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Sex hormones affect language lateralisation but not cognitive control in normally cycling women

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Abstract

Natural fluctuations of sex hormones during the menstrual cycle have been shown to modulate language lateralisation. Using the dichotic listening (DL) paradigm, a well-established measurement of language lateralisation, several studies revealed that the left hemispheric language dominance was stronger when levels of estradiol were high. A recent study (Hjelmervik et al., 2012) showed, however, that high levels of follicular estradiol increased lateralisation only in a condition that required participants to cognitively control (top-down) the stimulus-driven (bottom-up) response. This finding suggested that sex hormones modulate lateralisation only if cognitive control demands are high. The present study investigated language lateralisation in 73 normally cycling women under three attention conditions that differed in cognitive control demands. Saliva estradiol and progesterone levels were determined by luminescence immunoassays. Women were allocated to a high or low estradiol group. The results showed a reduced language lateralisation when estradiol and progesterone levels were high. The effect was independent of the attention condition indicating that estradiol marginally affected cognitive control. The findings might suggest that high levels of estradiol especially reduce the stimulus-driven (bottom-up) aspect of lateralisation rather than top-down cognitive control.

Keywords: Estradiol, progesterone, language lateralisation, dichotic listening, cognitive control

Introduction

Natural fluctuations of sex hormones, such as those that occur during the menstrual cycle, have been shown to exert a modulating effect on functional brain organisation. Cerebral lateralisation refers to the differential involvement of the left or the right hemispheres in specific cognitive process such as language, spatial abilities or face discrimination (Hellige, 1993). Although it is well-known that the left hemisphere is dominant for most language processes (Broca, 1861; Kimura, 1967), it has previously been suggested that sex differences exist with respect to the degree of language lateralisation, with males demonstrating a greater language lateralisation for specific tasks compared to women (e.g. Jaeger et al., 1998; Shaywitz et al., 1995); although results are inconsistent (e.g. Sommer et al., 2004, Voyer, 2011).

One reason for this inconsistency is that while language lateralisation is comparatively stable in men, it fluctuates within relatively short time periods across the menstrual cycle in women (Hampson, 1990a, 1990b; Rode et al., 1995; Weis et al., 2008). Indeed, a number of neuropsychological studies, across different modalities and cognitive processes (both verbal and non-verbal), have demonstrated reduced lateralisation during cycle phases associated with high levels of estradiol (i.e. follicular phase, Holländer et al., 2005; Weis et al., 2008) or high levels of both estradiol and progesterone (i.e. luteal phase, Hausmann, 2005; Hausmann et al., 2002; Hausmann and Güntürkün, 2000; Rode et al., 1995), and greater lateralisation (similar to men) during the low-hormone menstrual phase. Thus, the presence of a sex difference in lateralisation may partly be dependent on women's hormonal status at time of testing (see Hausmann and Bayer, 2010, for a review).

Regarding the mechanisms underlying this effect, it has been suggested that rather than selectively influencing activity in a particular hemisphere, lateralisation is influenced via

modulation of a central mechanism affecting both hemispheres. Specifically, it was proposed that lateralisation arises from interhemispheric inhibition of the non-dominant hemisphere by the dominant hemisphere (Chiarello and Maxfield, 1996; Cook, 1984). Furthermore, it has been suggested that it is especially progesterone (and its metabolites) that reduces corticocortical transmission via glutamatergic and GABAergic effects, resulting in reduced interhemispheric inhibition, and consequently reduced lateralisation (Hausmann and Güntürkün, 2000). More recent studies suggest that it is estradiol and progesterone (e.g. Hausmann et al., 2006) or estradiol alone (e.g. Hausmann, 2005; Holländer et al., 2005; Weis et al., 2008) that modulates the interhemispheric interaction between the left and right hemispheres (Hausmann et al., 2013; see Hausmann and Bayer, 2010 for a review).

Results from cycle-related studies of language lateralisation that used the dichotic listening (DL) paradigm are particularly inconsistent. The DL task is a well-established tool to investigate language lateralisation. It involves the presentation of two auditory stimuli, usually monosyllabic words (e.g. Alexander et al., 2002; Hampson, 1990a, 1990b) or consonant-vowel syllables (e.g. Cowell et al., 2011; Wadnerkar et al., 2008; Sanders and Wenmoth, 1998). One stimulus is presented to the left and the other is presented simultaneously to the right ear. Participants are required to verbally report the syllable/word they heard the most clearly. In healthy right-handed adults, this task typically reveals a bias towards stimuli presented to the right ear, indicative of left-hemispheric language lateralisation. It has recently been suggested that while there is a significant sex difference in the DL bias (i.e. males more lateralised than females), this effect is small (Hirnstein et al., 2014; Voyer, 2011). The right ear advantage (REA) results from several factors relating to the anatomy of auditory projections from the ear to the primary auditory cortex (Kimura, 1967). Firstly, although auditory information is relayed to both hemispheres via subcortical projections, contralateral projections are stronger than ipsilateral ones. Consequently, stimuli

presented to right ear have direct access to the language-dominant left hemisphere. In contrast, stimuli presented to the left ear are projected to the right hemisphere and have to be transferred, via the corpus callosum, for processing. Finally, under dichotic conditions (i.e. simultaneous stimulus presentation), the ipsilateral projections are suppressed in favour of processing contralateral stimuli (Hugdahl, 2003; Kimura, 1967; Pollmann et al., 2002, for a review see Westerhausen and Hugdahl, 2008).

