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Influence of sex, menstrual cycle, and hormonal contraceptives on egocentric navigation with or without landmarks

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ABSTRACT

This study examines the influence of sex, menstrual cycle, hormonal contraceptives (HC) and sex hormone levels in following egocentric navigation instructions with or without landmarks. Estradiol seem to bias the reference frame for navigation during estrous cycle of female rats. However, previous studies in humans found no differences in overall navigation between women in their early follicular and mid-luteal menstrual cycle phases, whose performance was worse than that of men. Our study hypothesis was that the performance of women would be improved during the peri-ovulatory phase and would remain the same during placebo and active phases of HC users. The study included 21 men, 62 women with natural menstrual cycle (21 during early follicular phase, 20 during peri-ovulatory phase, and 21 during mid-luteal phase), and 38 women that were receiving HC (13 during placebo phase and 25 during active phase). The men outperformed the women with a natural menstrual cycle when following egocentric instructions without landmarks. However, the women's performance varied according to the phase of their menstrual cycle, differing from men during early follicular and mid-luteal phases but not during the peri-ovulatory phase. The use of HC also improved the performance of women to the extent that the difference with men disappeared. No differences were observed between HC-placebo and HC-active user groups during egocentric navigation without landmarks and among all groups during egocentric navigation with landmarks. Analysis of salivary hormones showed that testosterone levels were higher in men and that estradiol levels in women were higher during peri-ovulatory and mid-luteal phases and also in HC users. Progesterone levels were higher in women during the mid-luteal phase. These results appear compatible with beneficial effect of testosterone and estradiol on egocentric navigation without landmarks and with a block of this effect produced by progesterone.

Keywords: Egocentric navigation; landmarks; sex differences; menstrual cycle phase; hormonal contraceptives; sex hormones.

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1. Introduction

Navigation or *wayfinding* refers to the ability of people and animals to orient themselves in physical space and navigate from place to place. It is measured by using goal-directed tasks in which participants receive instructions to follow a given path and reach a final destination (Dabbs et al., 1998; Gerber and Kwan, 1994; Lawton et al., 1996).

Information about distance and direction is needed to localize the target. The direction can be deduced from the starting position and body movement of the observer, which provide a so-called "egocentric reference frame". Examples include the use of such expressions as *"when I leave my house, I turn right, walk two blocks and turn left"* to describe the way to a destination (Ekstrom et al., 2018; Harris et al., 2019; Iaria et al., 2003; Lawton, 1994; O'Keefe and Nadel, 1978; Scheuringer and Pletzer, 2017). Landmarks in the environment can also be used as directional cues in egocentric navigation, in such expressions as *"turn right at the church, turn left when I see the bank"* (Ekstrom et al., 2018; Saucier et al., 2002; Scheuringer and Pletzer, 2017).

Sex hormones (testosterone, estradiol, and progesterone) have been related not only to the preference for one or other type of navigation strategy (Hussain et al., 2016) but also to the performance of the chosen strategy (Scheuringer and Pletzer, 2017). Release of testosterone, the main sex hormone in men, is under tonic control, but release of estradiol and progesterone, the main ovarian hormones in women, is much more complex. During the first week of the menstrual cycle (early follicular phase), levels of both estradiol and progesterone are low. Estradiol levels peak 2-3 days before ovulation (around 14 days before the onset of a new cycle), and progesterone levels are low (ovulatory phase). Finally, about a week before the onset of a new cycle (mid-luteal phase), estradiol and progesterone levels are high (Lenton et al., 1984; Sundström-Poromaa and Gingnell, 2014). Most hormonal contraceptives (HC) contain synthetic analogues of estrogen and progestins that disrupt the hypothalamic-pituitary-ovarian axis, prevent monthly sex hormone fluctuations and ovulation, and reduce the endogenous secretion of sex hormones (Aden, Jung-Hoffman and Khul, 1998; Batur et al., 2003; Griskiene et al., 2018; Mordecai et al., 2008; Warren et al., 2014).

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Initial studies on possible sex differences in navigation reported that men generally outperform women in both accuracy and response time and this result was related to higher levels of testosterone in men (see Andreano and Cahill, 2012 for review). However, these behavioral differences were reduced or reversed when an egocentric reference frame or landmarks were used (Dabbs et al., 1998; Galea and Kimura, 1993; Lawton, 1994). There has been little research on the influence of instructions that require participants to adopt an egocentric strategy, with or without landmarks, and the results have not been consistent (Saucier et al., 2002; Scheuringer and Pletzer, 2017). For example, when egocentric instructions with landmarks were followed, men were found to be slower by Saucier et al. (2002) and faster by Scheuringer and Pletzer (2017). The latter also found women to be slower when egocentric instructions without landmarks were followed, while this type of instruction was not included in the study by Saucier et al. (2002).

The menstrual cycle and female sex hormones influence on egocentric navigation has been extensively investigated in animals. Estradiol, whether naturally fluctuating across the cycle or administered to ovariectomized rats, bias the reference frame for navigation (see Hussain et al., 2014 for review). In addition, this hormone increases dopaminergic transmission in striate, a key neurochemical/neuroanatomical component of egocentric navigation (see Sotomayor-Sarate et al., 2014). However, in humans, although egocentric navigation depends on a similar substrate (Ekstrom et al., 2018; Iaria, 2003) no evidence suggesting the involvement of estradiol has been observed. Thus, a preference for the use of an egocentric reference frame during early follicular and ovulatory phases, but not during the luteal phase, which has been associated with progesterone, has been observed (Hussain et al., 2016). The sole published investigation of the menstrual cycle's influence on the capacity to follow egocentric instructions reported no overall differences between early-follicular and mid-luteal phases (Scheuringer and Pletzer, 2017). However, because only these two phases were examined, menstrual cycle-dependent improvement following egocentric instructions might be related to a rise in estradiol during the ovulatory phase (Scheuringer and Pletzer, 2017).There has been no study on egocentric navigation in women using HC, which offers an opportunity to explore in greater depth the influence of hormonal variations associated with the menstrual cycle.

