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Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity

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ABSTRACT

At rest, brain activity can be characterized not by an absence of organized activity but instead by spatially and temporally correlated patterns of activity. In this experiment, we investigated whether and to what extent resting state functional connectivity is modulated by sex hormones in women, both across the menstrual cycle and when altered by oral contraceptive pills. Sex hormones have been shown to have important effects on task-related activity, but few studies have investigated the extent to which they can influence the behavior of functional networks at rest. These hormones are dramatically altered by the use of hormonal contraception, which is used by approximately 100 million women worldwide. However, potential cognitive side effects of hormonal contraception have been given little attention. Here, we collected resting state data for naturally-cycling women ($n = 45$) and women using combined oral contraceptive pills ($n = 46$) and evaluated the differences in resting state activity between these two groups using independent component analysis. We found that in the default mode network and in a network associated with executive control, resting state dynamics were altered both by the menstrual cycle and by oral contraceptive use. Specifically, the connectivity of the left angular gyrus, the left middle frontal gyrus, and the anterior cingulate cortex were different between groups. Because the anterior cingulate cortex and left middle frontal gyrus are important for higher-order cognitive and emotional processing, including conflict monitoring, changes in the relationship of these structures to the functional networks with which they interact may have important consequences for attention, affect, and/or emotion regulation.

Introduction

Sex hormones are neuroactive steroids that influence cognitive function in a variety of domains. These hormones change significantly over the course of the menstrual cycle, and are strongly suppressed by synthetic hormones in women who use hormonal contraceptives. Potential cognitive side effects of hormonal contraceptives have been only minimally explored, despite their widespread use. Effects of hormonal contraceptives on mate selection have been documented, both as they affect women's preferences for men (Wedekind et al., 1995) and men's preferences for women (Kuukasjärvi et al., 2004). Differences in long-term relationship outcomes have also been observed in hormonal contraceptive users (Roberts et al., 2012).

Although the intended purpose of hormonal contraceptives is primarily reproductive, and it might be assumed that behavioral effects of these medications are limited to the reproductive domain, recent studies have suggested that more widespread cognitive changes may be associated with hormonal contraceptive use. Performance on verbal memory (Mordecai et al., 2008), verbal fluency, and mental rotation tasks differ in women using oral contraceptives (OCs; Griksiene and Ruksenas, 2011), as does the pattern of memory retention for recall of an emotional story (Nielsen et al., 2011).

Hormonal contraceptive use has been shown to significantly increase gray matter volume in prefrontal and temporal regions of the brain (Pletzer et al., 2010). Differences have also been observed in the white matter tracts of hormonal contraceptive users, specifically in the fornix (De Bondt et al., 2013). These differences in brain structure suggest that hormonal contraceptives may have functional effects on the brain as well, and indeed recent evidence has emerged suggesting that this is the case. Women using oral contraceptive pills have a larger blood-oxygen-level dependent (BOLD) response in the fusiform face area to images of faces compared to naturally-cycling women (Marečková et al., in press), but a reduced BOLD response in the precentral gyrus to some categories of erotic stimuli relative to women in the follicular phase of the menstrual cycle (Abler et al., 2013). On a verb generation task, women using OCs showed different patterns of activation than naturally-cycling women, and the localization of the differences depended on the cycle phase of the naturally-cycling women (Rumberg et al., 2010).

Hormonal contraceptives have been shown to alter the endocrine response to stressors (Kirschbaum et al., 1999; Maes et al., 1992), and more recently this has been linked to differences in the neural activity

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associated with fear learning. Cortisol administration reduces hippocampal response to a learned fear stimulus in naturally-cycling women, but increases the hippocampal BOLD signal in women using OCs (Merz et al., 2012). Finally, OC use has also been associated with differences in the BOLD response that underlies emotional reactivity, with OC users showing less activity in several prefrontal regions and failing to show the attenuation of activity in the amygdala observed in naturally-cycling women (Gingnell et al., 2012).

With the emergence of these studies indicating that OCs may affect brain activity in response to the demands of specific tasks, we hypothesized that they may have important effects on the resting state of the brain. The brain at rest, rather than entering a quiescent state, has a characteristic pattern of synchronous low-frequency oscillations (Biswal et al., 1995, 1997). A number of functional networks have been observed that show correlated patterns of activity at rest, most notably the default mode network (DMN; Greicius et al., 2003), but also a number of other networks and subnetworks that have since been correlated with specific behavioral domains (Laird et al., 2011; Smith et al., 2009). Previous studies have shown sex influences on resting state functional connectivity (Biswal et al., 2010; Tian et al., 2011), but to our knowledge neither menstrual cycle nor OC use has been explored as potential modulators of resting state dynamics.

Because the tasks previously shown to be affected by OCs were cognitive and affective in nature, and as a result of preliminary data analysis, we hypothesized that the resting state networks most sensitive to OCs would be those associated with cognitive and affective domains. Thus, in this experiment we examined the dynamics of two resting state networks, both of which have been implicated in cognitive and affective tasks: the anterior portion of the default mode network (aDMN), and a network previously described as the executive control network (ECN; Smith et al., 2009).

