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# Brain activity during a post-stress working memory task differs between the hormone-present and hormone-absent phase of hormonal contraception

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ABSTRACT

Taking hormonal contraceptives (HCs) affects the magnitude of the hormonal stress response and cognition. HCs are usually administered in a monthly cycle with both synthetic-hormone-containing and synthetic-hormone-absent phases. The synthetic hormones contained in HCs affect a wide range of neurophysiological systems, suggesting that effects of the medication might only be observed during the synthetic-hormone-containing phase of the HC cycle. To test this, women were seen twice, once during the hormone-present phase and once during the hormone-absent phase of the HC cycle. In each session, women performed an n-back working memory task to assess pre-stress performance outside of the magnetic resonance imaging scanner, were then exposed to cold pressor stress, and again completed the n-back task during functional magnetic resonance imaging. The free cortisol response to stress remained the same across the HC cycle. Women also performed comparably on the n-back task after stress exposure across the two phases. However, despite these similarities, women displayed greater disengagement of default mode network as task demands increased during the hormone-present phase only, a pattern more in line with working memory-related brain activation under non-stressful conditions reported in other studies. The results suggest that the synthetic hormones contained in HCs may mitigate stress-related disruptions of typical brain activation patterns during the hormone-present phase of the HC cycle, despite exhibiting comparable cortisol responses across the HC cycle. Additional research is required to determine the mechanisms contributing to, and the extent of, such mitigating effects.

## 1. Introduction

Hormonal contraceptives (HCs) exert a range of neurophysiological effects beyond reproduction. For instance, women using HCs exhibit smaller free cortisol responses to psychological and physical stressors compared with women not using HCs (Kirschbaum et al., 1999; Nielsen et al., 2013, 2014). Using HCs has also been shown to affect implicit fear learning (Merz et al., 2012), emotional memory (Nielsen et al., 2014), response inhibition (Gingnell et al., 2016), structural brain volume (Pletzer et al., 2010; Lisofsky et al., 2016), and brain activation at rest (Petersen et al., 2014; Lisofsky et al., 2016; Engman et al., 2018) and during tasks (Gingnell et al., 2016).

Previous work in our lab suggests that the smaller free cortisol response to stress exposure exhibited by women using HCs might impact cognition. This work demonstrated that altering the internal hormonal milieu in post-menopausal women, via nearly 5 years of randomized estradiol administration, reduced the effects of stress on working memory performance (Herrera et al., 2017), compared with women receiving placebo. The findings suggest that changes in hormone status can lead to reduced cortisol responses to stress and can mitigate the magnitude of stress effects on some cognitive processes.

Combination HCs alter the typical hormone profile by inhibiting follicle maturation and ovulation, which in turn prevents ovarian synthesis and release of estradiol and progesterone, and by introducing synthetic analogs of these sex hormones. Accompanying these changes in hormone status are reductions in the free cortisol response to stress, as compared with women not using HCs (Kirschbaum et al., 1999; Nielsen et al., 2013, 2014). Based on our previous findings suggesting that similar hormone-status-induced changes in the cortisol response to stress can mitigate effects of stress on working memory performance

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(Herrera et al., 2017), we aimed to investigate whether HC-related reductions in the free cortisol response to stress were also associated with mitigated effects of stress on cognitive performance.

To date, minimal work has examined how HC-related changes in the free cortisol response to stress relate to cognitive performance under stressful conditions. A good candidate domain for testing how hormone-induced modifications to the cortisol response to stress can also subsequently change the magnitude of stress effects on cognition and cognition-related brain activation is working memory. Working memory is an executive function particularly sensitive to, and reliably impaired by, stress exposure (Schoofs et al., 2008, 2009; Duncko et al., 2009; Luethi et al., 2009; Shields et al., 2016), an effect likely due to the role of the prefrontal cortex in working memory performance (Curtis and D'Esposito, 2003) and the region's sensitivity to glucocorticoids (Arnsten, 2009).

In one study examining the effects of stress on working memory, HC-users were recruited as participants to prevent hormone fluctuations during the natural menstrual cycle from affecting the free cortisol response to stress and cognition in the study (Qin et al., 2009), not to test the effects of HCs on these systems. In this study, women exposed to stress during the final 2 weeks of their HC cycle performed similarly on a working memory task as no-stress controls but did show disruptions of typical working memory-related brain activation. Typically, working memory tasks lead to increased activation of the dorsolateral prefrontal cortex (dlPFC) and deactivation of the default mode network (DMN; Esposito et al., 2006; Hampson et al., 2006). However, acute stress exposure resulted in reduced increased activation of dlPFC and reduced deactivation of DMN during the working memory task, even though working memory performance was not affected by stress (Qin et al., 2009). Within the DMN, impaired deactivation after stress exposure was greatest in the posterior cingulate cortex and medial orbitofrontal cortex (Qin et al., 2009).