In contrast to studies using visual paradigms, the majority of DL studies looking at menstrual cycle effects reported increased language lateralisation when levels of estradiol and/or progesterone are high (Cowell et al., 2011; Hampson, 1990a, 1990b; Sanders and Wenmoth, 1998; Wadnerkar et al., 2008). However, there are also DL studies showing the opposite, a decreased REA during the luteal phase ("premenstrual week", Alexander et al., 2002; Altemus et al., 1989; midluteal phase, Mead & Hampson, 1996). Two recent DL studies did not find that the menstrual cycle affected language lateralisation (the non-forced condition in Hjelmervik et al., 2012; Can et al., 2012). These inconsistent finding of menstrual cycle effects in dichotic listening studies are summarized in Table 1.

Table 1: Studies of dichotic listening (DL) and menstrual cycle phase illustrating different methods and findings (adapted from Hausmann & Bayer, 2010).

Study	Number of participants	Mean age	Handedness	Cycle phases (cycle days)	Definition of phase	DL paradigm(s)	Main results
Alexander et al. (2002)	30	32.03 (SD = 8.9)	Right	Menstrual (1 - 7) Follicular (8 - 14) Midcycle (15 - 21) Premenstrual (22 - 28)	Day count	Verbal (rhyming nonsense syllables, rhyming monosyllabic words, negative words, positive words, neutral words).	Reduced REA during premenstrual phase compared to follicular phase across all tasks.
Altemus et al. (1989)	39	30 (range: 18-45)	Right	Follicular (6 - 12) Premenstrual (21 - 28)	Day count	Verbal (rhyming nonsense syllables, rhyming monosyllabic words, negative words, positive words, neutral words).	Reduced REA during premenstrual phase across all tasks.
Can et al. (2012)	32	25.23 (SD = 4.57)	Right	Menstrual (2 - 5) Follicular (8-11) Luteal (20-22)	Saliva assays (E, P)	Verbal (consonant-vowel).	No cycle effects.
Cowell et al. (2011)	21	25.24 (SD = 0.74)	Right	Menstrual (2 – 5) Periovulatory (8 – 11) Luteal (18 – 25)	Blood assays (E, P, LH, FSH)	Verbal (consonant-vowel).	Reduced REA during the menstrual phase.
Hampson (1990a)	45	23.7 (range: 19-39)	41 right-handed 4 non-right handed	Menstrual (3 – 5) Midluteal (18 -23)	Day count	Verbal (monosyllabic words)	Reduced REA during menstrual phase (only trend).
Hampson (1990b)	50	26.4 (range: 20-43)	43 right handed 7 non-right handed	Menstrual (3 – 5) Follicular (12 – 13)	Blood assays (E, P, LH)	Verbal (monosyllabic words)	Reduced REA during the menstrual phase
Hjelmervik et al. (2012)	15	23.47 (SD = 5.11)	Right	Menstrual (2 - 4) Follicular (8 – 12) Luteal (20 – 22)	Saliva assays (E, P)	Verbal (consonant-vowel, three forced-attention conditions).	Increased LEA during the follicular phase (forced-left condition only).
Mead & Hampson (1996)	36	23.7 (range: 20–36)	Right	Menstrual (3 – 5) Midluteal (18 – 23)	Saliva assays (E)	Verbal (emotional prosody, linguistic).	Linguistic: Reduced REA during midluteal phase (only session 1). Emotional prosody: Reduced LEA during menstrual phase.
Sanders & Wenmoth (1998)	32	24 (range: 18-37)	Right	Menstrual (3 – 5) Midluteal (20 - 22)	Day count	Verbal (consonant-vowel) Music (chord recognition)	Verbal: Reduced REA during menstrual phase. Music: Reduced LEA during midluteal phase.
Tillman (2010)	23	Mean not reported (range: 18-35	Right	Menstrual (onset of menstruation ± 1 day) Follicular (16 – 17 days prior to menstruation)	Saliva assays (E, P)	Verbal (semantic categorisation) Non-verbal (complex tones)	Reduced ERP latencies to the left hemisphere (from the right ear) during the follicular phase and to the right hemisphere (from the left ear) during the menstrual phase. No behavioural LEA/REA reported.
Wadnerkar et al. (2008)	25	22.56 (SD = 2.04)	Right	Menstrual (2 – 5) Midluteal (18 – 25)	Day count	Verbal (consonant-vowel, three attention conditions).	Reduced REA during menstrual phase (all attention conditions combined).

