)	44.19 (1.25)	42.68 (1.50)	43.00 (2.99)	44.59 (0.84)	44.00 (1.11)	44.57 (2.34)
Neuroticism	32.74 (1.03)	30.47(1.03)	32.69 (1.57)	33.47 (1.68)	30.86 (2.44)	30.47 (1.24)	30.68 (1.46)	29.86 (4.27)
Openness	33.55 (0.51)	34.09 (0.56)	34.31 (0.85)	32.58 (0.77)	34.43 (0.95)	33.88 (0.90)	34.58 (0.68)	33.29 (2.07)
LEIDS-R								
Total	39.4 (2.34)	38.09 (1.82)	39.69 (3.05)	38.00 (3.72)	42.57 (7.50)	37.29 (1.85)	38.37 (2.92)	39.29 (7.05)
PANAS								
PA S1	24.68 (0.80)	26.60 (.78)	25.06 (1.04)	24.22(1.17)	25.00 (2.81)	26.35 (1.16)	27.32(1.22)	25.29 (2.24)
PA S2	24.29 (0.95)	25.58 (1.04)	24.44 (1.29)	24.28 (1.70)	24.00 (2.07)	25.24 (1.83)	25.32 (1.39)	27.14 (3.01)
NA S1	12.93 (0.52)	12.30 (0.39)	14.31 (0.99)*	11.94 (0.53)	12.29 (1.34)	11.76 (0.63)*	12.89 (0.58)	12.00 (1.00)
NA S2	12.49 (0.34)	12.37(0.35)	13.31 (0.69)	12.11 (1.11)	11.57 (0.53)	11.88 (0.52)	12.74 (0.52)	12.57 (1.13)
IPANAT								
PA S1	47.33 (1.59)	46.35 (1.45)	46.25 (2.52)	45.95 (2.43)	53.57 (3.33)	48.41 (1.67)	45.68 (2.63)	43.14 (3.48)
PA S2	47.21 (1.56)	45.56 (1.62)	47.13 (2.30)	45.21 (2.45)	52.86 (4.00)	46.41 (2.44)	44.53 (2.81)	46.29 (3.06)
NA S1	133.29 (3.39)	134.33 (3.54)	129.50 (4.82)	132.84 (5.81)	143.14 (6.43)	137.12 (5.20)	131.79 (5.95)	134.43 (8.28)
NA S2	132.86 (4.10)	134.23 (3.43)	127.81 (6.82)	135.21 (6.26)	138.00 (9.52)	132.18 (4.75)	132.05 (5.32)	145.14 (10.21)

Notes: N (%) or means (SE). *p <0.05. Abbreviations: OC = oral contraceptive users; NC = naturally cycling women; MRHT 1/3 = mineralocorticoid receptor haplotype 1/3; MRHT 2 het = mineralocorticoid receptor haplotype 2 heterozygotes; MRHT 2 hom = mineralocorticoid receptor haplotype 2 homozygotes; NEO-FFI = NEO Five-Factor Inventory; LEIDS-R = Leiden Index of Depression Sensitivity; S1 = session 1; S2 = session 2; PANAS = Positive Affect Negative Affect Scale; IPANAT = Implicit Positive Affect Negative Affect Scale; PA = Positive Affect; NA = Negative Affect.

Theta beta ratio

Two NC participants had aberrant EEG signals (Z > 3) and were excluded from analyses on TBR scores. Eighty-four participants remained for frontal and parietal analyses. See for mean outcomes on TBR per session Table 3.

Repeated measures ANOVA

Repeated measures ANOVAs with log-normalized TBR as outcome, revealed no significant between-subject effects for hormonal status (OC, NC) on frontal [F (1, 77) = .016; p = .901; η_p^2 <.001; power = .05] or parietal sites [F (1, 77) = .304; p = .583; η_p^2 = .004; power = .09]. A main-effect of MR-haplotype on parietal TBR was observed, however [F (2, 77) = 4.03; p = .022; η_p^2 = .095; power = .70]. Contrast analyses confirmed lower parietal TBR scores in MR-haplotype 2 homozygotes than in MR-haplotype 2 heterozygotes (p = .006) and MR-haplotype 1/3 carriers (p = .052). Although not significant, the same pattern of results was observed on frontal sites [F (2, 77) = .829; p = .441; η_p^2 =.021; power = .19], see figure 1. There were no significant interaction effects between hormonal status and MR-haplotypes on frontal [F (2, 77) = .065; p = .937; η_p^2 =.002; power = .06] or parietal sites [F (2, 77) = .244; p = .784; η_p^2 =.006; power = .09].

Moderated regression analyses on estrogen and progesterone

Regression analyses on TBR scores revealed no significant main or interaction effects between estrogen, progesterone, and the MR-haplotypes (*srs* ranged between -.17 and .10; p > .122).