This work, as well as studies comparing naturally cycling women to women using HCs, leaves unclear the impact of HC cycle position on differences in the stress response, cognition, and the effects of stress on cognition. In work comparing naturally cycling women and women using HCs, there is careful consideration and systematic investigation of the menstrual cycle phase of naturally cycling women. In contrast, little attention is paid to position in the HC cycle for those women using HCs. This is unfortunate as common forms of contraception (such as the combined oral contraceptive pill) follow a 28-day cycle, similar to the menstrual cycle. During this 28-day cycle, synthetic hormones are typically administered for 21 days (hormone-present phase) followed by 7 days of no synthetic hormone administration (hormone-absent phase).

Failure to test across the HC cycle limits interpretations of how HCs lead to differences in the stress response and cognition. For instance, if physiological and cognitive differences are only observed during the hormone-present phase, then the effects may be related to direct action of the synthetic hormones on the stress response or brain function. On the other hand, if the differences remain consistent into the hormone-absent phase, when synthetic hormones are being metabolized out of the system and are no longer present, then the effects may be a result of low ovarian output of endogenously produced sex steroids, which remains low throughout the entire HC cycle.

Some of the limited work examining effects across the HC cycle suggests women do experience differences across the HC cycle. For instance, women show greater functional connectivity within the executive control network at rest during the hormone-absent phase compared with the hormone-present phase (Petersen et al., 2014). In contrast, women show enhanced verbal memory during the hormone-present phase (Mordecai et al., 2008). However, other work has failed to find differences in emotional memory retrieval across the HC cycle under control versus stress conditions (Mordecai et al., 2017), although it should be noted that the stressor did not significantly increase cortisol levels in the participants in that study. Though some work has begun investigating these effects of HC cycle on cognition and resting brain activation, differences in brain activation during a cognitive task, or effects of stress on such activity, have yet to be investigated.

In the current study, we aimed to expand on the Qin et al. (2009) findings by testing whether the effects of stress exposure on the free cortisol response to stress, working memory performance, and brain activation during a working memory task, remained consistent across the HC cycle. Based on previous work from our lab (Herrera et al., 2019), we did not anticipate the free cortisol response to stress to differ across the HC cycle (see Herrera et al. (2019) for discussion of factors that may influence effects of HCs and HC cycle phase on the free cortisol response to stress). Although we hypothesized that free cortisol response to stress would not differ between HC phases, we still anticipated differences in brain activation during the working memory task across the HC cycle based on other work showing that resting state functional connectivity (Petersen et al., 2014) does differ across the HC cycle. Based on patterns observed in Qin et al. (2009), we were particularly interested in the effects of stress on dlPFC activation and DMN/posterior cingulate cortex deactivation across the HC phases.

## 2. Materials and methods

### 2.1. Participants and sample size selection

A within-subjects design was selected to reduce variability attributable to various factors including synthetic hormone content, which we have shown can affect the magnitude of the free cortisol response to stress (Herrera et al., 2019). The only previous study to compare brain activation patterns across the hormone-present and hormone-absent phases of the HC cycle found a large effect on resting-state functional connectivity (Petersen et al., 2014). We selected a sample size ($N = 20$, within-subjects) to ensure sufficient power to detect moderate-to-large ($d = 0.67$) differences in brain activation patterns across the HC cycle (Faul et al., 2007). This N also provided ample power to detect the main effect of stress on cortisol responses. Based on the main effect of stress across the HC cycle in our previous work (Herrera et al., 2019), a power analysis indicated that only thirteen women would be necessary to achieve 95% power.

Twenty-four women provided written informed consent approved by the University of Southern California Institutional Review Board. Eligible participants were eighteen-to thirty-five-year-old females using a monophasic oral HC or vaginal ring containing seven hormone-absent days for a minimum of four months. Participants also were non-smokers, right-handed, had no known claustrophobia, metal implants, or other contraindications for exposure to the MRI scanner, and no cardiovascular diseases or other contraindications for cold pressor test exposure. Participants were also required to be fluent in English, not using beta-blockers, corticosteroids, antidepressants or other psychoactive drugs, not been pregnant or nursing for at least one year, and not being treated for any major chronic illness. See Table 1 for demographic and mood information.

