

40 Hz auditory steady-state response in females: When is it better to entrain?

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Auditory steady-state responses (ASSRs) are widely applied to test brain's ability to follow external stimulation in neuropsychiatric disorders. It is known that ASSRs are related to GABAergic transmission. Female sex steroid hormones – both estrogens and progesterone – affect functioning of GABAergic system. However, it is not known how these hormones affect brain's ability to entrain. This study was designed to test the ability to synchronize to 40 Hz stimulation during different phases of the menstrual cycle. Twenty-eight healthy females participated in the research during one of the menstrual cycle phases: (1) early follicular; (2) late follicular; (3) and mid-luteal. Auditory 40 Hz trains of 500 ms were delivered binaurally and EEG was recorded. Time-frequency analysis of the data was performed and phase-locking index, evoked amplitude and total intensity measures were extracted and decomposed by non-negative multi-way factorization. Additionally, alpha power of the baseline period was calculated. Parameters of ASSR were increasing in a linear manner with increasing levels of 17 β -estradiol and largest estimates of ASSR parameters were obtained in the late follicular phase, smallest – in the mid-luteal phase. Alpha power values were highest in the late follicular phase and lowest in the mid-luteal phase, pointing to lower arousal level in the late follicular phase. We speculate that increased 40 Hz ASSRs during mid-cycle might be related to the level of general arousal and specific GABA-mediated changes during the menstrual cycle. The results suggest that the ability to entrain to 40 Hz stimulation depends on the phase of menstrual cycle. This should be taken into account, particularly when ASSRs are used in clinical practice, comparing patients and healthy subjects.

Key words: auditory steady-state response, ASSR, menstrual cycle, estrogen, time-frequency analysis

The auditory steady-state response (ASSR) is observed when stimuli are presented periodically resulting in electroencephalographic entrainment (Picton et al. 2003), i.e. the frequency of the ASSR is close to the frequency of stimulation and the greatest magnitude is observed when stimuli are presented at 40 Hz (Galambos et al. 1981). Since its discovery in 1981, ASSRs have been employed by physiologists, psychologists, and physicians along with transient event-related potentials (ERPs); however, both types of EEG responses serve different functions. ASSRs are used for testing hearing sensitivity (Picton et al. 2003), as a marker of the state of consciousness during anesthesia (Picton et al. 2003) and as an index of the ability

for gamma band frequency generation in local cortical networks in neuropsychiatric disorders (van Deursen et al. 2011, Oda et al. 2012, Griskova-Bulanova et al. 2013a).

It has been suggested that when examining healthy brain development and aging or when investigating possible biological mechanisms of 'brain connectivity' diseases, such as depression, ADHD, autism and schizophrenia, the contribution of sex steroids should not be ignored (Peper et al. 2011). In general, estradiol induces excitatory actions while progesterone induces inhibitory actions on the CNS (Carta et al. 2012). Nevertheless, there is no data existing on the influence of female sex steroid hormones on the ability to synchronize to external stimulation. However, synchronization *per se* is hypothesized to be propagated through networks in a cycle of GABA-A-mediated inhibition (Gonzalez-Burgos and Lewis 2008), and both estro-

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gens and progesterone are known to affect functioning of GABAergic system: estrogens are reported to reduce GABAergic transmission (Huang and Woolley 2012), while progesterone has the contrary effect (Carta et al. 2012). This study aimed at identification of effects, produced by changing levels of female sex steroid hormones on phase-locked and non phase-locked measures of 40 Hz ASSRs.

