Weis, S., Hodgetts, S., Hausmann, M. (2017). Sex differences and menstrual cycle effects in cognitive and sensory resting state networks. *Brain and Cognition,* in press.

Sex differences and menstrual cycle effects in cognitive and sensory resting state networks

Susanne Weis[1,2,3,4], Sophie Hodgetts[3,4], Markus Hausmann[3,4]

[1]Institute of Systems Neuroscience, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

[2]Institute of Neuroscience and Medicine, Brain & Behaviour (INM-7), Research Centre Jülich, Jülich, Germany

[3]Department of Psychology, Durham University, UK

[4]Durham University Neuroimaging Centre (DUNIC), UK

Corresponding author:
Susanne Weis
Institute of Systems Neuroscience
Medical Faculty
Heinrich Heine University Düsseldorf
Düsseldorf, Germany
Institute of Neuroscience and Medicine
Brain & Behaviour (INM-7)
Research Centre Jülich
Jülich, Germany
S.Weis@fz-juelich.de

This study was supported by a grant from the Deutsche Forschungsgemeinschaft to MH (STU 263/3-3, HA 3285/4-1).

Highlights

- Sex differences in resting state (RS) brain connectivity in a cognitive and a sensory brain network
- RS fMRI acquired at different menstrual cycle phases in women and repeated testing sessions in men
- DMN connectivity was stable across repeated tests in men but varied across menstrual cycle in women
- A sensory network showed a retest reliable sex difference with increased RS connectivity in men
- Sex hormones can dynamically influence RS connectivity in cognitive networks in woman

Abstract

It has not yet been established if resting state (RS) connectivity reflects stable characteristics of the brain, or if it is modulated by the psychological and/or physiological state of the participant. Based on research demonstrating sex hormonal effects in task-related brain activity, the present study aimed to investigate corresponding differences in RS networks. RS functional Magnetic Resonance Imaging (RS fMRI) was conducted in women during three different menstrual cycle phases, while men underwent three repeated RS fMRI testing sessions. Independent component analysis was used to identify the default mode network (DMN) and an auditory RS network. For the DMN, RS connectivity was stable across testing sessions in men, but varied across the menstrual cycle in women. For the auditory network (AN), retest reliable sex difference was found. Although RS activity in the DMN has been interpreted as trait characteristic of functional brain organization, these findings suggest that RS activity in networks involving frontal areas might be less stable than in sensory-based networks and can dynamically fluctuate. This also implies that some of the previously reported effects of sex hormones on task-related activity might to some extent be mediated by cycle-related fluctuations in RS activity, especially when frontal areas are involved.

Keywords: fMRI, resting state, default mode network, sex differences, sex hormones

1. Introduction

Sex differences have repeatedly been demonstrated in specific cognitive tasks, with men outperforming women in certain tests of spatial ability (Linn & Petersen, 1985; Voyer, Voyer, & Bryden, 1995) and women outperforming men in certain aspects of verbal ability (Hyde & Linn, 1988). Such cognitive sex differences have been related to differences in functional brain organization which partly result from transient activating effects of sex hormones, such as those during the menstrual cycle (Luine, 2014). Sex hormone modulated changes in functional brain organization have repeatedly been shown in functional brain imaging data for a variety of cognitive domains (e.g. Marecková et al., 2012; Weis, Hausmann, Stoffers, & Sturm, 2011; Weis et al., 2008, Weis & Hausmann, 2010; Zhu, Kelley, Curry, Lal, & Joseph, 2015).

Functional brain imaging data in particular suggests that sex hormone-related changes in functional brain organization might be partly based on modulations of functional connectivity during task-related activity, for example during verbal (Weis et al., 2008) and spatial tasks (Weis et al., 2011). Furthermore, sex hormones, especially estradiol, seem to affect performance and functional brain organization in frontally mediated cognitive tasks, such as top-down cognitive control (Hjelmervik et al., 2012) and selective attention (Thimm, Weis, Hausmann, & Sturm, 2014), specifically.

In addition to task-related functional brain imaging studies, recent fMRI research has revealed several functionally relevant cortical networks that exhibit synchronous fluctuations in brain activity while participants are not undertaking a specific task. These studies have shown that the brain exhibits a pattern of low-frequency oscillations in the BOLD signal at approx. 0.01-0.1Hz (Damoiseaux et al., 2006) while the participant is at rest.

The so-called resting state fMRI (RS fMRI) approach was initially described by Biswal, Yetkin, Haughton, & Hyde (1995), who demonstrated temporally correlated low frequency fluctuations within the sensorimotor cortex during rest. Subsequent research using RS fMRI has identified a number of networks that are spatially comparable to task-related activations (Damoiseaux et al., 2006), such as networks related to executive function (Laird et al., 2011; Seeley et al., 2007), language (Arelin et al., 2015; Laird et al., 2011) and memory (Laird et al., 2011; Vincent et al., 2006).

Additionally, a default mode network (DMN), which is comprised of the dorsal and ventral medial prefrontal cortex (mPFC), the posterior cingulate cortex (PCC)/precuneus and lateral parietal cortex has been identified (Fox et al., 2005; Greicius, Krasnow, Reiss, & Menon, 2003; Laird et al., 2011). The DMN shows higher activity levels during rest and is differentially attenuated during tasks, according to task demands (Fox et al., 2005; Raichle, 2015b; Raichle et al., 2001). Initially, the function of this network was hypothesized to be stimulus-independent thought like daydreaming or mind wandering and 'spontaneous cognition' like thinking about the past/future (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010; Christoff, Gordon, Smallwood, Smith, & Schooler, 2009). More recently, however, it has been suggested (Raichle, 2015a) that RS activity in the DMN reflects spontaneous brain activity playing a "much more fundamental role" than spontaneous cognition. In line with this suggestion, hyperconnectivity and hyperactivity in the DMN has been demonstrated in psychiatric disorders, such as schizophrenia (Whitfield-Gabrieli et al., 2009). In addition to cognitive RS networks, a number of sensory RS networks have been identified, including auditory, sensorimotor, and visual networks (Laird et al., 2011).

Due to the unrestricted nature of RS fMRI, the degree of variability in RS connectivity is currently under debate. Specifically, it is unclear whether RS reflects trait-like structural characteristics of the brain, or whether it is dependent on the psycho- and/or

physiological state of the participant during scanning. Evidence to date has yielded mixed results. For example, some studies have provided evidence for the former notion by demonstrating a link between RS connectivity and anatomical connectivity via white matter pathways (Johnston et al., 2008), including in the DMN (van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009). Further studies have shown RS to be consistent across both multiple test sessions (Damoiseaux et al., 2006) and multiple test sites (Biswal et al., 2010), with medium (Braun et al., 2012; Guo et al., 2012) to high (Zuo et al., 2010) test-retest reliability. In contrast, other studies have suggested that RS connectivity is variable according to time of day (Blautzik et al., 2013), psychological factors, such as mood (Harrison et al., 2008), learning (Zhang et al., 2014) or prior task execution (Pyka et al., 2009; Waites, Stanislavsky, Abbott, & Jackson, 2005).