Four women were excluded from analyses: one for use of an antidepressant, one for early termination due to experiencing claustrophobia in the MRI bore, one for failing to continue beyond session 1, and one for missing too many days of her oral contraceptive requiring stopping the current pack and starting a new pack between sessions 1 and 2. A fifth woman was excluded from cortisol analyses because her baseline salivary cortisol levels exceeded 1.0 μg/dL.

### 2.2. HC cycle and phase definitions

Women completed two sessions, one during the hormone-present phase and one during the hormone-absent phase, order counterbalanced (11 women completed session 1 during the hormone-present phase). Day 1 of the HC cycle was defined as the first hormone-containing pill of a new pack of oral contraception or the day a new contraceptive vaginal ring was inserted, with the first hormone-absent

**Table 1**
Demographic and mood information of participants. Stable mood measures were collected during session 1 only. STAI-Y2, State-Trait Anxiety Inventory for Adults form Y2 (Spielberger et al., 1983); CES-D, Center for Epidemiologic Studies Depression (Radloff, 1977); PSS, Perceived Stress Scale (Cohen et al., 1983); MSPSS, Multidimensional Scale of Perceived Social Support (Zimet et al., 1988).

| | Mean (*range*) |
|---|---|
| *Age (years)* | 23.5 (*18–28*) |
| *Education (years)* | 15.9 (*12–20*) |
| *BMI* | 22.8 (*17.7–34.2*) |
| *Trait Anxiety (STAI-Y2)* | 38 |
| *Depression (CES-D)* | 11.7 |
| *Chronic Stress (PSS)* | 17.5 |
| *Social Support (MSPSS)* | 6.4 |
| *Ethnicity (n)* | Non-Hispanic (15), Hispanic (5) |
| *Race (n)* | American Indian/Alaska Native (0)<br>Asian (7)<br>Pacific Islander/Native Hawaiian (0)<br>Black or African American (1)<br>White (8)<br>More than one race (3)<br>Unknown or not reported (1) |

day being day 22. Women were seen between days 8–21 for the hormone-present phase and between days 24–28 for the hormone-absent phase. See Table 2 for participants' HC information .

### 2.3. Stress exposure and measurement

Women completed the cold pressor test (CPT; Lovallo, 1975) at both sessions, allowing for comparisons of free cortisol response to the CPT between HC phases. A no-stress control condition was not included, but pre-stress working memory performance was assessed for baseline cognitive performance outside of the MRI scanner. The CPT involved holding their dominant hand in ice-cold water (0–3 °C) for up to 3 min and has been show to effectively increase free cortisol levels in our (Lighthall et al., 2011; Herrera et al., 2017; among others) and other (Nielsen et al., 2013, 2014) labs.

Salivary samples are a reliable source for determining biologically available, unbound, levels of hormones (Vining et al., 1983; Tunn et al., 1992; Gozansky et al., 2005; Duplessis et al., 2010). Passive drool salivary samples were collected at baseline and post-scan to assess free cortisol, progesterone, and estradiol levels and were processed using Salimetrics, LLC (State College, PA) ELISA kits, without modifications to the manufacturers' protocol, and measured optically using Molecular Devices, LLC SpectraMax M3 Multi-mode Microplate Reader (Sunnyvale, CA). All other samples were collected using Salimetrics, LLC (State College, PA) oral collection swabs and processed for cortisol only. Swab samples were frozen and shipped to Salimetrics' SalivaLab (Carlsbad, CA) and processed using the Salimetrics Salivary Cortisol Assay Kit (Cat. No. 1–3002), without modifications to the manufacturers' protocol. For passive drool samples, the inter- and intra-assay variations for cortisol (7.9%; 6.9%), progesterone (8.1%; 15.9%), and estradiol (4.4%; 7.6%) were within the expected ranges from our lab. For oral collection swab samples, the inter- and intra-assay variations for cortisol were 6.0% and 4.6%. The Salimetrics High Sensitivity Salivary 17β-estradiol ELISA has a lower limit of sensitivity of 0.1 pg/mL and is very sensitive to 17β-estradiol. Cross-reactivity of this enzyme immunoassay kit is very low for the other natural estrogens and synthetic estrogens, with a percent cross-reactivity of 1.276% for estrone, 0.234% for estriol, and 0.189% for ethinyl estradiol. To control for the diurnal cycle of cortisol, and factors that might influence baseline cortisol levels, sessions were conducted in the afternoons between 1200 and 1800h and participants were asked to refrain from food/drink within one hour, sleep within three hours, and caffeine, alcohol, and exercise within twenty-four hours of their session start time.