Right ear advantage (REA), left ear advantage (LEA), estradiol (E), progesterone (P), lutenising hormone (LH), follicular stimulating hormone (FSH), event related potential (ERP).

One critical limitation and potential explanation for these inconsistencies is that the majority of DL studies (Altemus et al., 1989; Alexander et al., 2002; Sanders and Wenmoth, 1998; Wadnerkar et al., 2008) did not include direct hormone measurements but relied entirely on calendar methods to estimate cycle phases and the underlying estradiol and progesterone levels. Direct hormone measurements are a prerequisite for menstrual cycle research, as previous studies had to exclude large numbers of participants (up to 46%, Gordon et al., 1986) because hormone assays revealed that participants were not in the expected cycle phase. As a result, if some participants were tested just before or after the expected peak in estradiol and/or progesterone levels, the variability in the degree of lateralisation would be greater across participants.

Task instruction can also affect the REA and interact with sex and menstrual cycle effects in the DL task (Voyer and Ingram, 2005; Hjelmervik et al., 2012; Wadnerkar et al., 2008). In these studies, participants are required to selectively attend to and report from either the left or the right ear, in addition to the standard non-forced attention condition. In contrast to the non-forced condition, the forced-left condition requires top-down cognitive control, requiring participants to actively override the tendency to report stimuli presented to the dominant right ear (Hugdahl, 2003; Loberg et al., 1999; Hugdahl et al., 2009). In line with other reports of sex differences in auditory attention (Halley, 1975; Andersson and Hugdahl, 1987), Voyer and Ingram (2005) found that women had a higher number of intrusions from the uncued ear compared to men. This finding was interpreted by the authors as evidence that women experiencing greater difficulty in orienting their attention to the cued ear compared to men. Furthermore, this suggests that top-down factors could account for sex differences in the DL bias. Regarding menstrual cycle studies, while Wadnerkar et al. (2008) pooled data across all three conditions, Hjelmervik et al. (2012) found a cycle-related change only in the condition that required participants to shift attention to stimuli presented to the left ear. In

this condition, women in the follicular phase showed an increased left-ear advantage compared to both the menstrual and the luteal phase. As no menstrual cycle effect was observed in the non-forced condition, Hjelmervik et al. (2012) concluded that estradiol influences cognitive control as opposed to language lateralisation *per se*. This is in line with previous studies showing that estradiol has an enhancing effect on cognitive control in non-lateralised tasks, such as working memory, recognition memory, and response inhibition (Jacobs and D'Esposito, 2011; Keenan et al., 2001). Moreover, this indicates that the prefrontal cortex (PFC) is an important site of estrogen activity in the female brain, as has been proposed by others (Hampson and Morley, 2013; Joffe et al., 2006; Keenan et al., 2001; Wang et al., 2010). In addition, this suggests that cognitive control can be a potential confounder in studies of lateralisation.

As noted by Hjelmervik et al. (2012), lateralisation tasks may vary in the amount of cognitive control they require. For example, word-matching tasks ask participants to report whether two consecutively presented words are the same, which requires the updating component of working memory. In contrast, lexical decision tasks require participants to discriminate words from non-words; this does not require working memory. Indeed, studies using word matching typically show reduced language lateralisation during the luteal and follicular phase (Hausmann and Güntürkün, 2000; Weis et al., 2008, respectively), while lexical decision studies often report no cycle effects (Chiarello et al., 1989; Compton and Levine, 1997; Heister et al., 1989; Weekes and Zaidel, 1996). Together with the findings from Hjelmervik et al. (2012), this suggests that cognitive control demands may be a possible confound when investigating language lateralisation (and lateralisation more generally). This again may partly explain some of the aforementioned inconsistencies.

In the present study we investigated normally cycling women using three attention conditions of the Bergen DL test (Hugdahl, 1995, 2003), which is identical to the task used in

Hjelmervik et al. (2012). In contrast to previous studies, we adopted a between-subjects design, which is more conservative (Charness et al., 2012), and avoids potentially confounding carry-over effects due to the repeated measures design (e.g. Soveri et al., 2013; Hausmann and Güntürkün, 1999). Such carry-over effects were, for example, reported by Hampson (1990b) showing that cognitive performance can increase when participants are initially tested in a physiologically conducive state, compared to those who began testing in a less favourable physiological state for a particular task. Moreover, by comparing groups with high or low hormone levels (as opposed to cycle phases), we maximise the differences in estradiol (and progesterone) levels, allowing to test whether sex hormones affect language lateralisation directly, or indirectly via an estradiol effect on cognitive control. If gonadal steroid hormones affect the bottom-up process related to language lateralisation, it is predicted that estradiol and/or progesterone will reduce the DL bias across all attention conditions. However, if high levels of gonadal hormones selectively affect top-down cognitive control, estradiol-related changes are expected only in the forced-left DL condition (Hjelmervik et al., 2012).

Method

Participants

Seventy-three healthy, normally cycling women (out of 81 participants tested; see hormone assessment section for exclusion details) with a mean age of 23.00 years (SD = 4.86; range: 19 – 40 years) were assigned to either a High estradiol (n = 37) or Low estradiol (n = 36) group, based on saliva estradiol assays.