More recently, it has been suggested that RS connectivity may be influenced by varying levels of sex hormones across the menstrual cycle (Arelin et al., 2015; De Bondt et al., 2015; Hjelmervik, Hausmann, Osnes, Westerhausen, & Specht, 2014; Petersen, Kilpatrick, Goharzad, & Cahill, 2014). While a number of fMRI studies (e.g. Thimm et al., 2014; Weis et al., 2011; Weis et al., 2008) have demonstrated an association between sex hormones and task-related brain activity, it is so far unclear, whether such hormone effects are task-related, or partly mediated by an influence on underlying RS connectivity.

Despite some inconsistencies, several lines of evidence support the notion RS connectivity may be affected by fluctuations in sex hormones. While Weissman-Fogel, Moayedi, Taylor, Pope, & Davis (2010) did not find any significant sex differences in RS connectivity in the DMN, salience, and fronto-parietal networks, other studies suggest that such sex differences exist (Biswal et al., 2010; Filippi et al., 2013; Hjelmervik et al., 2014; Tian, Wang, Yan, & He, 2011). Using a very large sample (N = 603), Allen et al., (2011) revealed stronger RS connectivity within the DMN in females, but no sex difference in the

fronto-parietal network. On the other hand, (Filippi et al., 2013) demonstrated increased RS connectivity in men in parietal and occipital networks, while women showed increased RS connectivity in frontal and temporal regions. Similarly, Hjelmervik et al. (2014) reported higher RS connectivity in women in two fronto-parietal networks.

Such sex differences in RS connectivity might be related to differences in brain structure (Cosgrove et al., 2007). Moreover, sex differences which have been demonstrated in RS data for frontal and parietal regions are somewhat similar to those demonstrated using task-related fMRI during visuo-spatial processing, where men demonstrated increased activity in parietal regions while women typically activate prefrontal regions (Jordan, Wustenberg, Heinze, Peters, & Jancke, 2002). Therefore, it is possible that sex differences in RS activity might partly underlie sex differences in task-related activity, and in turn, behaviors underpinned by these regions.

Critically, results to date are inconsistent, possibly due to methodological differences between RS studies. For example, Bluhm et al., (2008) demonstrated that sex differences were revealed inconsistently, depending on the analytical method used. Furthermore, it is possible that sex differences are limited to specific RS networks. For example, Filippi et al., (2013) suggested that sex differences are more apparent in cognitive as opposed to sensory RS networks. Consequently, these authors suggest that sex differences in connectivity between cognitive RS networks and several frontal regions (such as cingulate cortex, dlPFC, and inferior frontal gyrus) may be related to sex differences in task-related activity during processes such as working memory, emotion regulation, and selective attention.

To date, there have been only very few studies investigating sex hormonal effects on RS connectivity, and similar to the findings on sex differences, there is significant heterogeneity across these studies, both in terms of methodology and results. Petersen et al.,

(2014) adopted a between-subjects design to investigate RS connectivity in the anterior section of the DMN under different hormonal conditions, both across the menstrual cycle in normally cycling women, and in oral contraceptive pill users. This study demonstrated increased RS connectivity between the right anterior cingulate cortex (ACC) and the executive control network, and reduced RS connectivity between the left angular gyrus and the anterior DMN during the luteal as compared to the menstrual phase (termed 'early follicular' by the authors). However, in this study, women in the menstrual phase had very unusually high progesterone levels, resulting in only a small difference in progesterone levels between the cycle groups. Moreover, no cycle difference in estradiol levels was found, suggesting that the women in this study were inaccurate in their cycle phase self-report (see also Gordon, Corbin, & Lee, 1986). Thus, it is possible that these results might be due to other individual differences between participants (e.g. personality traits), especially since a between-subjects design was used.

Hjelmervik et al., (2014) investigated four fronto-parietal (cognitive control) RS networks in a repeated measures design. While this study did not find any cycle-related effect on RS connectivity, sex differences were revealed in two networks. In the anterior fronto-parietal network, women showed greater RS connectivity in the left middle frontal gyrus (MFG), bilateral precuneus, and right inferior parietal lobe. In the right dorsal network, women showed higher connectivity in the left cerebellum. De Bondt et al. (2015) also did not find any effect of sex hormones in fronto-parietal networks (termed 'executive control networks' by the authors). However, in the DMN, an increase in RS connectivity between the network and the cuneus was found in the luteal phase, as compared to the follicular phase.

Finally, Arelin et al. (2015) conducted 32 RS scans in a single subject across four menstrual cycles. Initial analyses using eigenvector centrality revealed that high progesterone levels were associated with increased connectivity of the dorsolateral prefrontal cortex (dlPFC) and

the sensorimotor cortex to the RS network. A further region-of-interest analysis revealed that high progesterone levels were associated with higher RS connectivity between right dlPFC, bilateral sensorimotor cortex, and the hippocampus, as well as between the left dlPFC and bilateral hippocampi during rest. A potential explanation for these differing findings is that this study differed in its analytic approach (i.e., eigenvector centrality and ROI analysis as opposed to ICA), and consequently, does not identify specific RS networks.

The present study investigates sex and sex hormonal effects on RS connectivity in both a cognitive (DMN) and a sensory (auditory) RS network. The DMN was selected as previous studies have demonstrated sex differences in RS connectivity for this network (Allen et al., 2011; Filippi et al., 2013; Weissman-Fogel et al., 2010). In addition, previous behavioral and task-related fMRI evidence suggests that regions of the DMN might be affected by hormonal fluctuations.

The auditory network (AN) was selected as a number of studies using auditory tasks, such as the dichotic listening paradigm, have demonstrated sex hormone effects on functional asymmetry (Cowell, Ledger, Wadnerkar, Skilling, & Whiteside, 2011; Hodgetts, Weis, et al., 2015; Sanders & Wenmoth, 1998; Wadnerkar, Whiteside, & Cowell, 2008). However, it is currently unclear whether these effects are due to sex hormonal effects on task-related activity, or if they were partly dependent on changes to RS connectivity.

The present study is one of few to included both males and females with three repeated measures in every participants. In line with previous RS (Petersen et al., 2014) and task-related data in the same cohort as the data reported here (Thimm et al., 2014) we predicted hormone-related fluctuations in the DMN between the follicular/luteal phase and the menstrual phase. In addition, on the basis of a recent study suggesting that hormonal

effects in dichotic listening are stimulus-driven (Hodgetts, Weis, et al., 2015), we expected RS connectivity in the AN to be relatively stable across the menstrual cycle.