Prior to hand immersion participants were asked to rate the amount of stress and pain they were currently feeling from none to the "greatest possible stress" and the "worst possible pain" on separate visual analog scales using 10-cm long lines. Immediately after hand immersion participants were asked to rate the maximum amount of stress and pain they felt while their "hand was in the water" from none to the "greatest possible stress" and the "worst possible pain".

**Table 2**
Hormonal contraceptives used by participants. Number of participants using specific hormonal contraceptive formulations, synthetic hormone content, dosage, and progestin generation.

| Brand | Number of participants using brand | Ethinyl Estradiol dose (mg) | Progestin (Generation) | Progestin dose (mg) |
|---|---|---|---|---|
| Combined Oral Contraception | | | | |
| *Microgestin 1.5/30* | 1 | 0.03 | Norethindrone Acetate (1st) | 1.5 |
| *Microgestin 1/20* | 1 | 0.02 | Norethindrone Acetate (1st) | 1 |
| *Necon 1/35* | 1 | 0.035 | Norethindrone (1st) | 1 |
| *Kelnor* | 1 | 0.035 | Ethynodiol Diacetate (1st) | 1 |
| *Falmina-28* | 1 | 0.02 | Levonorgestrel (2nd) | 0.1 |
| *Ocella* | 1 | 0.03 | Drospirenone (4th) | 3 |
| *Reclipsen* | 2 | 0.03 | Desogestrel (3rd) | 0.15 |
| *Microgestin fe 1.5/30* | 3 | 0.03 | Norethindrone Acetate (1st) | 1.5 |
| *Levora* | 3 | 0.03 | Levonorgestrel (2nd) | 0.15 |
| *Mononessa* | 3 | 0.035 | Norgestimate (3rd) | 0.25 |
| Vaginal Ring | | | | |
| *NuvaRing* | 3 | 0.015 | Etonogestrel (3rd) | 0.12 |

### 2.4. Working memory task

The n-back task was adapted from Qin et al. (2009). Prior to stress exposure, women were trained on the n-back task and then completed one pre-stress block outside of the MRI scanner. Two blocks of the post-stress n-back task were completed in the MRI scanner, beginning approximately 20 min after CPT onset and lasting approximately 16 min (i.e., taking place between 20 min and 36 min post-stress onset). Timing of the n-back task was selected to target the predicted peak of the cortisol response, between 20 and 40 min after the stressor (Kirschbaum et al., 1992, 1999; Duplessis et al., 2010). Each block of the n-back task consisted of 10 alternating trials: five were 2-back trials and five were 0-back trials. Each trial lasted 27 s and consisted of 15 randomly selected single digits presented serially for 400 ms each and a 1400 ms interstimulus interval. The jittered intertrial interval was 8–12s (average = 10s). Immediately prior to each trial participants were shown a 2-s cue disclosing whether the upcoming trial was 0-back or 2-back. Two target digits (13%) were shown per trial. Position of the target digits within the serial array of presented digits was randomized.

During the pre-stress run of the n-back task outside of the scanner, women were told to press a key on a computer keyboard whenever a target digit was presented on the screen. During the post-stress runs of the n-back task inside the MRI scanner, women were told to press a button on a response button box located in their left (non-dominant) hand whenever a target digit appeared on the screen. Prior to beginning the post-stress MRI runs of the task, the response button box was tested to ensure women knew which buttons to press and that responses were being recorded by the computer. Women were able to take a break between the two post-stress n-back blocks and took an average of 16 min to

complete both MRI runs.

### 2.5. MRI data acquisition

Data were acquired on a 3 T Siemens MAGNETOM Prisma$^{FIT}$ Scanner with Tim using a 32-channel head array coil at the University of Southern California Dana and David Dornsife Neuroimaging Center. N-back stimuli were presented on a liquid crystal display monitor (1024 × 768), which participants viewed via a mirror attached to the head coil. Task-associated blood oxygen level-dependent signal was acquired using an echo planar imaging (EPI) sequence (41 interleaved slices, slice thickness = 3 mm, TR/TE = 2000/25 ms, flip angle = 90°, FOV = 192 mm, bandwidth = 2520 Hz/Px). Anatomical images were collected using a high-resolution 3D magnetization prepared rapid gradient echo (MPRAGE) sequence (slices = 176 axial, TR/TE = 2300/2.26 ms, bandwidth = 200 Hz/pixel, flip angle = 9°, slice thickness = 1.0 mm; FOV = 256 mm).