Thirty female subjects (mean age 20.68, SD 0.63) participated in the study. The study was approved by the Lithuanian Bioethics Committee and all participants gave their written informed consent. Only healthy, non pregnant, not using hormonal contraceptives, experiencing regular menstrual cycle (mean duration 28.59, SD 2.13) and showing normal binaural auditory thresholds females were included. Subjects were randomly asked to participate in the research during one of the phases: (1) early follicular (low 17β -estradiol, low progesterone); (2) late follicular (high 17β -estradiol, low progesterone); (3) and mid-luteal (slightly elevated 17β -estradiol, high progesterone). The time window for each phase was determined individually on the basis of the duration of subject's previous three months cycle. It is known that the second part of menstrual cycle (from the ovulation to the onset of next menses) does not show high inter-subject variability and lasts about 14 days (Mumford et al. 2012). Thus, the preliminary date of ovulation was determined (cycle duration minus 14 days) and menstrual cycle was divided into two parts – follicular and luteal. The beginning of the follicular part was considered as early follicular phase; last two or three days before the predicted ovulation were considered as late follicular phase; six to eight days after the predicted ovulation were considered as mid-luteal phase. The levels of salivary 17β -estradiol and progesterone were measured to validate phases retrospectively.

Due to technical reasons, EEG data of 2 subjects could not be used and the final sample consisted of 9 females in their early follicular phase, 9 females in their late follicular phase and 10 females in their mid-luteal phase.

Stimuli were 500 ms trains, consisting of 20 identical clicks (1.5 ms burst of white noise), interspersed with 20 Hz stimuli (data not reported here), delivered binaurally through headphones (peak SPL of 60 dB). The 40 Hz trains were presented sixty times in a pseudo-random order with an inter-train interval of 1–1.5 s. The whole stimulation run lasted about 4 min-

utes. Participants were instructed to let their thoughts wander during the presentation of auditory stimuli and to fix their gaze at a fixation cross approximately 1.5 m in front of them. To minimize the effect of diurnal variations, all experiments were performed between 10:00 and 12:00 AM (Griskova-Bulanova et al. 2013b).

Salivary sex steroid (17β -estradiol and progesterone) levels were assayed to validate self-reported cycle phases, to compare hormone levels between groups and to evaluate the effect on 40 Hz ASSRs. Salivary sampling is a non-invasive, simple, stress-free procedure approved as a useful method for the assessment of ovarian function (Lu et al. 1999, Lienen et al. 2010, Gatti and De Palo 2011).

Participants were asked not to eat, drink, chew gum or brush their teeth for 30 min before sampling, but to rinse their mouth with cold water 5 min prior to sample collection. To avoid blood contamination, samples were not collected when oral disease, inflammation or lesions were present. A minimum of 1 ml of saliva was collected into special tubes (IBL SaliCap). Tubes were stored at -24°C until assayed. The concentrations of free 17β -estradiol and free progesterone in saliva were determined by enzyme immunoassay for *in vitro* diagnostic quantitative determination in human saliva (IBL-International). The analytical sensitivity of the 17β -estradiol assay was 0.4 pg/ml and of the progesterone assay – 3.8 pg/ml. All samples were duplicated in the same assay.

The EEG was recorded with an EEG device (ANTneuro, The Netherlands) from F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 sites (10/20 International system) using Ag/AgCl electrodes. Averaged mastoid electrodes served as a reference; the ground electrode was attached close to Fz. The impedance was kept below 5 k Ω . Data was digitized at 512 Hz.

Off-line processing was performed in ERPWAVE-LAB and EEGLAB for MatLab® (Delorme and Makeig 2004, Mørup et al. 2007) in a manner described in detail by Griskova-Bulanova and coauthors (2011). After wavelet transformation (frequencies represented from 10 to 80 Hz, 1 Hz intervals between each frequency), evoked potential measure (evoked amplitude, corresponding to phase-synchronized wavelet-transformed amplitude measure) and phase-locking index [PLI, phase locking factor of the evoked oscillations from trial to trial ranging from 0 (random phase) to 1 (nearly identical phase)] were analyzed (Mørup et al. 2006). Additionally, the average amplitude of the