2. Method

2.1 Participants

Nineteen healthy women (out of 21 originally tested, see hormone assay section for exclusion criteria) with a mean age of 24.73 years (*SD* = 3.58; range: 18 – 34 years) and 19 healthy men with a mean age of 24.05 years (*SD* = 2.72; range 20 - 29) completed three sessions of RS fMRI. Age did not differ significantly between the sexes ($t_{(36)} = 0.66$, $p = .51$).

The data presented in this study were collected in the University Hospital, RWTH Aachen University, as part of a larger fMRI study of functional brain organization across the menstrual cycle (Thimm et al., 2014). All participants were native German speakers. All participants were consistent right-handers according to the Edinburgh Handedness Inventory (female LQ = 84.15, *SD* = 13.20; male LQ = 86.19, *SD* = 11.09). Handedness did not differ significantly between the sexes ($t_{(36)} = 0.52$, $p = .61$). Women who had taken hormonal contraceptives or other hormone regulating medications during the previous 6 months were excluded. Following approval by the Local Ethics Committee, all subjects gave their written informed consent according to the Declaration of Helsinki (1991).

The women were tested in three different cycle phases: the menstrual phase (cycle days 1 - 3), follicular phase (cycle days 10 - 12) and luteal phase (cycle day 20 - 22). Time points for each session were estimated according to self-reported menstruation onset, collected over the preceding 6 months, and considered individual average cycle length. To control for a possible session effect, testing order was randomized across subjects such that

all three cycle phases were equally distributed across the three time points. Men were tested three times with one to two weeks in between two testing sessions and were subsequently assigned into three groups, equivalent to the female cycle phases. To control for circadian influences on hormone levels, for every participant, experimental sessions were performed at the same time of day.

2.2 Hormone assays

Blood samples were taken from all women immediately before the test session. Estradiol and progesterone levels were assessed via electrochemiluminescence immunoassay to verify cycle phase. Only women with hormone levels within the expected range (see Table 1) were included in the analysis. As a result, two women were excluded from further analysis. Of the 19 women included, six began testing in their menstrual phase, eight in their follicular phase, and five in their luteal phase.

2.3 Resting state fMRI

Functional magnetic resonance imaging was performed on a 3-Tesla Phillips Systems Achieva scanner, using an eight-channel SENSE head coil and T2*-weighted axial EPI sequences. Each run comprised 250 scans (plus three initial dummy scans) with the following parameters: number of slices: 37 continuous slices parallel to the AC–PC line comprising the whole brain; slice thickness: 3 mm; no interslice gap; matrix size: 64 × 64; field of view: 192 ×192 mm; echo time: 30 ms; repetition time: 2500 ms; flip angle: 81°. The duration of the resting state scan was 10 minutes and 32.5 seconds.

Participants were instructed to relax with their eyes closed during scanning. In addition to the RS scans, two attention tasks were administered (Thimm et al., 2014),

however, RS data was always acquired first. A full debriefing about the goals of the study was performed after the last session of the experiment.

2.4 Data analysis

The data were pre-processed using SPM 8 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/) implemented in MATLAB 2013b (The Mathworks Inc., Natick, MA, USA). After discarding the first three volumes (dummy scans), functional images were realigned to the first scan to correct for head movement. Unwarping was used to correct for the interaction of susceptibility artefacts and head movement. Volumes were normalized to a standard EPI template based on the Montreal Neurological Institute reference brain using default settings for normalization in SPM8 with 16 nonlinear iterations. Finally, all images were smoothed with a Gaussian kernel of 8-mm full-width half-maximum.

GIFT (Group ICA of fMRI Toolbox; v1.3i/2.0e)(Calhoun, Adali, Pearlson, & Pekar, 2001) was used to conduct group level Independent Component Analysis (ICA). In a pre-processing step, the individual data was mean corrected by subtracting the image mean per time point. Thereafter, using default setting, individual data dimensionality was firstly reduced using principal component analysis (PCA) on each participant, separately. The data were then group concatenated and subjected to two further PCA data reduction steps. Secondly, the infomax algorithm was used to estimate forty independent components from the reduced data. Thirdly, back-reconstruction of individual spatial maps from the components estimated at group level was conducted using GICA. The values of each individual map and time courses were scaled to represent percent signal change. No temporal filtering was applied on the data in GIFT.

Spatial sorting was used to identify the networks of interest from the forty components. The DMN and AN were identified via spatial sorting and statistical comparison to the intrinsic connectivity networks (ICNs) described by (Laird et al., 2011).

The component identified as the DMN (Figure 1(a)) correlated primarily with Laird et al.'s ICN 13 (r = 0.397). This component is widespread, bilateral network comprised of areas within the frontal, temporal and parietal lobes as well as medial temporal areas, the posterior cingulate and the precuneus. Laird et al. note that this component is strongly associated with theory of mind tasks, as well as episodic recall, imagining scenes and fixation.

The component identified as the AN (Figure 1(b)) correlated primarily with Laird et al.'s ICN 16 (r = 0.356). This component is comprised of the primary auditory cortices and adjacent areas, and is strongly associated with music and speech perception, as well as tone and pitch discrimination (Laird et al., 2011).

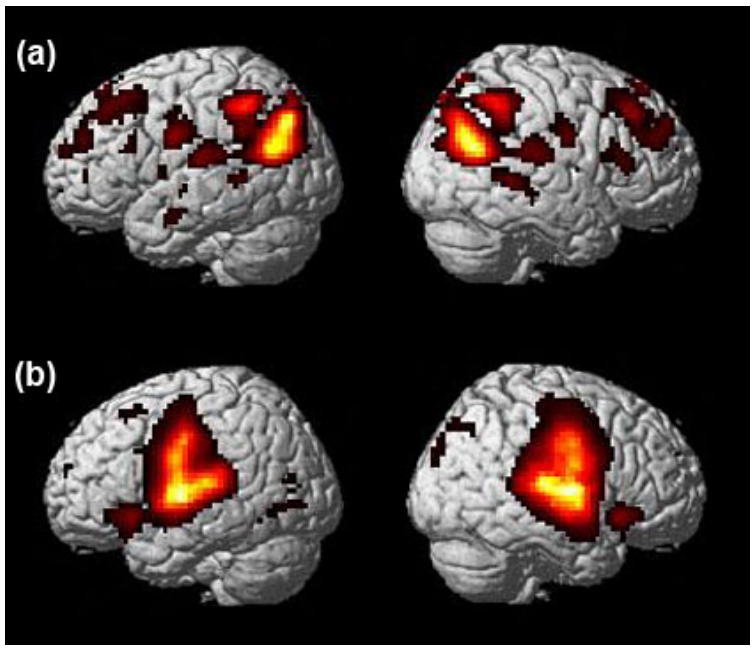

Figure 1. Maps of the DMN (a) and the AN (b). Networks are shown at a threshold of $p < 0.01$, corrected for multiple comparisons across the whole brain at $p < 0.01$ (FWE).