All women were native English speakers and right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). The laterality quotient (LQ) provided by this hand preference measure is calculated as $[(R - L)]/(R + L)] \times 100$, resulting in values between -

100 and +100, indicating consistent sinistrality and dextrality respectively. The mean LQ was 88.10 (SD = 13.83). There were no differences in age nor handedness between the estradiol groups (all $t_{(71)} < 1.21$, ns).

All participants reported no hearing difficulties, were not pregnant and did not currently, or in the previous 6 months, use hormonal contraceptives or other hormone regulating medications.

Procedure

The day of testing was arranged according to participants' self-reported cycle day (days 1-4, 7-12, 15-23, corresponding to the menstrual, follicular or luteal phase; respectively). Saliva samples were collected at the beginning of the test session. Saliva estradiol was used as the findings of Hjelmervik et al. (2012) identified saliva estradiol levels as significantly related to DL laterality shifts. This method of classification is based on objective quantification of estradiol levels, as opposed to inaccurate self-reports of current menstrual cycle phases. However, varying the cycle day of testing between women ensured a maximum range of estradiol levels. The majority of women allocated to the High estradiol group were in the luteal phase according to self-reports (n = 23). Consequently, it was expected that the High estradiol group would also yield higher progesterone levels. The Low estradiol group was primarily comprised of participants in the self-reported menstrual (n = 16) or follicular (n = 14) phases. Given that the follicular phase is characterised by a high level of estradiol, this demonstrates cycle phase estimation (based on day counts) did not correspond with directly measured hormone levels in the present study. This suggests either that participants' estimation of their current cycle phase was inaccurate, or that the majority of participants experienced an anovulatory cycle.

To facilitate collection of saliva samples, women were asked to avoid eating, drinking, smoking and brushing teeth for 30 minutes prior to the testing session. One sample (2 × 1 ml) was collected at the beginning of the test session. The saliva was stored at -20°C until completion of the study. Samples were assayed by an independent professional hormone laboratory with commercially available luminescence immunoassays for estradiol and progesterone. The sensitivity of the estradiol assay was 0.3 pg/ml, the sensitivity of the progesterone assay was 2.6 pg/ml. Intra-assay coefficients for estradiol and progesterone were 13.3 % and 6 %, respectively. The allocation of participants to either the High or Low estradiol groups was based on a median-split (split score: 3.4 pg/ml). Eight women were excluded from further analyses due to contamination (sample was semi-fluid and/or discoloured, suggesting blood contamination).

Table 2: Estradiol, progesterone, handedness and age (mean ± standard deviation and range) for all women in each estradiol group.

	Low estradiol (n =36)	**High estradiol (n = 37)**
	M ± SD (range)	M ± SD (range)
Estradiol (pg/ml)	2.07 ± 0.76 (0.6 – 3.30)	5.83 ± 3.5 (3.40 – 20.30)
Progesterone (pg/ml)	77.25 ± 59.58 (20.6 – 327.5)	147.82 ± 107.04 (21.30 – 366.7)
Handedness LQ	90.04 ± 13.72 (60-100)	86.2 ± 13.87 (52.94 – 100)
Age	22.31 ± 4.49 (19 – 40)	23.68 ± 5.18 (19 – 38)

The Bergen Consonant-Vowel Dichotic Listening Test

The Bergen Consonant-Vowel Dichotic Listening Test was included as part of a larger test battery of cognitive tasks. The stimuli set was six consonant-vowel syllables (/ba/, /da/, /ga/, /ka/, /pa/, /ta/), spoken with constant intonation and intensity by an English male voice. The stimuli were presented as 30 dichotic pairs (e.g. /ba/ - /pa/), and six additional homonymic

pairs (e.g. /ba/ - /ba/). The syllable duration was 400 – 450 ms with an inter-stimulus interval of 4000 ms. The stimuli were administered through a computer using Windows Media Player, and participants listened to the stimuli through supra-aural headphones (K271, AKG Acoustics, Vienna, Austria). Participants were required to give an oral response to each trial, which was recorded by the experimenter. The 36 trials were presented three times, each time with a different randomised order of trials, totalling 108 trials. Each block of trials began with a different instruction, in line with three attention conditions. All participants began with the non-forced condition, in which they were instructed to report the sound they heard the most clearly. This was followed by the forced-right/forced-left condition, in a counterbalanced order between participants, in which participants were respectively asked to attend to and report from the right or left ear. The non-forced condition was always completed first and was not randomised between participants so as to avoid biasing participants' responses regarding the attended ear. For each condition, the percentage of correct left-ear reports and correct right-ear reports were scored separately, and used to calculate LQs using the following formula: [(RE - LE)/(RE + LE) × 100]. Homonymous pairs were excluded from the analysis.

Data analysis

Non-parametric tests were used where assumptions of normality were not met. Greenhouse-Geisser adjustments were used whenever sphericity was violated.