Table I

| | | Phase-locking index | Evoked amplitude | Total intensity |
|-----------------------|----------------|---------------------|------------------|-----------------|
| Early follicular | Mean | 0.52 | 0.82 | 0.57 |
| | SD | 0.13 | 0.21 | 0.08 |
| Late follicular | Mean | 0.57 | 0.94 | 0.60 |
| | SD | 0.13 | 0.25 | 0.11 |
| Mid-luteal | Mean | 0.42 | 0.67 | 0.51 |
| | SD | 0.07 | 0.12 | 0.07 |
| 17 β -estradiol | R ² | 0.16* | 0.24* | 0.23* |
| Progesterone | R ² | 0.02 | 0.04 | 0.03 |

R² values for 17 β -estradiol and progesterone concentrations and phase-locking index, evoked amplitude and total intensity, significant values are marked with asterisk (*)

oscillation (both non phase-locked and phase-locked) was investigated to obtain a measure of the total intensity increase induced by the stimuli.

The measures were decomposed through non-negative multi-way factorization (NMWF) (Mørup et al. 2006, 2007), indicating how the parameters vary with experimental manipulation by giving the subject-specific strength to the activity that is most common across subjects, conditions and runs (Morup et al. 2006, 2007). This has proven useful in the analysis of event-related potentials (Arnfred et al. 2008, Griskova-Bulanova et al. 2012) and in the analysis of ASSRs (Griskova-Bulanova et al. 2011, 2013a, b). The window for mathematical decomposition of ASSRs was set as 30–46 Hz and 0 to +500 ms.

For the evaluation of arousal level, baseline EEG alpha (8–12 Hz) power was monitored. The power measures (Fast Fourier transformation) were obtained from 40 epochs of 5 s interstimulus intervals of a simple auditory stimulation from the same experimental session (not reported here) of each subject (data was available from 8 subjects in early follicular phase, 9 subjects in late follicular phase and 9 subjects in mid-luteal phase).

PLI, evoked amplitude and total intensity values, 17 β -estradiol and progesterone concentrations from all

subjects were subjected to univariate analysis of variance (ANOVA) with factor “phase” (early follicular, late follicular and mid-luteal) investigated. Separate ANOVA was performed for alpha power measures with factors “phase” (early follicular, late follicular and mid-luteal) and “electrode” (F3, Fz, F4, C3, Cz, C4, P3, Pz and P4); Bonferroni corrected *P* values are presented. Post hoc analyzes were performed using Least Significant Difference (LSD) test. In order to evaluate the relationship of PLI, evoked amplitude and total intensity with steroid hormone concentrations and alpha power, regression analysis was performed.

ASSRs were detected for all subjects. In line with previous studies, the non-negative multi-way factorization decomposition of selected measures of ASSRs resulted in the observation of a single component, maximal over Cz at about 300 ms and 38–40 Hz following 40 Hz stimulation (40 Hz ASSR) (Griskova-Bulanova et al. 2013a, b). As indicated by univariate ANOVA, 17 β -estradiol concentrations changed with menstrual cycle phase ($F_{2,27}=7.45$, $P=0.003$, observed power 0.912). As expected, significant increase of 17 β -estradiol concentration was obtained during the late follicular phase (mid-cycle, mean 8.89, SD 3.29), slight increase was observed during the mid-luteal phase (mean 5.70, SD 1.72; lower in comparison to late folli-

cular phase, $P=0.013$) and the lowest level of 17β -estradiol concentration was observed during the early follicular phase (mean 4.18, SD 1.13; lower in comparison to late follicular, $P=0.005$). Effect of menstrual cycle phase was also significant for progesterone concentration ($F_{2,27}=4.97$, $P=0.015$, observed power 0.760). In accordance with previous observations, progesterone concentrations during mid-luteal phase (mean 205.1, SD 134.7) was significantly higher ($P=0.013$) as compared to early follicular phase (mean 76.5, SD 15.1) and did not differ from the late follicular phase (mean 134.4, SD 64.7) (Celec et al. 2009, Griksiene and Rukšenas 2011, Mumford et al. 2012).