Group analyses of the spatial maps of both components were estimated using the individual back-reconstructed components to investigate whether changes in connectivity between the networks of the components and the rest of the brain varied according to sex or cycle phase. As the spatial maps represented the whole brain, the analysis estimates statistical parameter maps both for voxels within the core region of the component, as well as for those in distant, non-core regions.

A 2 (Sex) × 3 (Cycle Phase) ANOVA was carried out for each of the two components, using the full factorial design setup in SPM8, with Sex specified as being independent and of unequal variance and Cycle Phase specified as dependent and of equal variance. The results were explored at a significance threshold of $p < 0.001$, uncorrected for multiple testing. A Monte-Carlo simulation of the brain volume was employed to establish an appropriate voxel contiguity threshold (Slotnick, Moo, Segal, & Hart, 2003). This correction has the advantage of higher sensitivity, while still correcting for multiple comparisons across the whole brain volume. Assuming an individual voxel type I error of $p < 0.001$, a cluster extent of 17 contiguous resampled voxels was indicated as necessary to correct for multiple voxel comparisons across the whole brain at $p = 0.05$ (based on 10,000 simulations).

3. Results

3.1 Hormone concentrations

Hormone concentrations for each cycle phase are given in Table 1.

In the following analyses, Greenhouse-Geisser adjustments were applied whenever sphericity was violated. A repeated measures ANOVA for estradiol levels across the three cycle phases revealed a significant main effect of cycle phase ($F_{(2, 36)} = 22.55$, $p < .001$, $\eta_p^2 = .56$). Bonferroni-corrected post-hoc tests revealed significant differences between the

menstrual and follicular phase (p = .002), the menstrual and luteal phase (p < .001), and between the follicular and luteal phase (p = .03). Estradiol levels were lowest during the menstrual and highest during the luteal phase.

A repeated measures ANOVA for progesterone levels across the three cycle phases also revealed a significant main effect of cycle phase ($F_{(1.003,\ 18.05)}$ = 35.44, p < .001, η_p^2 = .663). Bonferroni-corrected post-hoc tests revealed a significant difference between the menstrual and luteal phase (p < .001), and between the follicular and luteal phase (p < .001). As expected, progesterone levels were relatively low in the menstrual and follicular phased and increased during the luteal phase.

Table 1. Means, standard deviations, and range (in parentheses) of estradiol and progesterone levels from blood samples of the female sample for each cycle phase.

	Menstrual	**Follicular**	**Luteal**
Estradiol (pmol/l)	104.53 ± 64.35 (25.80 – 252.00)	336.87 ± 270.95 (62.10 – 967.00)	500.84 ± 316.69 (238.00 – 1460.00)
Progesterone (nmol/l)	2.08 ± 0.91 (1 – 4.40)	1.89 ± 0.79 (0.9 -3.5)	32.74 ± 22.06 (8 -81.50)

3.2 Default mode network

The back-reconstructed individual connectivity maps were entered into 2 × 3 ANOVA to identify effects of sex and cycle phase (in women) / repeated tests (in men) on spatial connectivity patterns with the DMN. No significant main effects of sex or cycle phase / repeated tests were identified at the chosen level of significance.

However, the ANOVA revealed a significant interaction between Sex and Cycle phase, located in left middle frontal gyrus (centered in Brodmann Area (BA) 46, cluster size = 33 voxels, Figure 2). Pairwise post-hoc tests were conducted to compare DMN connectivity between the three cycle phases in women (menstrual vs. follicular phase, menstrual vs. luteal

phase, follicular vs. luteal phase) and repeated testing sessions in men (test 1 vs. test 2, test 1 vs. test 3, test 2 vs. test 3). These analyses revealed significantly increased connectivity (Bonferroni – corrected) for the DMN with the left middle frontal area in the menstrual phase as opposed to the follicular phase ($p < 0.05$, corrected for multiple comparisons across the whole brain, see above). All other pairwise comparisons in women and men were non-significant (all $p > 0.001 / 6$). Specifically, no significant differences in DMN connectivity with this area was found between any of the repeated testing sessions in men.

Thus, in summary, the interaction was due to the lack of any significant changes in males across repeated tests in contrast to the significant differences seen in women across cycle phases.

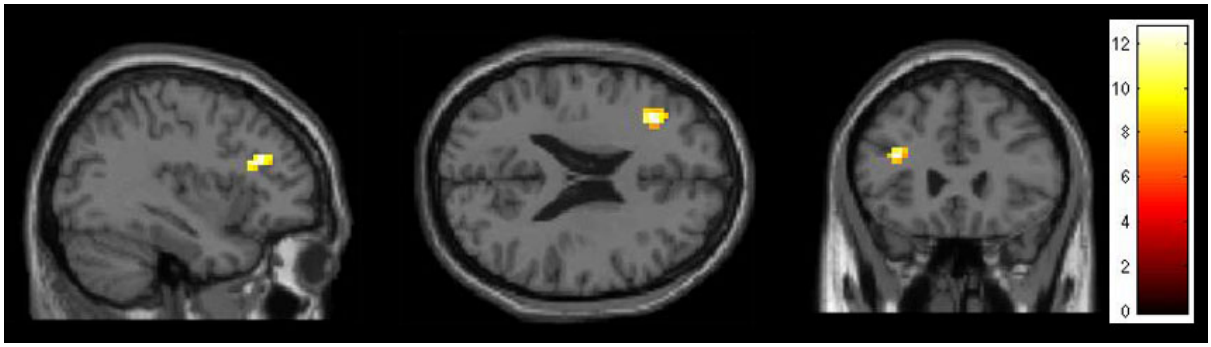

Figure 2. Sex x Cycle interaction effect on RS connectivity with the DMN. Results are shown corrected for multiple comparisons across the whole brain at $p < 0.05$. Blobs represent areas of higher connectivity in the left prefrontal cortex for women in the menstrual phase relative to the follicular and luteal phases.

3.3 Auditory network

The back-reconstructed individual connectivity maps were entered into 2 × 3 ANOVA to identify effects of sex and cycle phase / repeated tests on spatial connectivity patterns with the AN. The analysis identified a main effect of sex in two regions of the left hemisphere, one located in the left superior temporal gyrus (centered at BA 22, cluster size = 66 voxels, Figure 3(a)) and one in the left postcentral gyrus (centered at BA 40, cluster size =

30 voxels, Figure3(b)). Both these areas showed higher connectivity with the AN in men as compared to women.