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With this background, the objectives of this study were to determine sex differences in the ability to follow egocentric instructions with or without landmarks and to examine the influence of the menstrual cycle and the use of HC on this ability. The study hypotheses were that the overall performance of women in egocentric navigation tasks would be improved during the ovulatory phase of the menstrual cycle, reducing the differences with men, in comparison to early follicular and mid-luteal phases. In addition, these fluctuations in navigation performance during menstrual cycle phases should disappear in women with HC because they have fewer hormonal changes associated with menstrual cycle.

2. Material and methods

2.1 Participants

Students at University of Granada were recruited for this study. Exclusion criteria were: uncorrected visual problems; any kind of hearing, language, neurological, or psychiatric impairment; the use of anabolic steroids, or medication for chronic or neurological diseases; and substance abuse (Colzato et al., 2010; Sundström-Poromaa and Gingnell, 2014). Additional exclusion criteria for the women were the use of abortion pill in previous 4 months and the presence of dysphoric emotional disorder (Sacher et al., 2013), which was based on their responses to a premenstrual dysphoric disorder questionnaire (see below), with one volunteer being excluded for this reason. The following groups were enrolled: 26 healthy men aged 18–32 years (mean±standard error of the mean [SEM] of 22.2± 0.5 yrs), 62 healthy women with a natural menstrual cycle aged 18–32 years (mean of 21.2±2.6 yrs), and 38 healthy women using HC aged 18–30 years (mean, 22.0± 2.6 yrs). As a reward for their participation in the experiment, volunteers obtained university course credits.

2.2 Determination of menstrual cycle phase in women with natural menstrual cycles and HC groups.

Classification of the women's natural menstrual cycle as regular was based on self-reports of the onset date of the last four cycles (Becker et al., 2005), only including women with cycle duration of 28 ± 7 days. The women with a natural menstrual cycle were randomly assigned to one of three experimental

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groups (early follicular, peri-ovulatory, or mid-luteal phase). Selection of the day of the experiment for individuals in each group was determined calculating the mean duration of their menstrual cycle and estimating the onset date for their next cycle. The real date and actual duration of the menstrual cycle were determined posteriorly when the women informed the onset date of the mense. Women in the early follicular group (n=21) were tested on days 1–7 of the cycle, those in the peri-ovulatory group (n=20) were tested on days 16–12 before their next menses, and those in the mid-luteal group (n=21) on days 9 - 3 days before their next menses, calculating the last two phases from the date of the next menses because the luteal phase is considered more stable (Sundström-Poromaa and Gingnell, 2014).

Women in the HC group were tested during the placebo (n=13) or active (n=25) phase, established according to self-reports. HC were combinations of a synthetic estrogen with a progestin (see Supplement A).

2.3 Experimental procedure and material

Written informed consent was first obtained from all volunteers that participated in the study, which was approved by the Granada University ethical committee. Next, women filled in the premenstrual dysphoric disorder questionnaire and salivary samples were obtained from all participants. Finally, participants completed the navigation task and an intelligence task.

2.3.1 Premenstrual dysphoric disorder questionnaire

This dysphoric disorder questionnaire, based on the APA classification (American Psychiatric Association, 2013), was applied to exclude any women with this disorder, which could affect their performance during the experimental tasks (Sacher et al., 2013). The women responded to 14 items related to symptoms during their last premenstrual phase: whether they had felt depressed, whether any state of depression was repeated for at least two consecutive months during the past year, whether it interfered with their work, and whether it disappeared shortly after the onset of menstruation.

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2.3.2 Saliva sample collection and immunoassay protocols and analysis

Participants were asked to avoid alcohol consumption during 24 hours prior to the saliva sample collection, food intake during 1 hour prior to the collection and tooth brushing during 3 hours prior to the collection (Colzato et al., 2010). Saliva samples were collected by passive drool into 10-ml polypropylene tubes, which were then centrifuged and stored at −20 ºC until further analysis. Salivary testosterone, estradiol, and progesterone concentrations were analyzed by an independent laboratory using high-sensitivity salivary enzyme immunoassay kits from LDN (Nordhorn, Germany); the sensitivity for testosterone, estradiol, and progesterone was 2.2 pg/ml, 0.2 pg/ml, and 5.0 pg/ml, respectively.

2.3.3 Navigation task

All participants performed the 2-D Matrix Navigation Task (see Figure 1). Each trial comprised a 10 x 10 matrix (18.5 cm × 18.5 cm), with each cell containing one of 10 repeated, nameable symbols (Scheuringer and Pletzer, 2017; adapted from a paper-pencil version by Saucier et al., 2002). The placing of each symbol was random, with the exception that a symbol could not appear more than once per row.

An arrow indicates the location in the matrix for starting navigation in accordance with egocentric instructions with or without landmarks (see Figure 1). An example of egocentric instruction with landmarks would be: “Starting at the ❤ (indicated by the ⇨), go up until the 🔑, turn right until the ★, and go up until you see the 🚗. Which symbol is to your immediate left?”. The same instruction in egocentric terms without landmarks would be: “Starting from the symbol indicated by the arrow, go up two squares, then go right six squares, then go up three squares. Which symbol is to your immediate left?” The difference between the two types of instruction is the use of picture symbols. The egocentric instructions with landmarks were formulated as in Saucier et al. (2002), whereas words were preferred in Scheuringer and Pletzer (2017). The egocentric instructions without landmarks were formulated in accordance with the egocentric perspective-Euclidean strategy of Scheuringer and Pletzer (2017), whereas these instructions were not used by Saucier et al. (2002).