Univariate ANOVA revealed the significant effect of menstrual cycle phase on phase-locking index values ($F_{2,25}=4.705$, $P=0.018$, observed power 0.736) and on evoked amplitude measures ($F_{2,25}=4.547$, $P=0.021$, observed power 0.720). The PLI and evoked amplitude of the ASSR (maximal at Cz) were highest during the late follicular phase, intermediate during the early follicular phase and lowest during the mid-luteal phase (PLI: larger in late follicular as compared to mid-luteal, $P=0.006$; evoked amplitude: larger in late follicular as compared to mid-luteal, $P=0.007$). A trend towards significant effect of menstrual cycle phase was seen for the total intensity of 40 Hz ASSRs

($F_{2,25}=2.634$, $P=0.092$, observed power 0.475). Means and standard deviations of the measures are presented in Table I. Head plot of phase-locking index collapsed across subjects (A) for the 40 Hz ASSR, time-frequency plots as a weighted collapse across subjects and electrodes for the 40 Hz ASSR (B) and means and standard deviations of NMWF scores of phase-locking index for early follicular, late follicular and mid-luteal phases (C) are presented in Figure 1.

Univariate ANOVA resulted in significant effect of menstrual cycle phase on alpha power measures ($F_{2,207}=33.412$, $P<0.001$, observed power 1.000), indicating largest alpha power in late follicular (22.73, SD 18.35) as compared to early follicular phase (11.58, SD 7.43, $P=0.024$) and mid-luteal phase (9.71, SD 7.56, $P<0.001$). Significant effect of “electrode” for alpha power estimates emerged ($F_{8,207}=7.007$, $P<0.001$, observed power 1.000), showing larger power values over parietal electrodes. Significant interaction of the factors was detected ($F_{26,207}=2.056$, $P=0.011$, observed power 0.967), indicating largest alpha power values in late follicular phase over the parietal electrodes ($P<0.05$).

Curve-fit analyses revealed significant linear increase of all the measures with increasing 17β -estradiol levels: $R^2=0.158$, $F_{1,27}=4.894$, $P=0.036$ for PLI