No significant main effect of cycle phase / testing session and no interaction was found.

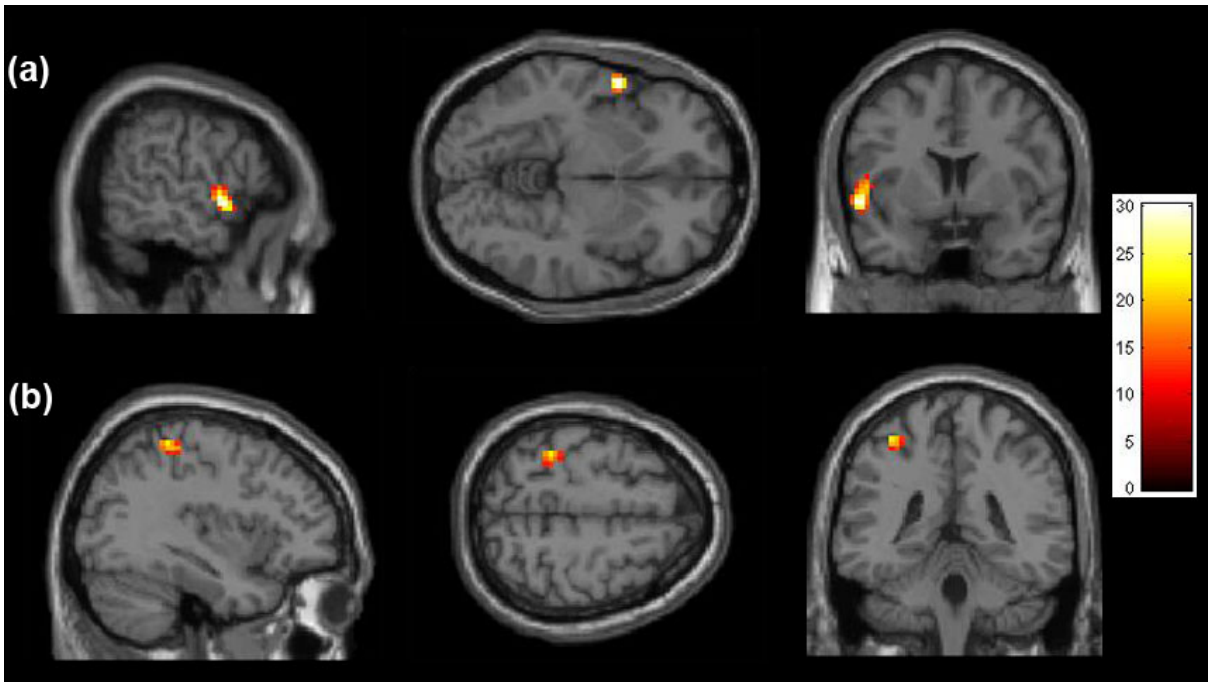

Figure 3. Effect of sex on RS connectivity with the AN. Results are shown corrected for multiple comparisons across the whole brain at $p < 0.05$. Blobs represent areas of higher connectivity in males, relative to females, in the superior temporal gyrus (a) and in the post-central gyrus (b).

4. Discussion

The present study investigated sex differences and sex-hormone effects in the DMN and an auditory RS network. In the DMN, RS connectivity was stable across repeated testing sessions in men but varied across the menstrual cycle in women. Specifically, during the menstrual phase, women showed increased RS connectivity of a specific region of left frontal cortex and the DMN. No sex difference in RS connectivity was found. In contrast, a stable sex difference was found in the AN. Here, men showed increased RS connectivity between

the superior temporal gyrus, the postcentral gyrus, and the AN compared to women. However, no change in RS connectivity was identified across the menstrual cycle in women, or across testing sessions in men.

4.1 Menstrual cycle effect on default mode network

The present study demonstrated that RS connectivity with the DMN was influenced by the natural fluctuations in estrogen and progesterone levels that occur across the female menstrual cycle. This finding has implications for further neuroimaging studies, both with respect to task-related fMRI and RS fMRI.

Firstly, the findings suggest that the test-retest reliability of RS connectivity in DMN is sex dependent. Specifically, the present study revealed that RS connectivity of the DMN was stable in males only. In contrast, in women, DMN RS connectivity fluctuated within relatively short time periods across the menstrual cycle. This is in line with two recent RS fMRI studies: Petersen et al. (2014) demonstrated fluctuations across the menstrual cycle both for the DMN and an executive control network, and De Bondt et al. (2015) who also found sex hormone related changes of RS connectivity in the DMN.

It should be noted that another study found no menstrual cycle effects in fronto-parietal RS networks (Hjelmervik et al., 2014). This might suggest that RS connectivity depends not only on participant's sex but also on the specific RS network. On the other hand, Hjelmervik et al. (2014) did not find any hormone-related variability in any of the studied networks, possibly indicating that the lack of menstrual cycle effect in their study might at least partly due to a methodological issues, for example a lack of sensitivity of the RS data to reliably detect hormone level related changes in RS connectivity.

The finding of a menstrual cycle effect on DMN connectivity also has implications for previous findings on cycle-related changes in functional brain organization. Specifically, the present findings suggest that sex hormone effects on behaviors underpinned by regions of the DMN might be dependent on intrinsic RS connectivity as opposed to actual task-related activity.

Several studies have suggested that DMN deactivation is required for successful task performance (Weissman, Roberts, Visscher, & Woldorff, 2006; for a review, see Sonuga-Barke & Castellanos, 2007). Therefore, menstrual-cycle related changes within the DMN might be reflected in task-related fMRI. For example, Weissman et al. (2006) investigated the relationship between task-related brain activity, RS activity in the DMN, task-related deactivation of the DMN, and task performance. This study showed that poor performance in a selective attention task was associated with reduced task-related activity in ACC and right prefrontal cortex, as well as less deactivation of the DMN. The authors suggest that this reflects a less efficient suspension of task-irrelevant cognitive processes (such as daydreaming), which in turn, interferes with task-related activity. The present data might support this claim, and might suggest that high levels of sex hormones (as present in the luteal and follicular phase) result in reduced RS connectivity of prefrontal regions to the DMN. Thus, these regions are available to be recruited during task-related activity, resulting in better task performance.

In line with previous research (Petersen et al., 2014), the present study demonstrated an increase in RS connectivity with the DMN during the menstrual phase. However, the present study demonstrated increased connectivity between the DMN and a region of left frontal cortex while Petersen et al (2014) showed increased connectivity between the right ACC and the DMN during this phase. Although the findings do not correspond in terms of the specific location of the effect, it is interesting to note that especially frontal areas of the

DMN network were affected hormonally. This is in line with task-related fMRI studies which also found cycle-related changes in functional brain connectivity across the menstrual cycle especially in frontal areas (e.g. Thimm et al., 2014; Weis et al., 2008), possibly due to the high density of estradiol receptors located in these regions (Montague et al., 2008), as compared to other cortical regions (Bixo, Backstrom, Winblad, & Andersson, 1995).