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At the beginning of the task, a test block appeared with two examples of each strategy. The instructions remained on the screen (below the matrix) throughout the trial to avoid the influence of working memory abilities on performance (see Scheuringer and Pletzer, 2017).

Participants were not allowed to touch or point at the screen at any time to avoid the possible use of an unintended strategy (Saucier et al., 2002). For each type of instruction (egocentric with or without landmarks), the arrow appeared five times on each side of the matrix. Each participant therefore completed 40 trials, 20 with landmark-based and 20 with non-landmark-based egocentric instructions, with blocked presentation and in counterbalanced order. Feedback was shown after each trial on the accuracy of responses and the time spent. Stimuli were presented using E-prime 2 software (Psychology Software Tools, Pittsburgh, PA). Each trial begun with the appearance of a fixation point for 500 ms, followed by a blank screen for 500 ms, and the appearance of the matrix, which then stayed on screen until the participant pressed a symbol on the keyboard. Response times (RTs) and accuracy in selecting the correct alternative among 10 symbols labeled on the keyboard were recorded for each item. The duration of the navigation task was around 20 min.

2.3.4 Raven standard progressive matrices

This widely-used reasoning-based test was applied to determine the intelligence scores of participants (Raven et al., 1988). It consists of 60 incomplete figures arranged according to their complexity (maximum score = 60). Participants had to use a keyboard key to select a piece from among several alternatives that correctly completed the figure. The duration of this task was 20 minutes.

2.4 Statistical analysis

The ANOVA module of STATISTICA software (StatSoft Inc., Tulsa, OK, USA) was used for data analyses. Results were first compared between the men and the women with a natural cycle using an ANOVA 2x(2) of the proportion of correct answers and an ANOVA 2x(2) of the response times for egocentric instructions both with and without landmarks. Results for six study groups (men vs. women in early follicular phase vs. women in peri-ovulatory phase vs. women in mid-luteal phase vs.

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HC users in placebo phase vs. HC users in active phase) were compared using an ANOVA 6x(2) of the proportion of correct answers and ANOVA 6x(2) of the response times for both egocentric instructions with and without landmarks. Testosterone, estradiol, and progesterone levels were analyzed by ANOVAs of the six groups. Significant effects were then analyzed with a *post-hoc* Fisher`s LSD test. Pearson`s correlation coefficients were computed to test whether behavioral differences among groups were linearly related to saliva hormone levels. Correlations were first calculated for all the participants and when significant we analyzed it for each group separately. All data were expressed as means±SEM, and statistical significance was set at the 5% level.

3. Results

3.1 Demographic data and sex hormone levels

Demographic data are exhibited in Table 1. No significant differences were observed among the six study groups in age, $F(5,120)=1.11$, $p=.36$, or Raven's test result, $F(5,120)=1.04$, $p=.40$. No significant difference was found in menstrual cycle duration among the three groups of women with natural menstrual cycle, $F < 1$. Women in the early follicular group performed their tasks 3.8±0.3 days after the beginning of the cycle (M1) and 25.3±0.5 days before the next cycle (M2). Women in the peri-ovulatory group performed the task 14.9±0.7 days after M1 and 14.2±0.6 days before M2. Women in the mid-luteal group performed the task 23.6±0.5 days after M1 and 5.5±0.6 days before M2.

Table 1 also exhibits the mean±SEM values for sex hormones, showing significant differences among the experimental groups in levels of testosterone, $F(5,120)=31.01$, $p<.001$, estradiol, $F(5,120)=2.39$, $p<.05$, and progesterone, $F(5,120)=8.19$, $p<.01$. Testosterone levels were higher in the men than in the other groups (all *ps*<.001) and were higher in the peri-ovulatory group than in the HC-active group ($p<.05$). Estradiol levels were higher in the peri-ovulatory, mid-luteal, and HC groups than in the early follicular phase group (all *ps*<.05). Progesterone levels were higher in the mid-luteal group than in any other group (all *ps*<.01). No significant difference in sex hormone levels was found

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between the HC groups. The levels of testosterone, estradiol, or progesterone of the participants did not correlate with their performance in egocentric navigation with or without landmarks (all *ps*> .05).

3.2 Sex differences in navigation and correlations with sex hormone levels

3.2.1 Accuracy

A significant effect of group, $F(1,86)= 6.39$, $p<.01$, $\eta_p^2= .07$; condition, $F(1,86)= 30.48$, $p<.01$, $\eta_p^2= .26$; and group x condition interaction, $F(1,86)=7.10$, $p<.01$, $\eta_p^2= .08$, were found for the accuracy of men and women with a natural menstrual cycle in following egocentric instructions with or without landmarks. According to the *post-hoc* analysis of interaction, the performance of the women was worse in the egocentric task without landmarks than with them (0.77±0.03 *vs.* 0.95±0.01; $p =.001$) and they performed worse than the men in the egocentric task without landmarks (0.77±0.03 *vs*.0.89±0.02; $p =.001$) (Figure 2a).

Testosterone levels of men and women with a natural menstrual cycle together correlated positively with the accuracy following egocentric navigation instructions without landmarks (r= 0.23, $p< .04$). However, no correlations were observed when each group was considered separately (r= 0.15, $p=.46$ for men and r= -0.11, $p=.93$ for women).

3.2.2 Response Time

Response times were longer for egocentric instructions without landmarks than with them (21.9±0.7 *vs.* 16.1±0.4; $F(1,86)=84.45$, $p<.01$, $\eta_p^2= .49$ (Figure 2b). Response times were longer for the women than for the men, although statistical significance was not reached, $F(1,86)= 3.48$, $p=.066$, $\eta_p^2= .04$. The effect of group x condition interaction was not significant, $F(1,86)= 2.52$, $p=.11$, $\eta_p^2= .03$.