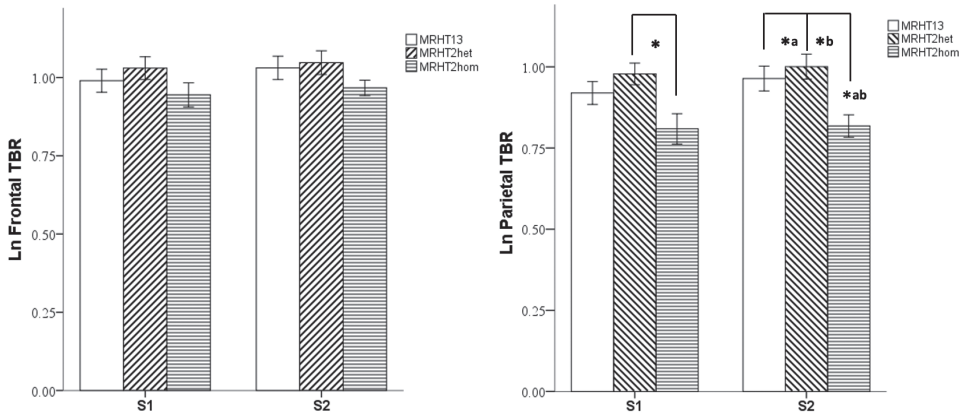

Figure 1. Log-normalized frontal (left graph) and parietal (right graph) TBR scores by MR-haplotype.
Abbreviations. Ln = log-normalized; MRHT = mineralocorticoid receptor haplotype; het = heterozygotes; hom = homozygotes; Error bars: +/- 1 SE.* p < .05.

Table 3. Means (SE) for frontal and parietal EEG data per session

	Hormonal status		**NC**			**OC**			**MRHT**		
	NC	**OC**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**
Frontal											
TBR S1	1.13 (0.09)	1.06 (0.09)	1.16 (0.16)	1.19 (015)	0.91 (0.13)	1.02 (0.13)	1.14 (0.17)	0.91 (0.13)	1.08 (0.10)	1.17 (0.11)	0.91 (0.09)
TBR S2	1.17 (0.10)	1.14 (0.10)	1.26 (0.17)	1.21 (0.16)	0.87 (0.07)	1.12 (0.14)	1.22 (0.17)	0.99 (0.09)	1.19 (0.11)	1.21 (0.11)	0.93 (0.06)
Parietal											
TBR S1	1.03 (0.08)	0.90 (0.06)	1.05 (0.16)	1.14 (0.12)	0.73 (0.13)	0.95 (0.11)	0.92 (0.08)	0.72 (0.10)	1.00 (0.09)	1.03 (0.07)*	0.73 (0.08)*
TBR S2	1.11 (0.10)	0.97 (0.07)	1.21 (0.20)	1.17 (0.14)	0.73 (0.09)	1.04 (0.10)	1.01 (0.11)	0.70 (.07)	1.12 (0.11)*[a]	1.09 (0.09)*[b]	0.71 (0.06)*[ab]

Notes: Means without univariate outliers (Z > 3). Abbreviations: OC = oral contraceptive users; NC = naturally cycling women; MRHT 1/3 = mineralocorticoid receptor haplotype 1/3; MRHT 2 het = mineralocorticoid receptor haplotype 2 heterozygotes; MRHT 2 hom = mineralocorticoid receptor haplotype 2 homozygotes ; TBR = theta/beta ratio. * $p < .05$.

Discussion

We investigated the impact of OC and menstrual cycle phase and the moderating effect of the MR-haplotypes on rsEEG theta/ beta ratio (TBR) in a counterbalanced within-subject design.

Contrary to our expectations, neither OC-use nor menstrual cycle phase were associated with TBR. Comparable resting state functional connectivity patterns in both OC-users and NC women were not only observed in earlier studies (DeBondt et al., 2013), but also in a recently published study (20) using a double-blind randomized placebo-controlled design, investigating the influence of OC (.03 EE; .15 LNG) on amygdala and salience network resting-state functional connectivity. In this study menstrual cycle phase (in the placebo condition) and (in)active OC-use influenced amygdala and salience resting-state networks comparably. Higher endogenous and exogenous hormone levels resulted in higher rs functional connectivity, although the effect of endogenous hormones was slightly more pronounced (Engman et al., 2018).