The findings from the present study can also be directly linked to those reported by Thimm et al. (2014), who report task-related fMRI data acquired in the same sample acquired in the same fMRI scanning session (although two participants had to be excluded in the present study). The present findings might suggest that the cycle effect on task-related activity during a selective attention task reported by (Thimm et al., 2014) are to some extent mediated by the sex hormonal effects on RS. It is of interest to note that the region of left middle frontal gyrus showing increased connectivity to the DMN in the menstrual phase of the present study is similar to that highlighted in the functional connectivity analysis of (Thimm et al., 2014).

Specifically, these authors reported that a region of left middle frontal gyrus yielded a stronger negative correlation with a region of left medial frontal gyrus during the menstrual phase. Therefore it is possible that together these findings might reflect DMN interference, with the left middle frontal gyrus being more connected to the DMN during the menstrual phase, and thus, less connected to the left medial frontal region during task-related activity.

4.2 Sex differences in the auditory resting state network

In contrast to the DMN, a similar analysis of the AN revealed a sex difference, with men showing stronger connectivity between regions of the network and left superior temporal gyrus, and left postcentral gyrus. A similar sex difference in an auditory RS network has been reported previously (Filippi et al., 2013), which demonstrated that men showed higher

connectivity between the network and the left insula and right cuneus. In contrast, women showed higher connectivity between the network and the left middle frontal gyrus. While this finding contrasts those of the present study, where no regions of increased connectivity were found in women, it should be noted that the results of (Filippi et al., 2013) did not survive grey matter volume correction, as was applied throughout the present analyses.

The lack of menstrual cycle effect on the auditory RS network has several implications. Firstly, it might suggest that the previously observed hormonal effects on auditory-based tasks, such as dichotic listening (Cowell et al., 2011; Hodgetts, Weis, et al., 2015; Wadnerkar et al., 2008) are possibly due to a hormonal influence on task-related activity, as opposed to underlying intrinsic RS connectivity. Moreover, the present study suggests that sex differences in sensory RS connectivity (at least in the AN) occur independently of female hormonal state at the time of testing. One implication might be that sensory RS networks, as opposed to the DMN and possibly other cognitive RS networks, rather reflect structural differences between males and females.

There are several studies supporting the existence of sex differences in brain structure. Such differences have been shown for grey matter volume in specific brain regions (e.g. Schlaepfer et al., 1995; Good et al., 2001; Im et al., 2006) as well as for structural brain connectivity (e.g. Hsu et al., 2008). Furthermore, both grey matter volume (Lisofsky et al., 2015) and structural brain connectivity (Barth et al., 2016) have been shown to be modulated by sex hormones.

Thus, both hormone-related changes and sex differences in RS connectivity as discussed here might at least partly be driven by structural brain changes. Further studies combining RS connectivity with measures of structural connectivity, such as DTI, may help address this

issue (e.g. Greicius, Supekar, Menon, & Dougherty, 2009) and shed light on the mechanisms underlying sex differences and hormonal influences on RS connectivity.

4.3 Sex hormone effects in cognitive as opposed to sensory networks

In the RS fMRI study by Hjelmervik et al. (2014), the authors concluded that RS networks might be less modulated by sex hormonal fluctuations than task-related fMRI. The present study does not support this claim, as RS connectivity in the DMN changed across the menstrual cycle in women. Instead, findings from the present study might suggest that inconsistent findings and inter-individual differences in RS networks differs not only between the sexes, but also between different RS networks with sensory networks being less susceptible to sex hormonal changes than cognitive networks.

As the DMN and other cognitive networks (such as the executive control network and salience network) predominantly involve prefrontal areas (including medial PFC, dorsolateral PFC, orbitofrontal cortex, and anterior cingulate), it is possible that these networks are more susceptible to menstrual cycle effects due to their higher sensitivity to sex hormonal actions. Indeed, physiological evidence suggests that prefrontal regions have particularly high estradiol levels as compared to other cortical regions, including parietal, temporal and cingulate cortices (Bixo et al., 1995) and a large number of estrogen receptors (Montague et al., 2008). Moreover, evidence from task-related fMRI (Jacobs & D'Esposito, 2011; Joffe et al., 2006) and behavioral studies (Hjelmervik et al., 2012; Keenan, Ezzat, Ginsburg, & Moore, 2001) suggests that the prefrontal cortex is a key target for estrogenic activity in the cortex, and an important site for estrogenic effects on cognition.

In contrast, sensory networks appear to be less affected by hormones, but more susceptible to sex differences in brain structure. This might suggest that sex hormone effects on cognitive performance and/or functional brain organization, at least for auditory-based

tasks such as dichotic listening, are due to sex hormone effects on task-related brain activity and not due to changes in underlying sensory activity.

Whether these findings can generalize to other cognitive and sensory RS networks or whether they are specific to the RS networks investigated here should be clarified in future studies.

4.4 Conclusion

In conclusion, the present study demonstrated that, although RS connectivity in the DMN revealed satisfactory test-retest reliability across repeated testing sessions in men, it is susceptible to hormonal fluctuations in women across the menstrual cycle. Moreover, these findings suggest the effects of sex hormones on task-independent RS connectivity might, at least in part, mediate sex-hormonal effects on tasks involving frontal brain regions (e.g. cognitive control and selective attention).

In contrast, RS connectivity in the auditory RS network was stable across three fMRI sessions in both men and women, suggesting higher test-retest reliability in sensory RS networks, as compared to DMN. However, a sex difference in RS connectivity was found in this network, possibly reflective of structural sex differences in the superior temporal and postcentral gyri.

Altogether, if more studies were to take the activity in RS networks into account when investigating task-related activity in the brain, we might be able to develop a better understanding of the interaction between them.

5. References

Allen, E. A., Erhardt, E. B., Damaraju, E., Gruner, W., Segall, J. M., Silva, R. F., et al. (2011). A baseline for the multivariate comparison of resting-state networks. *Front Syst Neurosci, 5*, 2.

Andrews-Hanna, J. R., Reidler, J. S., Sepulcre, J., Poulin, R., & Buckner, R. L. (2010). Functional-anatomic fractionation of the brain's default network. *Neuron, 65*(4), 550-562.

Arelin, K., Mueller, K., Barth, C., Rekkas, P. V., Kratzsch, J., Burmann, I., et al. (2015). Progesterone mediates brain functional connectivity changes during the menstrual cycle-a pilot resting state MRI study. *Front Neurosci, 9*, 44.