Results

Salivary hormone concentrations

The mean saliva estradiol and progesterone concentrations are given in Table 2. Estradiol levels in the High estradiol group were significantly higher than those in the Low estradiol group, $U = 1332.0$, $p < .001$, as were progesterone levels, $U = 958.5$, $p < .001$. There was no

significant correlation between estradiol and progesterone in either the High estradiol (r_s = .02, p = .88), or the Low estradiol group (r_s = .23, p = .18).

Dichotic listening task

The laterality quotients (Table 3) were subjected to a 3 × 2 mixed model ANOVA, with Condition (non-forced, forced-right, forced-left) as the within-subjects, and Group (High estradiol, Low estradiol) as the between-subjects factor. The significant intercept effect revealed an REA across the whole sample ($F_{(1, 71)}$ = 33.07, p <.001, η_p^2 = .32). The main effect of Condition was significant, ($F_{(1.37, 97.58)}$ = 187.83, p <.001, η_p^2 = .73). Post-hoc pairwise comparisons revealed that LQs in the forced-right condition were significantly greater than in the non-forced condition (p <.001), indicating an increased REA in the forced-right condition. Furthermore, LQs in the forced-left condition were significantly smaller (i.e. negative) than in the non-forced and forced-right conditions, indicating a shift to a LEA in the forced-left condition (both p < .001). The Condition × Group interaction was not significant ($F_{(1.37, 97.58)}$ = 3.37, p = .056, η_p^2 = .045).

Table 3. Mean LQ and standard deviations across attention conditions (non-forced, forced-right, forced-left, all conditions combined) in each group.

	Low estradiol N = 36	**High estradiol N = 37**
Non-forced	17.78 ± 18.67	11.73 ± 20.57
Forced-right	49.00 ± 20.95	39.51 ± 24.19
Forced -left	-34.53 ± 25.21	-25.24 ± 22.99
All combined	10.75 ± 11.38	8.67 ± 16.86

It is important to note that the main effect of Group on LQ was not significant, probably because of positive LQs and negative LQs being averaged across all three attention conditions, thereby masking a general, condition-independent reduction in ear asymmetries.

We therefore conducted a second analysis in which we included the absolute LQs for all conditions (see Figure 1).

The absolute LQs were subjected to a 3 × 2 mixed model ANOVA with Condition (non-forced, forced-right, forced-left) as the within-subjects factor, and Group (High estradiol, Low estradiol) as the between-subjects factor (see Figure 1). The main effect of Condition was significant ($F_{(1.53, 108.90)} = 36.17$, $p < .001$, $\eta_p^2 = .34$). Post-hoc pairwise comparisons revealed that the absolute LQ in the forced-right condition was significantly greater than in both the non-forced and forced-left conditions (both $p < .001$). Moreover, the absolute LQ was larger in the forced-left condition than in the non-forced condition ($p < .002$). More importantly, and in contrast to the previous analysis, the main effect of Group was significant ($F_{(1, 71)} = 4.52$, $p < .037$, $\eta_p^2 = .06$), indicating that the absolute LQ in the High estradiol group ($M = 25.50$, $SD = 17.21$) was significantly reduced as compared to the Low estradiol group ($M = 33.77$, $SD = 15.99$). The Condition × Group interaction was not significant ($F_{(1.53, 108.90)} = 0.155$, $p = .80$, η_p^2 .002).

It should be noted that, if absolute LQs were analysed according to a progesterone median split, neither the main effect of Cycle phase ($F_{(1, 71)} = .29$, $p = .59$, $\eta_p^2 = .004$), nor the Cycle phase × Condition interaction ($F_{(1.53, 108.76)} = .28$, $p = .69$, $\eta_p^2 = .004$) approached significance. Similarly, conducting the same analysis using estimated cycle phases based on day count (i.e. menstrual/cycle day 1-4, follicular/cycle day 7-12, luteal/cycle day 15-23) rather than using High versus Low estradiol groups, neither the main effect of Cycle phase ($F_{(2, 70)} = 2.77$, $p = .07$, $\eta_p^2 = .07$), nor the Cycle phase × Condition interaction ($F_{(3.04, 106.27)} = .59$, $p = .62$, $\eta_p^2 = .02$) reached significance. Subjecting the standard LQs to the same analysis revealed similar results for both the main effect of Cycle phase ($F_{(2, 70)} = .06$, $p = .94$, $\eta_p^2 = .002$) and the Cycle phase × Condition interaction ($F_{(2.76, 96.47)} = 2.59$, $p = .06$, $\eta_p^2 = .07$). However, as previously stated, it is important to note that participants' cycle phase estimation

did not correspond with salivary hormone levels. Therefore, drawing conclusions based on cycle phase is highly problematic in the current study.