### 2.6. Session structure

Upon arrival, participants completed the informed consent, MRI safety screening form, an incidental-findings consent form and consumed an 8-oz bottle of water. Questionnaires for demographics, emotional state (Positive and Negative Affective Scale; PANAS; Watson et al., 1988), state anxiety (State-Trait Anxiety Inventory for Adults form Y1; STAI-Y1; Spielberger et al., 1983), perceived chronic stress (Perceived Stress Scale; PSS; Cohen et al., 1983), perceived social support (Multidimensional Scale of Perceived Social Support; MSPSS; Zimet et al., 1988), depression (Center for Epidemiologic Studies Depression Scale; CES-D; Radloff, 1977), and trait anxiety (STAI-Y2; Spielberger et al., 1983) were administered throughout the remainder of the session.

The first saliva sample (baseline) was collected after at least 10 min had elapsed since finishing the 8 oz of water, followed by the practice and pre-stress n-back blocks. Next came the second saliva sample (pre-stress-onset), the CPT, and entering the MRI scanner. Once in the scanner, participants provided the third saliva sample (16 m-post-onset) followed by completion of the two post-stress n-back blocks. After the second post-stress n-back block, women provided the fourth saliva sample (39 m-post-onset) and completed a resting state arterial-spin labeling scan not reported here. Next the structural MPRAGE scan was collected and was immediately followed by a fifth saliva sample (53 m-post-onset) and an auditory oddball task not reported here. After completing the auditory oddball task, women were taken out of the scanner and provided the final saliva sample (69 m-post-onset).

### 2.7. Statistical analyses

#### 2.7.1. Hormone analyses

Baseline and end-of-session (69 m-post-onset) sex steroid values were averaged together to approximate estradiol and progesterone levels across the session. There was insufficient saliva to assess progesterone levels in one woman during the hormone-present phase and insufficient saliva to assess estradiol levels in three women, one during the hormone-present phase and two during the hormone-absent phase. Sex steroid levels between the HC phases were compared using two-tailed, paired t-tests.

Salivary cortisol levels were submitted to a 2 (HC phase: hormone-present vs. hormone-absent) x 6 (time: baseline vs. pre-stress-onset vs. 16 m-post-onset vs. 39 m-post-onset vs. 53 m-post-stress vs. 69 m-post-onset) repeated-measures ANOVA to test changes in free cortisol levels in response to CPT exposure between the hormone-present and hormone-absent HC phases. Bonferroni-corrected pairwise comparisons were completed for each of the factors. Two women did not provide enough saliva to measure cortisol in duplicate for a subset of the saliva samples. Due to the within-subject nature of the analyses, these women were removed from cortisol analyses, leaving a total of 17 women in the salivary cortisol analyses.

#### 2.7.2. Subjective pain and stress ratings analyses

Change in subjective feelings of stress and pain from immediately before CPT completion to immediately after CPT completion were analyzed using separate 2 (HC phase: hormone-present phase vs. hormone-absent phase) x 2 (time: pre-to post-stress) repeated-measures ANOVAs for stress and pain.

#### 2.7.3. Behavioral analyses

For the n-back task, hits (button presses to the correct digit during the 0-back and 2-back) and reaction time for hits were tested using 2 (HC phase: hormone-present vs. hormone-absent) x 2 (time: pre-to post-stress) x 2 (load: 0-back vs. 2-back) repeated-measures ANOVAs. Reaction time data for each participant were cleaned of potential outliers by removing any reaction times that exceed ± 2.5 SD of each participant's mean reaction time for 0-back trials and for 2-back trials, separately. Bonferroni-corrected pairwise comparisons were completed for each of the factors.

#### 2.7.4. Cortisol-behavioral analyses

Separate regression analyses were completed to determine the influence of change in cortisol (i.e., *post-stress salivary cortisol level just before the n-back task – baseline salivary cortisol level*) on 0-back and 2-back performance in each HC phase.