MR-haplotype 2 homozygotes had the lowest parietal TBR compared with MR-haplotype 2 heterozygotes and MR-haplotype 1/3 carriers. Low TBR is associated with better attentional control, resilience to stressful events (Putman et al., 2010; Tortella-Feliu, 2014; Angelidis et al., 2016) and a reduced tendency to worry (van Son et al., 2018c). Worry is sometimes referred to as a 'negative form' of mind wandering and can be seen as self-generated off-task thought (Ottaviani et al., 2015). Improved cognitive control over the affective system results in a more adaptive appraisal of stressors, decreasing the odds on developing a major depressive disorder and other stress-related psychopathology (Joormann & Stanton, 2016; Mogg & Bradley, 2016). This is in line with reports of lower vulnerability to major depressive disorder, less ruminative cognitions and higher explicit optimism scores in MR-haplotype 2 carriers in a cohort study (Klok et al., 2011) and higher implicit happiness scores in MR-haplotype 2 homozygotes in an experimental study (Hamstra et al., 2017a). The finding of lower self-reported chronic stress in MR-haplotype 2 homozygotes (van Leeuwen et al., 2011) and resilience to childhood trauma (Vinkers et al., 2015) supports the hypothesis of an improved coping ability with stressors in this group as well.

Interpretations on the clinical and cognitive implications of differences in TBR may diverge. Lower TBR in MR-haplotype 2 homozygotes might represent a lower baseline reactivity to affective stimuli (theta) and an improved ability to down-regulate amygdala reactivity (beta), thus a more 'cognitive' way of coping with potential stressors. Consistently, in a study of Wirz et al. (Wirz et al., 2017) MR-haplotype 2 carriers showed in two experiments less hippocampal activity and reduced amygdala connectivity with the parahippocampal cortex after stress. Only in MR-haplotype 2 carriers stress led to a more pronounced shift from hippocampal to dorsal striatal learning, and was followed by distinctive alterations in memory networks, reflecting a stress-induced shift toward habit memory.

Additionally, MR-haplotype 2 carriers had reduced amplitudes on ERP components associated with early attentional processing, irrespective of stress. Wirz et al (2017) treated homo- and heterozygous carriers of MR-haplotype 2 as one group, however. Our study revealed that all MR-haplotypes constitute distinct phenotypes on TBR, with significant lower base-line TBR in MR-haplotype 2 homozygotes. Earlier studies, which found lower self-reported chronic stress and higher implicit happiness only in MR-haplotype 2 homozygotes, but not in MR-haplotype 2 heterozygotes (van Leeuwen et al., 2011; Hamstra et al., 2017a), agree on this idea. Future studies may benefit from treating MR-haplotype 1/3, MR-haplotype 2 heterozygotes and homozygotes as separate groups or adding the amount of MR-haplotype 2 alleles (0, 1, 2) as a covariate to the model.

Our research has a number of potential limitations. We did not exclude NC participants for prior OC-use. Although TBR was previously related to performance on an emotion regulation task (e.g., Tortella-Feliu et al., 2014) we did not assess self-reported or behavioral emotion regulation abilities in our sample. Furthermore, we have no direct neuroimaging evidence of the assumption that TBR reflects prefrontal control over subcortical areas. This should be investigated with methods with a higher spatial resolution than EEG, such as magnetoencephalography, or supplement rs EEG with rs fMRI. Lastly, our sample was small for a genetic association study. Our sample size was too small to detect if differences on TBR, theta and beta power between MR-haplotype 2 hetero- and homozygotes were driven by the G-allele. This would have been of scientific interest, because MR-haplotype 2 homozygotes lack the G-allele from the rs5522 (A/G) and rs2070951 (C/G) SNPs. Carriers of G-allele from the rs5522 SNP displayed higher stress-reactivity (Kuningas et al., 2007; Van Leeuwen et al., 2011), and increased threat-related amygdala reactivity (Joels et al., 2007; Bogdan et al., 2010).

The strengths of our study include the fact that we assessed TBR in a counterbalanced, mixed between-subject (OC/NC) design. We observed subtle physiological effects in emotional processing which remained unnoticed in self-reports on affective state and personality, as the MR-haplotypes did not differ in scores on neuroticism, depressive cognitions, implicit anxiety or negative affect. This is the first report on TBR in a homogenous sample of OC-users during (in) active use and NC women on personalized time-points in their menstrual cycle. Additionally, we verified hormonal status with the saliva concentration of estrogen and progesterone. To conclude, our work suggests that MR-haplotype 2 homozygotes had lower TBR than the other haplotype groups, possibly reflecting an improved regulatory cortical activity over the affective system. MR-haplotype 2 homozygotes may therefore be better in regulating their emotions, allowing them to cope better with stress and negative events.

Conclusion

Neither OC-use nor menstrual cycle phase were associated with rsEEG theta/ beta ratio (TBR) in healthy premenopausal women. Lower parietal TBR in MR-haplotype 2 homozygotes may reflect improved PFC-mediated effortful control and resilience to stressful events. Genetic variation associated with enhanced MR expression may modulate regulation of arousal in females.