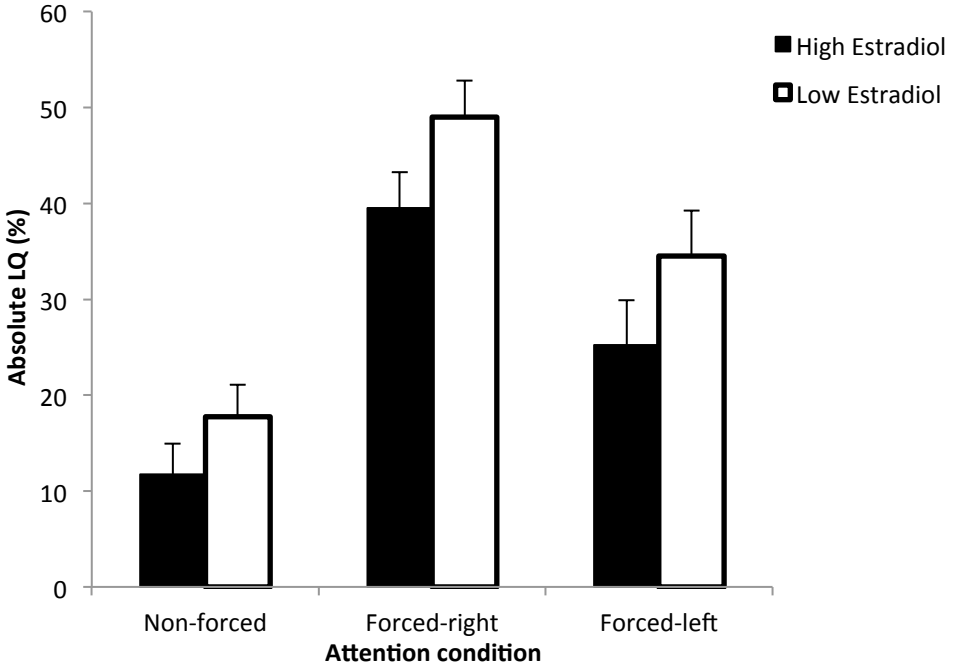

Figure 1. Mean absolute laterality quotient (LQ) and standard error means according to estradiol group (high, low) for each attention condition. LQs represent the degree of ear advantage by the dominant versus non-dominant ear.

Relationship between absolute laterality quotients and sex hormones

Spearman's correlation revealed a small but significant relationship between mean absolute LQs (all attention conditions combined) and estradiol levels (r_s = -.24, p = .04) but not with progesterone levels (r_s = -.15, p = .19). However, due to the significantly reduced absolute DL biases across all conditions in women with higher levels of estradiol, and the possibility that estradiol and progesterone may have interactive effects on language lateralisation, we conducted a moderated multiple regression to investigate the relationship between sex hormone levels and absolute LQs. The mean absolute LQ (across all three attention conditions) was used as dependent variable. To avoid multicolinearity, independent (predictor) variables were centered. The interaction variable was calculated as the product of

estradiol and progesterone (both centered). The regression analysis did not revealed a significant model, $F_{(3, 69)} = 1.94$, $p = .13$, $R^2 = .078$. The only predictor that approached significance was estradiol ($\beta = -.269$ p = .06). Progesterone and the estradiol × progesterone interaction did not approach significance ($\beta = -.113$, p = .34, $\beta = -.222$ p = .12, respectively). Together with the significant Spearman's correlation, the trend effect in the regression suggests a weak relationship between high levels of estradiol and reduced language lateralisation across all conditions (see Figure 2). Notably, participants with the highest estradiol levels were in the follicular cycle phase, according to self-report.