3.3 Influence of menstrual cycle and HC and correlations with sex hormone levels

3.3.1 Accuracy

Significant effects of group, $F(5,120)= 3.34$, $p<.01$, $\eta_p^2= .12$; condition, $F(1,120)= 67.91$, $p< .01$, $\eta_p^2= .36$; and group x condition interaction, $F(5,120)=3.66$, $p<.01$, $\eta_p^2= .13$, were found for the accuracy of the six study groups (men, early follicular, peri-ovulatory, mid-luteal, HC-placebo, and HC-active

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groups) in following egocentric instructions with or without landmarks. According to *post-hoc* analyses of interaction, the performance of all groups of women was significantly better when egocentric instructions were given with landmarks *vs.* without landmarks ($p < .01$, 0.96±0.01 *vs.* 0.74±0.05 for early follicular, 0.94±0.02 *vs.* 0.72±0.06 for mid-luteal and 0.96±0.01 *vs.* 0.86±0.03 for HC-active groups; $p < .03$, 0.94±0.01 *vs.* 0.86±0.02 for peri-ovulatory group; and $p < .05$, 0.97±0.01 *vs.* 0.89±0.02 for HC-placebo group) but not in the group of men (0.96±0.1 *vs.* 0.89±0.02). The performance of egocentric navigation task without landmarks was significantly worse for early follicular and mid-luteal groups than for the other four groups (all *ps*< 0.01); however, no significant differences were observed among experimental groups in the egocentric navigation task with landmarks (Figure 3a).

No significant correlations were observed between sex hormone levels of all participants or groups and accuracy during egocentric navigation without landmarks**.**

3.3.2 Response time

Response times were significantly longer for egocentric navigation without landmarks than with them, $F(1,120)= 171.81$, $p<.01$, $\eta_p^2= .59$. The effect of group was not significant, $F(5,116)= 1.13$, $p=.35$, $\eta_p^2= .04$.The group x condition interaction was only close to significant, $F(5,120)=2.18$, $p=.061$, $\eta_p^2= .08$. *Post-hoc* analyses of this interaction revealed that response times for egocentric navigation without landmarks were significantly longer for the early follicular (23.9±1.9 s.) and mid-luteal (23.1±1.6 s.) groups than for the other four groups (19.8±0.9 s. for men, 21.2±1.4 s. for peri-ovulatory, 20.8±1.0 s. for HC-placebo and 20.6±1.2 s. for HC-active group; $p < .01$ early follicular *vs.* others; $p < .05$ mid-luteal *vs*. others).

A negative correlation between testosterone levels and response times following egocentric navigation instructions without landmarks was close to significant when all the participants were considered (r= -0.17, $p=.06$). However, no significant correlations were observed when each group was considered separately (r= -0.28, $p=.17$ for men; r= -0.37, $p=.11$ for early follicular; r= -0.15, $p=.53$ for peri-

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ovulatory; r= 0.24, *p*=.31 for mid-luteal; r= -0.20, *p*=.*54* for HC-placebo and r= -0.02, *p*=.94 for HC-active groups).

4. Discussion

This study examined the influence of sex, menstrual cycle phase, and HC use on the execution of a navigation task using egocentric instructions with or without landmarks. The relationship of the results with sex hormone levels was also investigated. When no landmarks were used, greater accuracy was observed for the men (with high testosterone levels), for women performing the task during the peri-ovulatory phase of their menstrual cycle, and for women using HC in either placebo or active phase, i.e., three groups of women with higher estradiol levels. A lower accuracy was observed for women in early follicular phase, when levels of all three sex hormones are reduced, and for those in mid-luteal phase, with the highest progesterone levels. Response times for all groups were faster in navigation tasks with landmarks *versus* without landmarks and, with the exception of the men, fewer errors were made in the former. There was no difference in performance (accuracy or response time) among experimental groups during egocentric navigation with landmarks.

4.1. Behavioral differences

The finding of longer response times in the absence of landmarks is in agreement with Scheuringer and Pletzer (2017) and may be attributable to the need to count squares to determine distances. The accuracy of responses by the women was also worse without landmarks than with them, this is consistent with traditional studies that reported that performance of women was facilitated by landmarks in the environment when navigating (Dabbs et al., 1998; Galea and Kimura, 1993; Lawton, 1994). However, neither the accuracy nor the response times were significantly different between women and men when landmarks were used. This lack of a statistically difference between the sexes in our study may be explained by the very high level of accuracy (>93%) achieved by all groups. It has been observed that sex differences are lower when the spatial task makes a lesser cognitive demand (Coluccia et al., 2004). In fact, sex differences in navigation disappeared in environments

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containing multiple landmarks (Andersen et al., 2012), unlike the observations of significantly shorter (Saucier et al., 2002) or longer (Scheuringer and Pletzer, 2017) response times for women during egocentric navigation with landmarks.

Following egocentric instructions without landmarks, Scheuringer and Pletzer (2017), observed longer response times for women than for men. This effect was only close to significant in the present study, perhaps due to the influence of the menstrual cycle phase in women. Thus, while Scheuringer and Pletzer (2017) only included early follicular and mid-luteal women, our study also contained a peri-ovulatory group. In fact, we observed a trend for longer response times (p= .061) for women in the early follicular and mid-luteal groups than for those in any other group during egocentric navigation without landmarks. In addition, the women in early follicular and mid-luteal phase were less accurate than the men, but this was not the case for those in peri-ovulatory phase. This worse performance by women in the early follicular and mid-luteal groups during egocentric navigation without landmarks, appears compatible with the existence of a true difficulty with this type of navigation frame.

Our results showed no differences between early follicular and mid-luteal groups. This observation differs from Scheuringer and Pletzer (2017) who reporter faster navigation times following egocentric instructions without landmarks and higher accuracy following egocentric instructions with landmarks for the mid-luteal group. However, in Scheuringer and Pletzer (2017) study, participants had to remember the instructions as these disappeared before the presentation of the navigation matrix, therefore menstrual cycle differences in verbal working memory (Rosenberg and Park, 2002) may have influenced the effect.