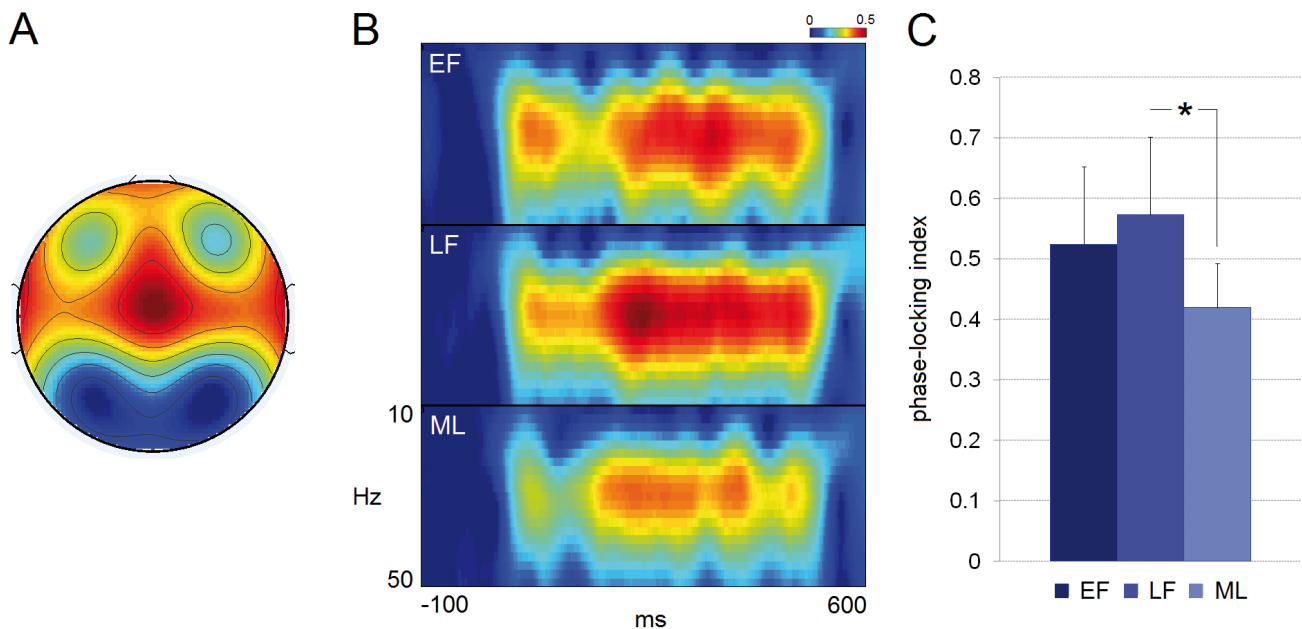


Fig. 1. (A) Head plot of phase-locking index collapsed across subjects for the 40 Hz ASSR. (B) Time-frequency plots as a group collapse across subjects and electrodes for the 40 Hz ASSR in early follicular EF, late follicular LF and mid-luteal ML phase. (C) Means and standard deviations of NMWF scores of phase-locking index for the 40 Hz ASSR in early follicular, late follicular and mid-luteal phases. * $P<0.05$.

(Fig. 2A); $R^2=0.188$, $F_{1,27}=6.021$, $P=0.021$ for evoked amplitude and $R^2=0.169$, $F_{1,27}=5.271$, $P=0.03$ for the total intensity). No relationship of any measures to progesterone concentrations was observed. A significant relationship, that was best described by logarithmic regression was detected between average alpha power and phase-locking index ($R^2=0.290$, $F_{1,25}=9.817$, $P=0.005$, Fig. 2B) and between alpha power and evoked amplitude ($R^2=0.261$, $F_{1,25}=8.480$, $P=0.008$). Interestingly, no significant relationship between 17β -estradiol levels and alpha power was observed.

The main findings of the current paper are diminished phase-locked activity in response to 40 Hz auditory stimulation in mid-luteal phase as compared to late follicular phase and significant positive correlation between 17β -estradiol levels and parameters of ASSR.

ASSR is an index of brain's ability to entrain, or to "follow" the stimulation. Functionally, synchronous oscillations, and particularly ASSRs in the gamma frequency range, were related to GABAergic transmission (Whittington et al. 2000, Lewis et al. 2008, Gonzalez-Burgos et al. 2011).

Importantly, both estrogens and progesterone are known to affect functioning of GABAergic system: estrogens are reported to reduce GABAergic transmission (Huang and Woolley 2012), while progesterone has the contrary effect (Carta et al. 2012). However, existing data points to the fact that hormonal-GABAergic effect might be more complex (Epperson et al. 2002). Taken abovementioned into account, reduction

of 40 Hz ASSR in mid-luteal phase, as observed in the current study, could be related to diminished levels of GABA.

Alternatively, our finding of diminished ASSRs in mid-luteal phase is in line with Walpurger and coworkers (2004): they showed that during luteal phase, the involuntary cortical arousal responses to incoming stimuli were reduced (Walpurger et al. 2004). Along with diminished parameters of 40 Hz ASSR, we found lowered alpha power estimates in mid-luteal phase in our subjects. Alpha power is considered a measure of arousal – increased alpha power indicates decreased arousal (Barry et al. 2007, 2011). We have shown previously that 40 Hz ASSRs were larger in low arousal/activation level conditions as compared to high activation/arousal level conditions (Griskova et al. 2007). This is in agreement with our current finding of higher ASSRs during late follicular phase when the largest alpha power values are obtained, and lower ASSRs in mid-luteal phase, when lowest alpha power values are measured. However, findings of alpha power over the menstrual cycle in the literature are very inconsistent. Vasil'eva (2005) showed decrease in alpha power during ovulatory phase as compared to follicular phase (Vasil'eva 2005). Krug and colleagues (1999) reported increased alpha power during ovulation and menses and decreased during the luteal phase (Krug et al. 1999). Solis-Ortiz and others (1994) found increased alpha1 (8–10 Hz) power during premenstrual phase and increased alpha2 (10–12 Hz) power during the menstrual phase (Solís-Ortiz et al. 1994). Thus, the