Barth, C., Steele, C.J., Mueller, K., Rekkas, V.P., Arélin, K., Pampel, A., Burmann, I., Kratzsch, J., Villringer, A., Sacher, J. (2016). In-vivo Dynamics of the Human Hippocampus across the Menstrual Cycle. *Sci Rep*, 6, 32833.

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med, 34*(4), 537-541.

Biswal, B. B., Mennes, M., Zuo, X. N., Gohel, S., Kelly, C., Smith, S. M., et al. (2010). Toward discovery science of human brain function. *Proc Natl Acad Sci U S A, 107*(10), 4734-4739.

Bixo, M., Backstrom, T., Winblad, B., & Andersson, A. (1995). Estradiol and testosterone in specific regions of the human female brain in different endocrine states. *J Steroid Biochem Mol Biol, 55*(3-4), 297-303.

Blautzik, J., Vetter, C., Peres, I., Gutyrchik, E., Keeser, D., Berman, A., et al. (2013). Classifying fMRI-derived resting-state connectivity patterns according to their daily rhythmicity. *Neuroimage, 71*, 298-306.

Bluhm, R. L., Osuch, E. A., Lanius, R. A., Boksman, K., Neufeld, R. W., Theberge, J., et al. (2008). Default mode network connectivity: effects of age, sex, and analytic approach. *Neuroreport, 19*(8), 887-891.

Braun, U., Plichta, M. M., Esslinger, C., Sauer, C., Haddad, L., Grimm, O., et al. (2012). Test-retest reliability of resting-state connectivity network characteristics using fMRI and graph theoretical measures. *Neuroimage, 59*(2), 1404-1412.

Calhoun, V. D., Adali, T., Pearlson, G. D., & Pekar, J. J. (2001). A method for making group inferences from functional MRI data using independent component analysis. *Hum Brain Mapp, 14*(3), 140-151.

Christoff, K., Gordon, A. M., Smallwood, J., Smith, R., & Schooler, J. W. (2009). Experience sampling during fMRI reveals default network and executive system contributions to mind wandering. *Proc Natl Acad Sci U S A, 106*(21), 8719-8724.

Cosgrove, K. P., Mazure, C. M., & Staley, J. K. (2007). Evolving knowledge of sex differences in brain structure, function, and chemistry. *Biol Psychiatry, 62*(8), 847-855.

Cowell, P. E., Ledger, W. L., Wadnerkar, M. B., Skilling, F. M., & Whiteside, S. P. (2011). Hormones and dichotic listening: evidence from the study of menstrual cycle effects. *Brain Cogn, 76*(2), 256-262.

Damoiseaux, J. S., Rombouts, S. A., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., et al. (2006). Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A, 103*(37), 13848-13853.

De Bondt, T., Smeets, D., Pullens, P., Van Hecke, W., Jacquemyn, Y., & Parizel, P. M. (2015). Stability of resting state networks in the female brain during hormonal changes and their relation to premenstrual symptoms. *Brain Res, 1624*, 275-285.

Filippi, M., Valsasina, P., Misci, P., Falini, A., Comi, G., & Rocca, M. A. (2013). The organization of intrinsic brain activity differs between genders: a resting-state fMRI study in a large cohort of young healthy subjects. *Hum Brain Mapp, 34*(6), 1330-1343.

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A, 102*(27), 9673-9678.

Good, C. D., Johnsrude, I., Ashburner, J., Henson, R. N., Friston, K. J., & Frackowiak, R. S. (2001). Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage, 14*(3), 685-700.

Gordon, H. W., Corbin, E. D., & Lee, P. A. (1986). Changes in specialized cognitive function following changes in hormone levels. *Cortex, 22*(3), 399-415.

Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A, 100*(1), 253-258.

Greicius, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb Cortex, 19*(1), 72-78.

Guo, C. C., Kurth, F., Zhou, J., Mayer, E. A., Eickhoff, S. B., Kramer, J. H., et al. (2012). One-year test-retest reliability of intrinsic connectivity network fMRI in older adults. *Neuroimage, 61*(4), 1471-1483.

Harrison, B. J., Pujol, J., Ortiz, H., Fornito, A., Pantelis, C., & Yucel, M. (2008). Modulation of brain resting-state networks by sad mood induction. *PLoS One, 3*(3), e1794.

Hjelmervik, H., Hausmann, M., Osnes, B., Westerhausen, R., & Specht, K. (2014). Resting states are resting traits--an FMRI study of sex differences and menstrual cycle effects in resting state cognitive control networks. *PLoS One, 9*(7), e103492.

Hjelmervik, H., Westerhausen, R., Osnes, B., Endresen, C. B., Hugdahl, K., Hausmann, M., et al. (2012). Language lateralization and cognitive control across the menstrual cycle assessed with a dichotic-listening paradigm. *Psychoneuroendocrinology, 37*(11), 1866-1875.

Hodgetts, S., Weis, S., & Hausmann, M. (2015). Sex hormones affect language lateralisation but not cognitive control in normally cycling women. *Hormones and Behavior, 74*, 194-200.

Hsu, J. L., Leemans, A., Bai, C. H., Lee, C. H., Tsai, Y. F., Chiu, H. C., et al. (2008). Gender differences and age-related white matter changes of the human brain: a diffusion tensor imaging study. *Neuroimage, 39*(2), 566-577.

Hyde, J. S., & Linn, M. C. (1988). Gender Differences in Verbal-Ability - a Meta-Analysis. *Psychological Bulletin, 104*(1), 53-69.

Im, K., Lee, J. M., Lee, J., Shin, Y. W., Kim, I. Y., Kwon, J. S., et al. (2006). Gender difference analysis of cortical thickness in healthy young adults with surface-based methods. *Neuroimage, 31*(1), 31-38.

Jacobs, E., & D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J Neurosci, 31*(14), 5286-5293.

Joffe, H., Hall, J. E., Gruber, S., Sarmiento, I. A., Cohen, L. S., Yurgelun-Todd, D., et al. (2006). Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause, 13*(3), 411-422.

Johnston, J. M., Vaishnavi, S. N., Smyth, M. D., Zhang, D., He, B. J., Zempel, J. M., et al. (2008). Loss of resting interhemispheric functional connectivity after complete section of the corpus callosum. *J Neurosci, 28*(25), 6453-6458.

Jordan, K., Wustenberg, T., Heinze, H. J., Peters, M., & Jancke, L. (2002). Women and men exhibit different cortical activation patterns during mental rotation tasks. *Neuropsychologia, 40*(13), 2397-2408.

Keenan, P. A., Ezzat, W. H., Ginsburg, K., & Moore, G. J. (2001). Prefrontal cortex as the site of estrogen's effect on cognition. *Psychoneuroendocrinology, 26*(6), 577-590.