Differences in the connectivity of these networks were examined across four hormonally distinct groups: (1.) early follicular naturally-cycling women, (2.) luteal naturally-cycling women, (3.) OC users during the inactive week of pill use, and (4.) OC users during the active phase of pill use. These four groups represent, respectively: women with low endogenous sex hormones; women with high endogenous sex hormones; women with low endogenous hormones and low synthetic hormones; and finally, women with high synthetic hormones and low endogenous hormones. The two groups of naturally-cycling women were selected to maximize the contrast between endogenous hormones, as the early follicular phase is characterized by low levels of sex hormones, and the luteal phase is characterized by relatively elevated levels of both estrogen and progesterone. Further, by including both naturally-cycling women and OC users, we were able to examine the extent to which the synthetic hormones in OCs mimicked the effects of endogenous hormones, and by including OC users during the inactive pill week, we were able to examine whether the effects of synthetic hormones (if any) were acute or chronic.

Methods and materials

Participants

Participants were recruited from the University of California, Irvine student population and the surrounding community. Signed, informed consent was obtained before beginning the experiment. Participants were screened by phone and excluded for age under 18 or over 40; a reported history of drug or alcohol abuse; a previous diagnosis of psychiatric, endocrine, or neurological disorders; epilepsy; strokes; brain tumors; current pregnancy or breastfeeding; irregular periods; left-handedness; or non-removable metal implants. Participants did not differ on basic demographic characteristics (see Table S1 in Supplementary materials).

Naturally-cycling women were excluded if they had used any form of hormonal contraception in the previous 3 months. Women assigned to the early follicular group were scanned during cycle days 2 to 6, and women assigned to the luteal group were scanned during cycle days 18 to 24. These days were selected in accordance with those in previous literature (Andreano and Cahill, 2010; Andreano et al., 2008; Maki et al., 2002). We elected to narrow the follicular window very slightly in light of unpublished data from our laboratory showing that salivary hormone levels are sometimes higher on cycle day 1 compared to cycle day 2, and our goal was to minimize hormone levels in the early follicular group. We also sought to avoid the possibility that women in this group were near enough to ovulation to be experiencing the pre-ovulatory estrogen surge, hence the cutoff of cycle day 6.

Women in the birth control group were excluded if they had used OCs for fewer than 3 consecutive months prior to scanning, if their OCs were progestin-only, or if they were not using a 28-day cycle. Inactive pill users were scanned during days 2–6 of inactive pill use, and active pill users were scanned during the 11th to 17th days of active pill use. We treated the beginning of the inactive week from the preceding month's pack as cycle day 1, rather than treating the beginning of the active pill week as cycle day 1. This way, in both the naturally-cycling and the OC group, cycle day 1 corresponded approximately with the onset of menses, and thus days 11 to 17 of pill use corresponded to cycle days 18 to 24 in the naturally-cycling group.

One participant was consented but withdrew before scanning due to feelings of claustrophobia, another was consented but could not be scanned due to technical problems at the imaging center, and three participants in the naturally-cycling group were scanned but later excluded for menstrual irregularities leading to abnormal cycles within the study cycle; their data were not analyzed. After data pre-processing, an additional 2 subjects were excluded for excessive head motion (>4 mm or 4°). Data from 91 subjects was available for analysis: 20 follicular, 25 luteal, 22 inactive pill users, and 24 active pill users.

Saliva collection and assay

Saliva was collected immediately before and after scanning via direct expectoration into 15 mL Falcon tubes. Each sample was approximately 2 mL. Samples were frozen at −20 °C until the day of assaying, when they were defrosted and centrifuged for 15 min at 3000 rpm. The supernatant was decanted into a clean Falcon tube and centrifuged again for 10 min at 3000 rpm before assaying.

Salivary progesterone and 17β-estradiol assays were performed using commercially available immunoassay kits (Salimetrics, State College, PA, USA). The detection sensitivity levels reported for these kits are 5 and 0.1 pg/mL, respectively.

Each of the two samples from each participant was assayed in duplicate. The two samples were averaged together to provide the average hormone level at the time of the resting scan. The average inter-assay coefficient of variance for the progesterone assays was 9.86%, and for the estradiol assays, 7.18%. The intra-assay coefficient of variance was 9.32% and 3.71% for the progesterone and estradiol assays, respectively.

MRI data collection and preprocessing

Data was collected on a Philips Achieva 3T MR scanner (Eindhoven, The Netherlands) equipped with an 8-channel SENSE head coil. Functional echoplanar imaging data was collected in 30 slices with an 80×79 acquisition matrix size, a 70° flip angle, 2 s repetition time, 30 ms echo time, and $3.0 \times 1.5 \times 3\text{mm}^3$ voxel size. Two hundred and fourteen volumes were collected. Structural T1-weighted data was collected in 160 slices with $1.0 \times 1.0 \times 0.67$ mm^3 voxel size.

Once positioned in the scanner, participants were instructed, "Relax, and try to stay as still as you can until the scan is over. The scan will take about 8 minutes." After the resting data was collected, participants remained in the scanner to participate in a separate experiment.

Imaging data was preprocessed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) modules via the toolbox Data Processing