The use of HC also modulated the sex differences observed during egocentric navigation without landmarks. To the best of our knowledge, this is the first study to demonstrate HC-dependent changes in navigation. The results obtained demonstrate that the use of HC improves the performance of women, which was superior for HC users than for the women in the early-follicular and mid-luteal groups, eliminating the difference with men or peri-ovulatory group. No difference in the performance

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of HC users was observed between those in placebo and active phase. Hence, the use of HC, which prevents monthly fluctuations in sex hormones, eliminates the fluctuations in egocentric ability.

4.2. Sex hormone levels

As expected, salivary testosterone concentrations were higher in the men than in the women, higher estradiol levels and lower progesterone levels were observed in peri-ovulatory group when compared to early-follicular and mid-luteal group respectively. HC groups showed no fluctuation over time in sex hormone levels and lower testosterone and progesterone levels when compared to men and mid-luteal group respectively (Aden, et al., 1998; Griskiene and Ruksenas, 2011; Keevil et al., 2014; Marečková et al., 2014; Sundström-Poromaa and Gingnell, 2014; Mordecai et al., 2008). A less expected result in our women with natural menstrual cycle (as in Hussain et al., 2016) and HC users (as in Griskiene et al., 2018; see Pletzer and Kerschbaum, 2014 for review) was the absence of any significant difference in estradiol concentrations among the peri-ovulatory, mid-luteal, and HC groups. This may be attributable to the large between differences in hormone levels during the menstrual cycle (Sundström Poromaa and Gingnell, 2014), or it may be that the women in peri-ovulatory and mid-luteal groups were not sampled on days when their estradiol levels were the highest.

4.3. Behavior and sex hormone levels during egocentric navigation without landmarks

Better performance of men, women during peri-ovulatory phase and women using HC on egocentric navigation without landmarks are compatible with a possible involvement of endogenous sex hormones in the participants' behavior. However, the ability to perform well on egocentric navigation tasks does not appear to be influenced by the exogenous hormones contained in HC (see Supplement B for more details).

Men have the highest levels of testosterone, and this hormone has frequently been related to their spatial abilities in different tasks (see Silverman et al., 2007, for a cross-cultural study and Voyer et al., 1995, for a meta-analysis), including navigation (see Andreano and Cahill, 2009 for review;

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Scheuringer and Pletzer, 2017). In this study, testosterone may have contribute to the improved of egocentric navigation performance without landmarks. Thus, we observed a positive correlation between testosterone levels of men and women with natural menstrual cycle and the accuracy in this task and a marginally significant negative correlation between testosterone levels of all participants and the response time.

Women performing the task in the peri-ovulatory phase of their menstrual cycle, have shown better performance and higher estradiol levels than early follicular group. These results support for first time a possible role for estradiol in facilitating egocentric navigation. This hormone has been considered responsible for biasing the navigation reference frame in animals, but in humans the role of estradiol remains unclear (Hussain et al., 2014, 2016). The finding that peri-ovulatory and HC groups show similar performance and endogenous sex hormone levels strengthen this possible role for estradiol in facilitating egocentric navigation. But, more importantly, it suggests that it is not necessary to reach the peak of estradiol levels to observe this effect because HC reduce the concentration of the sex hormones and block the ovulation (Aden, Jung-Hoffman and Khul, 1998; Batur et al., 2003; Griskiene et al., 2018; Mordecai et al., 2008; Warren et al., 2014). In the same way, the administration of low but not high doses of estradiol to ovariectomized rodents facilitates the use of egocentric reference frames (Quinlan et al., 2008, see also Hussain et al., 2014).

Conversely, women performing the task in the mid-luteal phase of their menstrual cycle, have shown worse performance and higher progesterone levels in comparison with peri-ovulatory group. These results supports a possible role for progesterone in hindering egocentric navigation. Similary, Hussain et al. (2016), observed that the preference for the egocentric reference frame was reduced during the luteal phase of menstrual cycle and related this effect with the increases in progesterone levels. On the contrary, Scheuringer and Pletzer (2017) found an effect of facilitation of progesterone on egocentric navigation but their participants also had to remember the description of the route to follow. In fact, in a similar but 3-D navigation task in which the instructions were kept on the screen, no influence of sex hormones on the performance of women, tested during their mid-luteal cycle phase, or men was observed (Harris, Scheuringer and Pletzer, 2019).

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Nevertheless, we found no statistically significant correlation between the levels of the three sex hormones and performance when considering each group separately, in agreement with Hussain et al. (2016). In this regard, studies on the relationship between sex hormones and cognitive performance have yielded contradictory results (see Halari et al., 2015). An alternative explanation for the impact of sex hormones may be related to the activation/inactivation of brain processes responsible for egocentric navigation. Accordingly, these brain processes could be activated by testosterone, favoring egocentric navigation in a tonic manner in men, and they could be "switched on" by increased estradiol levels in women during the pre-ovulatory phase of their cycle and subsequently 'switched off" by increased progesterone levels during the luteal phase.

The present behavioral data are consistent with the influence of these sex hormones on the striatal release of dopamine (DA), a key neurochemical/neuroanatomical component of egocentric navigation in animals and humans (Burgess et al., 2001; Ekstrom et al., 2018; Epstein and Kanwisher, 1998; Iaria et al., 2003; Maguire et al., 1998; Packard and White, 1991; Weniger et al., 2010). Thus, striatal DA is increased by both testosterone and estradiol, whose levels were higher in the groups showing greater accuracy in egocentric navigation (Becker, 2000; Becker and Ramirez, 1981; see Sotomayor-Sarate et al., 2014 for review). In addition, progesterone administration in estradiol-primed animals inhibits DA release in the striatum (Dluzen and Ramirez, 1984; see Yoest et al., 2018, for review), and lesser accuracy was achieved by the women in the mid-luteal phase, who had elevated levels of estradiol and progesterone.