Laird, A. R., Fox, P. M., Eickhoff, S. B., Turner, J. A., Ray, K. L., McKay, D. R., et al. (2011). Behavioral interpretations of intrinsic connectivity networks. *J Cogn Neurosci, 23*(12), 4022-4037.

Linn, M. C., & Petersen, A. C. (1985). Emergence and characterization of sex differences in spatial ability: a meta-analysis. *Child Dev, 56*(6), 1479-1498.

Lisofsky, N., Mårtensson, J., Eckert, A., Lindenberger, U., Gallinat, J., Kühn, S. (2015). Hippocampal volume and functional connectivity changes during the female menstrual cycle. *Neuroimage,* 118,154-62.

Luine, V. N. (2014). Estradiol and cognitive function: Past, present and future. *Hormones and Behavior, 66*(4), 602-618.

Marecková, K., Perrin, J.S., Nawaz Khan, I., Lawrence, C., Dickie, E., McQuiggan, D.A., Paus, T. (2012). Hormonal contraceptives, menstrual cycle and brain response to faces. *Soc Cogn Affect Neurosci.* 2014 Feb;9(2):191-200.

Montague, D., Weickert, C. S., Tomaskovic-Crook, E., Rothmond, D. A., Kleinman, J. E., & Rubinow, D. R. (2008). Oestrogen receptor alpha localisation in the prefrontal cortex of three mammalian species. *J Neuroendocrinol, 20*(7), 893-903.

Petersen, N., Kilpatrick, L. A., Goharzad, A., & Cahill, L. (2014). Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity. *Neuroimage, 90*, 24-32.

Pyka, M., Beckmann, C. F., Schoning, S., Hauke, S., Heider, D., Kugel, H., et al. (2009). Impact of working memory load on FMRI resting state pattern in subsequent resting phases. *PLoS One, 4*(9), e7198.

Raichle, M. E. (2015a). The brain's default mode network. *Annu Rev Neurosci, 38*, 433-447.

Raichle, M. E. (2015b). The restless brain: how intrinsic activity organizes brain function. *Philos Trans R Soc Lond B Biol Sci, 370*(1668).

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proc Natl Acad Sci U S A, 98*(2), 676-682.

Sanders, G., & Wenmoth, D. (1998). Verbal and music dichotic listening tasks reveal variations in functional cerebral asymmetry across the menstrual cycle that are phase and task dependent. *Neuropsychologia, 36*(9), 869-874.

Schlaepfer, T. E., Harris, G. J., Tien, A. Y., Peng, L., Lee, S., & Pearlson, G. D. (1995). Structural differences in the cerebral cortex of healthy female and male subjects: a magnetic resonance imaging study. *Psychiatry Res, 61*(3), 129-135.

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci, 27*(9), 2349-2356.

Slotnick, S. D., Moo, L. R., Segal, J. B., & Hart, J., Jr. (2003). Distinct prefrontal cortex activity associated with item memory and source memory for visual shapes. *Brain Res Cogn Brain Res, 17*(1), 75-82.

Sonuga-Barke, E. J., & Castellanos, F. X. (2007). Spontaneous attentional fluctuations in impaired states and pathological conditions: a neurobiological hypothesis. *Neurosci Biobehav Rev, 31*(7), 977-986.

Thimm, M., Weis, S., Hausmann, M., & Sturm, W. (2014). Menstrual cycle effects on selective attention and its underlying cortical networks. *Neuroscience, 258*, 307-317.

Tian, L., Wang, J., Yan, C., & He, Y. (2011). Hemisphere- and gender-related differences in small-world brain networks: a resting-state functional MRI study. *Neuroimage, 54*(1), 191-202.

van den Heuvel, M. P., Mandl, R. C., Kahn, R. S., & Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Hum Brain Mapp, 30*(10), 3127-3141.

Vincent, J. L., Snyder, A. Z., Fox, M. D., Shannon, B. J., Andrews, J. R., Raichle, M. E., et al. (2006). Coherent spontaneous activity identifies a hippocampal-parietal memory network. *J Neurophysiol, 96*(6), 3517-3531.

Voyer, D., Voyer, S., & Bryden, M. P. (1995). Magnitude of sex differences in spatial abilities: a meta-analysis and consideration of critical variables. *Psychol Bull, 117*(2), 250-270.

Wadnerkar, M. B., Whiteside, S. P., & Cowell, P. E. (2008). Dichotic listening asymmetry: sex differences and menstrual cycle effects. *Laterality, 13*(4), 297-309.

Waites, A. B., Stanislavsky, A., Abbott, D. F., & Jackson, G. D. (2005). Effect of prior cognitive state on resting state networks measured with functional connectivity. *Hum Brain Mapp, 24*(1), 59-68.

Weis, S., Hausmann, M. (2010). Sex Hormones: modulators of interhemispheric inhibition in the human brain. *Neuroscientist*, 16, 132-138.

Weis, S., Hausmann, M., Stoffers, B., & Sturm, W. (2011). Dynamic changes in functional cerebral connectivity of spatial cognition during the menstrual cycle. *Hum Brain Mapp, 32*(10), 1544-1556.

Weis, S., Hausmann, M., Stoffers, B., Vohn, R., Kellermann, T., & Sturm, W. (2008). Estradiol modulates functional brain organization during the menstrual cycle: an analysis of interhemispheric inhibition. *J Neurosci, 28*(50), 13401-13410.

Weissman-Fogel, I., Moayedi, M., Taylor, K. S., Pope, G., & Davis, K. D. (2010). Cognitive and default-mode resting state networks: do male and female brains "rest" differently? *Hum Brain Mapp, 31*(11), 1713-1726.

Weissman, D. H., Roberts, K. C., Visscher, K. M., & Woldorff, M. G. (2006). The neural bases of momentary lapses in attention. *Nat Neurosci, 9*(7), 971-978.

Whitfield-Gabrieli, S., Thermenos, H. W., Milanovic, S., Tsuang, M. T., Faraone, S. V., McCarley, R. W., et al. (2009). Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proc Natl Acad Sci U S A, 106*(4), 1279-1284.

Zhang, H., Long, Z., Ge, R., Xu, L., Jin, Z., Yao, L., et al. (2014). Motor imagery learning modulates functional connectivity of multiple brain systems in resting state. *PLoS One, 9*(1), e85489.

Zhu, X., Kelly, T.H., Curry, T.H.Jr., Lal, C., Joseph, J.E. (2015). Altered functional brain asymmetry for mental rotation: effect of estradiol changes across the menstrual cycle. *Neuroreport,* 26(14), 814-819.

Zuo, X. N., Kelly, C., Adelstein, J. S., Klein, D. F., Castellanos, F. X., & Milham, M. P. (2010). Reliable intrinsic connectivity networks: test-retest evaluation using ICA and dual regression approach. *Neuroimage, 49*(3), 2163-2177.