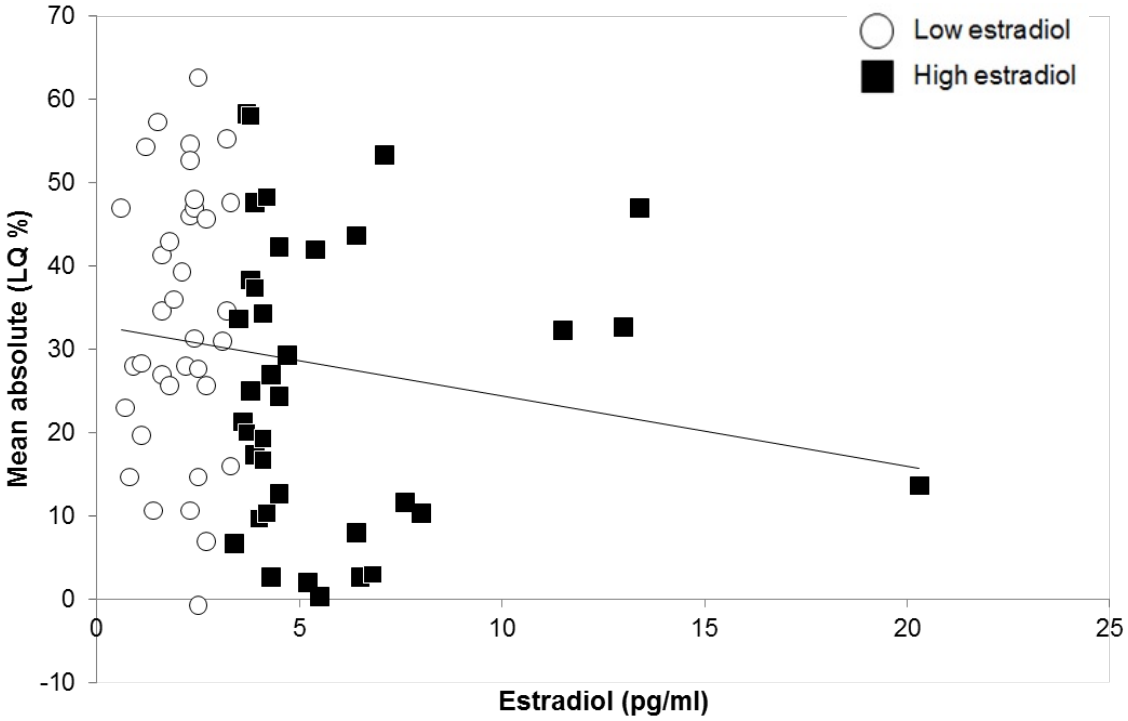

Figure 2. Scatterplot of the relationship between the estradiol levels and the mean absolute LQs in the dichotic listening task averaged across all attention conditions. Black squares represent Participants of the High estradiol group; white circle represent participants of the Low estradiol group. NB: Graph depicts non-centered estradiol levels, centering was used in the moderated multiple regression analysis.

Discussion

The present study demonstrated a reduction in the absolute DL bias across all attention conditions for the High estradiol group, compared to the Low estradiol group. This suggests that high levels of estradiol are related to reduced language lateralisation as measured with the DL paradigm. However, both groups differed significantly in estradiol *and* progesterone levels. The correlation analysis suggested that the absolute DL bias was reduced with increasing estradiol levels. Further analysis using moderated multiple regression basically confirmed that estradiol alone was the best predictor (although only approaching significance) for the reduced absolute DL bias. Although the estradiol × progesterone interaction was also negatively related to the DL bias, this effect was not significant. These results suggest that high levels of estradiol reduce language lateralisation. These findings are fundamentally different to Hjelmervik et al. (2012) who found a positive relationship between estradiol levels and the DL bias in the forced-left condition only. This finding was interpreted by the authors as an estradiol-related improvement in cognitive control, as opposed to an effect on language lateralisation per se.

It is noteworthy that Hjelmervik et al. (2012) and the present study investigated normally-cycling women with the identical Bergen DL paradigm, though stimuli were spoken by a native Norwegian (Hjelmervik et al.) or English (present study) male speaker. However, there are some important differences between the studies that might partly account for the conflicting findings. Firstly, the current study revealed consistently larger DL biases across all conditions. Specifically, the mean REAs in the non-forced and forced-right conditions (averaged across High/Low estradiol groups) in the current study are about twice as large as those in Hjelmervik et al. (2012). The difference in average LEAs in the forced-left condition between studies is even larger (greater in the current study). The comparatively small ear advantages in Hjelmervik et al. (2012) may partly explain why there was no further reduction

in DL biases when hormone levels were high. Secondly, women in the present study showed higher mean concentrations and also a larger range of estradiol levels, possibly partly due to the larger sample size in the current study. This, together with the fact that the present study compared extreme groups (low/high estradiol), instead of testing women during different cycle phases, might have promoted a hormone effect on language lateralisation. Third, it should be noted that participants in the present study with high estradiol levels also had high progesterone levels. This is different to the hormone profiles of the follicular phase reported by Hjelmervik et al. (2012) and suggests that while estradiol alone improves cognitive control, the effect on language lateralisation may also depend on progesterone levels. Finally, Hjelmervik et al. (2012) adopted a within-subjects design, which is potentially subject to carry-over effects (e.g. Hampson, 1990b; Hausmann and Güntürkün, 1999; Charness et al., 2012), as opposed to the between-subject design in the present study. As mentioned above, the forced-left condition requires cognitive control - the ability to override a stimulus-driven response in favour of an instruction-driven one (i.e. top—down process). Given that all participants in Hjelmervik et al. (2012) performed the DL task three times, during different cycle phases, it is possible that repeated testing enhanced participants' ability to cognitively control the stimulus-driven bottom-up process. Indeed, as previously discussed, Hampson (1990b) demonstrated that cognitive performance can increase when participants are initially tested in a physiologically conducive state, compared to those who began testing in a less favourable physiological state for a particular task (see also Mead & Hampson, 1996). Thus, in Hjelmervik et al. (2012), high levels of estradiol during the follicular phase may have provided a physiologically conducive state which may have promoted the LEA in the more demanding forced-left condition.

Although all previous DL studies counterbalanced the cycle phase in which participants were tested, an interactive effect of repeated testing and hormonal state on language

lateralisation cannot be ruled out, as shown by participants initially tested during high-hormone cycle phases (Hampson 1990a, b; Mead & Hampson, 1996). To determine the stability of the laterality bias, some studies repeatedly tested male or postmenopausal female controls at comparable time points (e.g. Hausmann and Güntürkün, 2000; Hjelmervik et al., 2012; Hjelmervik et al., 2014; Bayer et al., 2008; Weis et al., 2011) because their hormone levels are relatively stable. However, it has been argued that these procedures may not completely rule out carry-over effects (Hausmann and Güntürkün, 1999). Therefore, the present study is not subject to this potential confound.