4.4. Limitations

The current study takes into account diverse limitations that have been described (Scheuringer and Pletzer, 2017), as the inclusion of the peri-ovulatory group, and the control for general intelligence of participants and memory load in the route-descriptions. Nevertheless, a persistent limitation of the computerized 2-D matrix format used in our study is the mental rotation requirement with the egocentric instructions without landmarks. Thus, sex differences in mental rotation may have confounded sex differences in navigation (Andreano and Cahill, 2009; see also Scheuringer and

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Pletzer, 2017). In addition, we did not compare the same women across different cycle phases, increasing the inter-individual variability. A further study limitation was the small sample sizes for the different phases of the menstrual cycle and for HC user groups. Our experimental design, with no manipulation of the hormone levels of participants, prevented the drawing of definite conclusions on the involvement of sex hormones in the behavioral differences observed between men and women or among the groups of women. However, the inclusion of six study groups examining sex, menstrual cycle phase, and HC use may compensate to some degree for this limitation.

5. Conclusion

The present study shows sex differences on egocentric navigation without landmarks. Factors responsible for variations in the levels of sex hormones (sex, menstrual cycle phase, and HC use) were associated with these differences in egocentric navigation performance. Sex hormone levels and egocentric navigation results both differed between men and women and fluctuated among the women with natural menstrual cycle, but there was no fluctuation among HC users. A possible explanatory mechanism compatible with these findings may be related to the influence of sex hormones on the brain substrate involved in egocentric navigation. Further research is warranted to explore this possibility.

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Contributors

All authors participated in the research and article preparation. AB devised the concept of the study and drafted the first manuscript. DP designed the experiments and drafted the methods section, and RM was responsible for data acquisition. All authors critically reviewed the manuscript and approved the final version.

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Role of funding source

Funding source had no influence on the study design, or on the collection, analysis, or interpretation of the data. They had no influence on the writing of the report or the decision to submit the article.

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Conflict of interest

The authors declare no conflicts of interest.

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Table and figure legends:

Table 1: Demographic data and hormone concentrations.

Figure 1: Example of 2-D matrix for the navigation task.

Figure 2: Accuracy (A) and response time (B) during egocentric navigation with landmarks (E+L) or egocentric navigation without landmarks (E-L) for men and women with natural menstrual cycles (¶ $p<0.01$ vs. men; # $p< 0.01$ vs. E+L).

Figure 3: Accuracy (A) and response time (B) during egocentric navigation with landmarks (E+L) or egocentric navigation without landmarks (E-L) for men, women with natural menstrual cycle during early follicular, peri-ovulatory, and mid-luteal phases, and women with hormonal contraceptives (HC) during placebo and active phases (* $p<0.01$ vs. early follicular and mid-luteal groups; # $p< 0.05$ vs. E+L).