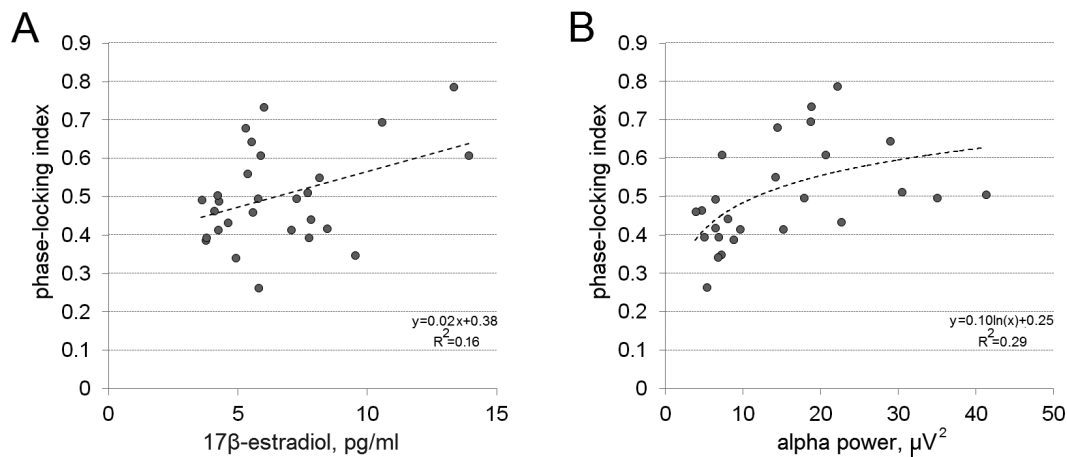


Fig. 2. (A) Scatter plot of 40Hz ASSR phase-locking index values *versus* 17β -estradiol concentrations. Linear regression significant at $P=0.036$. (B) Scatter plot of 40 Hz ASSR phase-locking index values *versus* alpha power averaged values. Logarithmic regression significant at $P=0.005$

question of arousal level during the menstrual cycle and its' effects on brain responses deserve further investigations.

The effect of steroid hormones on hearing function is a matter of debate with no firm conclusion. Some data exists that very small increase of hearing sensitivity occurs around the time of ovulation (Al-Mana et al. 2010). However, other authors did not find this effect (Yellin and Stillman 1999, Arruda and Silva 2008, Gurbuzler et al. 2012). We cannot exclude that results of the current study were in part caused by slight changes in hearing sensitivity of our subjects. Nonetheless, it has been shown that estrogen changes the overall level of activation, rather than modulating a specific activation pattern related to a specific function (Dietrich et al. 2001). Correspondingly, we have shown that both phase-locked and non phase-locked parameters of 40 Hz ASSR are related to the salivary 17β -estradiol but not progesterone concentration. The largest ASSRs, obtained during the high 17β -estradiol concentration in the late follicular period, are in accordance with Tillman (2010), who used different auditory task and showed a global increase in amplitude of ERPs during the high-estrogen phase. Tillman (2010) related this observation to the findings of fMRI (Dietrich et al. 2001) and PET (Berman et al. 1997), suggesting the global signal increase during cognitive tasks being associated with higher estrogen levels.

In conclusion, we speculate that increased 40 Hz ASSRs during mid-cycle might be related to the level of general arousal and specific GABA-mediated changes during the menstrual cycle. The complexity of the factors modulating the 40 Hz ASSR is not entirely solved; nevertheless, the current results suggest that the ability to synchronize to high frequency external stimulation depends on the phase of menstrual cycle. This should be taken into account, particularly when ASSRs are used in clinical practice, comparing patients and healthy subjects.

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