In contrast to the majority of studies investigating menstrual cycle effects on language lateralisation as measured by DL (Cowell et al., 2011; Wadnerkar et al., 2008; Sanders and Wenmoth, 1998), the current study found a reduction in lateralisation when estradiol and progesterone levels were high, regardless of the attention condition. As different attention conditions were used, resulting in either LEAs or REAs, it is unlikely that the general reduction in language lateralisation is due to sex hormones selectively affecting one hemisphere. It is also rather speculative that estradiol and/or progesterone modulated the efficacy of the ipsilateral/contralateral projections from the non-dominant/dominant ear to the right/left auditory cortices because sex hormonal effects on subcortical auditory pathways are not known (Al-Mana et al, 2008). We are therefore inclined to believe that the observed reduction in condition-specific DL biases occurred on the cortical level.

It has recently been proposed (see Hausmann and Bayer, 2010, for review) that sex hormones modulate lateralisation through their neuromodulatory effects on interhemispheric inhibition. It was originally proposed that progesterone reduces lateralisation by suppressing the excitatory responses of neurons to glutamate and increasing their response to GABA, leading to a 'decoupling' of the hemispheres by reducing corticocortical transmission and interhemispheric inhibition (Hausmann and Güntürkün, 2000). Subsequent research has

provided evidence that estradiol may also modulate interhemispheric interaction and, in turn, lateralisation (Hausmann et al., 2013, Weis et al., 2008; Weis et al., 2011; Hausmann et al., 2006; Holländer et al., 2005). In line with this hypothesis, the reduced REA found in the non-forced and forced-right condition in the High estradiol group may be explained by a reduction of inhibition of the subdominant right hemisphere by the dominant left. This would facilitate right hemisphere processing of stimuli presented to the left ear. Similarly, the reduced LEA in the forced-left condition for the High estradiol group may be viewed as a reduction of inhibition from the right hemisphere over the left hemisphere, which subsequently facilitates left hemisphere processing of stimuli presented to the right ear, which would consequently reduce the LEA.

Further analysis of the absolute LQs indicated that the reduction in the DL bias was mainly underpinned by estradiol. Although progesterone levels were also high in the high estradiol group, progesterone alone and the interaction between estradiol and progesterone did not predict the DL bias. Therefore, the results of the present study directly contribute to the debate concerning which sex hormone, estradiol and/or progesterone, drives menstrual cycle-related effects on language lateralisation (Hausmann and Bayer, 2010; Weis et al., 2008). Indeed, although estradiol and progesterone exact opposing influences on glutamatergic and GABAergic receptors, a transcranial magnetic stimulation study (Hausmann et al., 2006) revealed that estradiol and progesterone can have similar attenuating effects on interhemispheric inhibition, during the follicular and luteal phases, respectively. In addition, Smith et al. (1987) showed that combining estradiol with a high dose of progesterone leads to a decrease in excitatory neural responses to glutamate, similar to the effect of progesterone alone. Although progesterone was not directly linked to language lateralisation in the present study, it is important to note that the high estradiol group also had high progesterone levels. Thus, we cannot rule out that high levels of estradiol might have

reduced the interhemispheric inhibition in combination with progesterone, as was previously suggested (see Hausmann & Bayer, 2010; Weis & Hausmann, 2010 for reviews), thereby decreasing DL bias across all attention conditions in the present study. However, the exact mechanism underlying the interactive effect of estradiol and progesterone on absolute LQs remains an open question.

In conclusion, in contrast to previous studies (Hjelmervik et al., 2012; Cowell et al., 2011; Sanders and Wenmoth, 1998; Wadnerkar et al., 2008), the present study revealed a reduced lateralisation in women with high levels of estradiol and progesterone across all DL conditions. This suggests that the proposed estradiol-related improvements in cognitive control may be smaller than previously reported (Hjelmervik et al., 2012). The present findings rather support the notion that sex hormones affect language lateralisation directly, probably via a modulation of stimulus-driven bottom-up processes and interhemispheric inhibition. This finding also suggests that reduced lateralisation is related to high levels of estradiol. The present study highlights the need to consider interactions between sex hormones when investigating lateralisation across the menstrual cycle. Moreover, with respect to sex differences in language lateralisation, the present study provides further evidence to suggest that while women are less lateralised compared to men, the degree of sex difference in (language) lateralisation may partly dependent on women's hormonal state during the menstrual cycle phase. Finally, the present study suggests that the top-down and bottom-up aspects of lateralisation can be differently affected by hormonal fluctuations across the menstrual cycle. This might be an additional factor that may account for some of the inconsistencies in the literature on sex differences in the functional organisation of the brain.

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