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Fig. 1

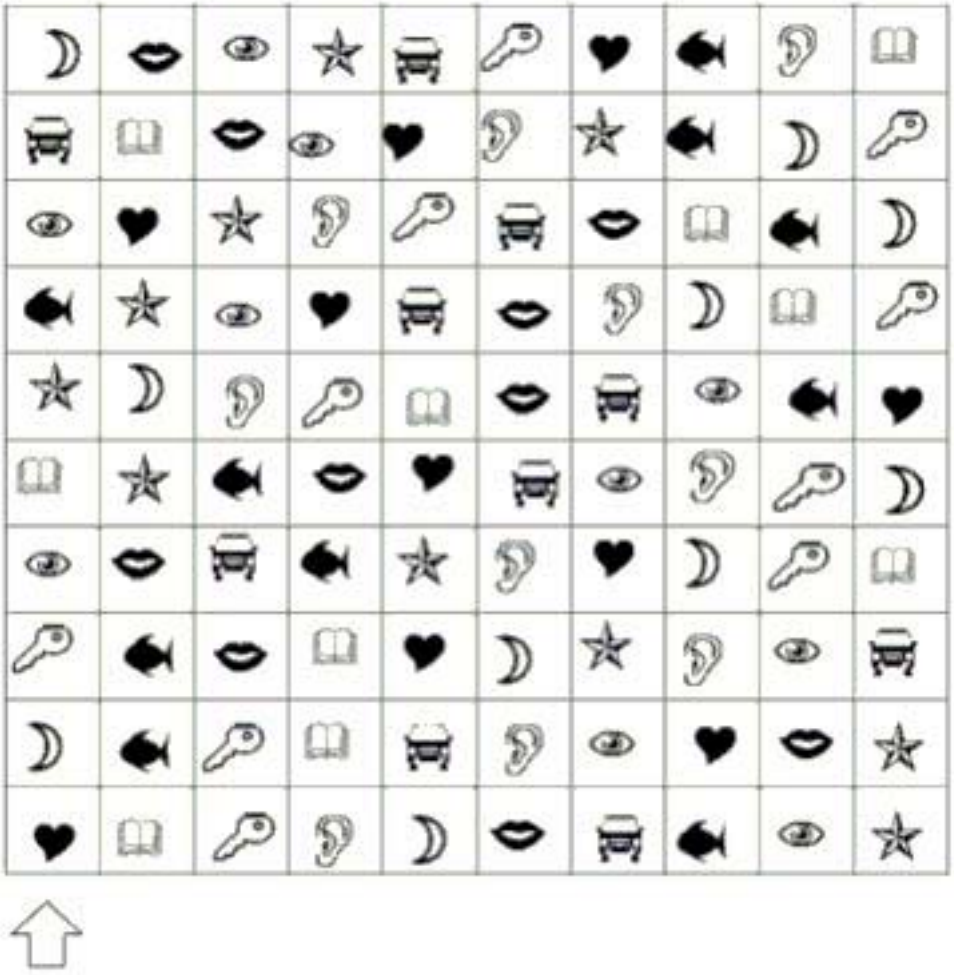

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Fig. 2

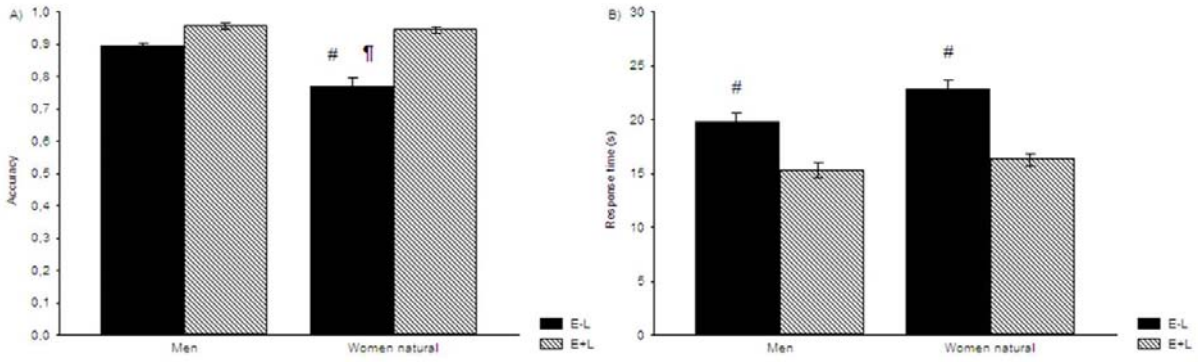

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Fig. 3

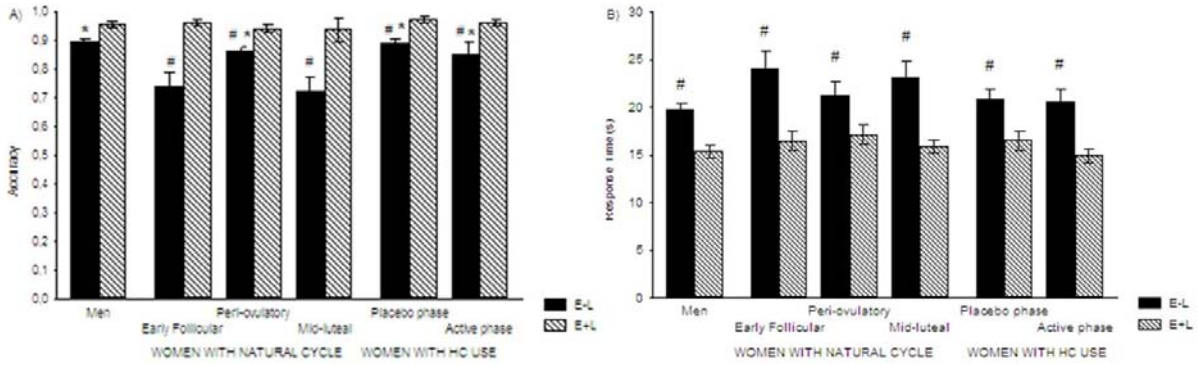

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Table 1

	N	Age	Raven	Menstrual cycle duration (days)	Testosterone (pg/ml)	Estradiol (pg/ml)	Progesterone (pg/ml)	Days from M1	Days M2
Men	26	22.2 (0.3)	49.4 (1.1)		132.4 (6.9)	3.4 (0.3)	67.5 (7.6)		
Natural cycle women									
Early follicular	21	20.9 (0.4)	45.2 (1.5)	29.1 (0.5)	64.7 (5.3)	3.5 (0.2)	91.8(14.5)	3.8 (0.3)	25.3 (
Peri-ovulatory	20	20.9 (0.5)	46.8 (1.5)	29.1 (0.6)	70.3 (4.0)	4.2 (0.3)	111.5(20.8)	14.9 (0.7)	14.2 (
Mid-luteal	21	21.9 (0.7)	47.0 (1.8)	29.1 (0.6)	69.6 (4.4)	4.4 (0.3)	350.2 (29.8)	23.6 (0.5)	5.5 (0
HC user									
Placebo	13	22.2 (1.0)	47.1 (1.4)	28.6 (0.4)	53.2 (6.1)	3.9 (0.3)	79.5 (6.8)		
Active	25	21.9 (0.4)	47.4 (1.1)	28.8 (0.5)	54.4 (5.7)	4.0 (0.4)	79.9 (6.6)		

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Supplement A. Characteristics of hormonal contraceptives (HC) of the present study.

No.	Variety	Phase	Estrogen type and concentration (mg)	Progestin type and concentration (mg)	Androgenic activity of pills	HC type*
1	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
2	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
3	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
4	oral	active	0.02 mg ethinyl estradiol	3 mg drospirenone	low	21/7
5	oral	active	0.03 mg ethinyl estradiol	2 mg chlormadinone acetate	low	21/7
6	oral	active	0.02 mg ethinyl estradiol	3 mg drospirenone	low	21/7
7	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
8	patch	placebo	0.6 mg ethinyl estradiol	6 mg norelgestromin		21/7
9	oral	placebo	0.03 mg ethinyl estradiol	0.15 mg levonorgestrel	high	21/7
10	oral	active	0.03 mg ethinyl estradiol	3 mg drospirenone	low	21/7
11	oral	active	0.03 mg ethinyl estradiol	0.15 mg levonorgestrel	high	21/7
12	vaginal ring	active	0.015 mg ethinyl estradiol	0.120 mg etonogestrel		21/7
13	vaginal ring	active	0.015 mg ethinyl estradiol	0.120 mg etonogestrel		21/7
14	vaginal ring	active	0.015 mg ethinyl estradiol	0.120 mg etonogestrel		21/7
15	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
16	oral	active	0.035 mg ethinyl estradiol	0.25 mg norgestimate	low	21/7
17	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
18	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
19	oral	placebo	0.035 mg ethinyl estradiol	0.25 mg norgestimate	low	21/7
20	oral	placebo	0.02 mg ethinyl estradiol	3 mg drospirenone	low	21/7
21	oral	placebo	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
22	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
23	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
24	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
25	oral	placebo	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
26	oral	placebo	0.02 mg ethinyl estradiol	3 mg drospirenone	low	24/4
27	oral	placebo	0.035 mg ethinyl estradiol	0.25 mg norgestimate	low	21/7
28	oral	placebo	0.02 mg ethinyl estradiol	2 mg dienogest	low	21/7
29	oral	active	1.5 mg estradiol hemihydrate	2.5 mg nomegestrol acetate	low	24/4
30	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
31	oral	active	0.02 mg ethinyl estradiol	3 mg drospirenone	low	21/7
32	oral	placebo	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
33	oral	active	0.035 mg ethinyl estradiol	0.25 mg norgestimate	low	21/7
34	oral	placebo	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7

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35	oral	placebo	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
36	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
37	oral	active	0.02 mg ethinyl estradiol	0.1 mg levonorgestrel	high	21/7
38	oral	placebo	0.03 mg ethinyl estradiol	2 mg dienogest	low	21/7

**Type of hormonal contraceptive: e.g., 21/7 = 21 days of active phase and 7 days of placebo phase.*

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Supplement B. Oral Contraceptives (OCs) users: statistics and analysis of the influence of the exogenous hormones contents in pills on egocentric navigation with or without landmarks

The degree to which HC affect cognition through the exogenous hormones they contain or through their impact on endogenous hormones remains unclear. The influence of hormonal contraceptives (HC) on mental rotation, a spatial ability related to navigation (Galea and Kimura 1993; Saucier et al. 2002), has been investigated (see Griksiene et al., 2018 for review) and their impact on this ability has been attributed to the androgenicity of their progestin content by some researchers (Griksiene and Ruksenas, 2011; Wharton et al., 2008) but to their ethinyl estradiol content by others (Beltz et al., 2015). However, present data showed no influence of these synthetic analogues of estrogen and progestins on the navigation behavior.

Excluding from the analysis the four participants that uses non oral hormonal contraceptives (three participant with vaginal ring and one that uses contraceptive patch, see Supplement 1), the results are very similar to the ones observed with all the HC users (for accuracy: significant effects of condition, $F(1,16)= 116.17$, $p< .01$; group, $F(5,116)= 6.72$, $p< .01$; and group x condition interaction, $F(5,116)=6.55$, $p<.01$, for response times a significant effect of condition $F(1,116)= 159.99$, $p<.01$ was observed. The effect of the group was not significant, $F(5,116)= 1.03$, $p= .39$, and the effect of group x condition interaction was only close to significant, $F(5,116)=2.10$; $p=.07$).

With this background, data on the behavior and sex hormone levels of the OC users are exhibited in Table B. Further analysis showed no differences in results related to the androgenic activity or ethinyl estradiol dose of the pill used. Analysis of users of androgenic (n=20) vs. antiandrogenic (n=14) OCs revealed a significant effect of task (egocentric with landmarks vs. egocentric without landmarks): $F(1,32)=12.69$ $p<.01$ for accuracy; and $F(1,32)=50.16$, $p<.01$ for response times, with accuracy being higher and response times lower for egocentric tasks with landmarks *versus* without them. However, no between-group differences or groups x task interaction (all $F<1$) were found. There were no between-group differences in hormone levels (estradiol and progesterone, both $F<1$; and testosterone, $F(1,32)=$

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2.73; p=.11). Regardless of the type of pill, users obtained high accuracy results when following egocentric instructions without landmarks (all ≥0.85 except for four participants).

Analysis of users of androgenic (n=18) vs. antiandrogenic (n=6) pills with 0.02 mg of ethinyl estradiol revealed the same effect of task, showing higher accuracy and lower response times for egocentric instructions with landmarks *versus* without them (F(1,22)= 4.51, p< .05 for accuracy; F(1,22)= 19.30, p<.01 for response times) and finding no between-group differences or groups x task interaction (all F<1). There were no statistically significant between-group differences in hormone levels (F(1,22)= 2.47, p= .13 for testosterone levels; F<1 for estradiol levels and F(1,22)= 1.05, p=.31 for progesterone levels).

Table B. Mean ± SEM accuracy and response time (RT) during egocentric navigation without landmarks (E-L) and egocentric navigation with landmarks (E+L), and testosterone, estradiol and progesterone levels for oral contraceptive (OC) users of androgenic or antiandrogenic pills. The first two rows show the mean values for all users of androgenic or antiandrogenic pills (independently of the ethinyl estradiol dose), and the next two rows show the mean values for users of androgenic or antiandrogenic OCs with 0.02 mg of ethinyl estradiol (see Supplement A for more details).

	n	Accuracy E-L	RT E-L	Accuracy E+L	RT E+L	Testosterone (pg/ml)	Estradiol (pg/ml)	Progesterone (pg/ml)
Androgenic OC users	20	.85 (.04)	21.4 (1.4)	.96 (.01)	15.9 (0.9)	62.0 (6.8)	4.0 (.4)	85.5 (7.0)
Anti-androgenic OC users	14	.88 (.01)	20.4 (1.0)	.96 (.01)	15.7 (1.0)	47.2 (4.6)	3.6 (.3)	75.0 (8.0)
Users of androgenic pills with 0.02 ethinyl estradiol	18	.86 (.05)	21.3 (1.5)	.96 (.01)	16.1 (1.0)	63.2 (7.5)	4.1 (.4)	83.8 (7.7)
Users of anti-androgenic pills with 0.02 ethinyl estradiol	6	.88 (.03)	18.4 (.6)	.97 (.02)	15.0 (07)	41.5 (7.2)	3.7 (.5)	67.9 (13.8)