Differences in regression outcomes were further compared to determine if cortisol change x 2-back performance relationships significantly differed between HC phases (Lee and Preacher, 2013). To accomplish this, we first had to calculate the correlations for change in cortisol and 2-back performance in each phase, as well as cross correlations for each factor (i.e., all combinations of HC phase, 2-back performance, and cortisol change). Next, these 6 correlation coefficients were converted to *z*-scores using an *r*-to-*z* transformation and further processed using equations (2) and (11) from Steiger (1980) (Lee and Preacher, 2013).

### 2.8. Image processing

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl).

#### 2.8.1. Preprocessing

Registration of the functional data to the high-resolution structural images was carried out using the boundary based registration algorithm (Greve and Fischl, 2009). Registration of the high resolution structural image to the 2 mm MNI-152 standard space images was carried out using FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002). Motion correction was applied using MCFLIRT (Jenkinson et al., 2002); slice-timing correction using Fourier-space time-series phase-shifting; non-brain removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel of full-width half-maximum of 5 mm, grand mean intensity normalization of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50.0s) of 100s. Noise components due to physiological processes or movement, such as extreme motion and physiological artifacts located across the whole brain, were identified and removed using a single-session Independent Component Analysis (ICA) using MELODIC version 3.14 (Beckmann and Smith, 2004). Methods and criteria for identifying and removing noise components have been described in more detail elsewhere (Clewett et al., 2013).

#### 2.8.2. Whole-brain analysis

For both runs of the n-back task, blood oxygen-level dependent (BOLD) signal changes during the 0-back and 2-back blocks were modeled for each participant in each HC phase. Two regressors of

interest were included: 0-back and 2-back. Contrasts for 0-back minus 2-back and 2-back minus 0-back were also modeled into the first-level analyses. The regressors were convolved with a double-gamma hemodynamic response function with added temporal derivative and temporal filtering. The whole-brain contrast images for 0-back minus 2-back and vice versa for each of the two n-back runs were then passed up into a second-level fixed-effects analysis to estimate the average activation for 0-back > 2-back and vice versa across the two runs. The resulting 0-back > 2-back and 2-back > 0-back contrast images provided input to a third-level analysis, where paired differences between the hormone-present and hormone-absent phases for the two contrasts-of-interest were modeled. Significant BOLD signal more associated with 0-back > 2-back and 2-back > 0-back performance within each HC phase and between HC phases were tested using one-sample and paired-differences t-tests, respectively. Clusters in the resultant Z-statistic images were thresholded at $z > 2.3$ with a corrected significance threshold of $p = 0.05$.

#### 2.8.3. Regions of interest (ROI)

Task-related brain networks and DMN are often in competition, with one network showing decreased activation as the other exhibits increased activation (Shulman et al., 1997; Mazoyer et al., 2001). This same competition is observed during working memory performance, which engages dlPFC and disengages the DMN, particularly the posterior cingulate cortex (PCC; Hampson et al., 2006; Esposito et al., 2006). This brain network response to working memory demands is disrupted by acute stress exposure in women tested during the final 2 weeks of the HC cycle (Qin et al., 2009). To examine differences in stress effects on network competition during a working memory task across the HC cycle within subjects, ROI analyses were conducted for the dlPFC and the PCC using Featquery in FSL. The dlPFC mask was created by combining "association test" clusters retrieved from neurosynth.org using the search term "working memory". These cluster maps contain brain-wide activation. In order to limit activation to dorsolateral frontal regions, the "working memory" cluster map was combined with masks of the middle frontal gyrus, inferior frontal gyrus (pars opercularis), and frontal pole. Masks for frontal gyri and the frontal pole were made using the Harvard-Oxford Cortical Atlas in FSL using a threshold of 25% to remove voxels with a lower probability of being within these regions. The PCC mask was created using the Harvard-Oxford Cortical Atlas in FSL using a threshold of 50%. Since dlPFC is defined both structurally and functionally, less conservative thresholding was utilized for the frontal gyri in order to capture the full extent of the region for the dlPFC mask.

Mean parameter estimates were extracted from each participant's first-level functional images for the 0-back and 2-back loads and were converted to percent signal change. These values for both ROIs were then statistically compared using a 2 (HC Phase: hormone-present vs. hormone-absent) x 2 (ROI: dlPFC vs. PCC) x 2 (Load: 0-back vs. 2-back) repeated-measures ANOVA.

## 3. Results

### 3.1. Sex hormone levels

Estradiol and progesterone levels were markedly lower than salivary levels reported for the natural menstrual cycle (Choe et al., 1983), regardless of HC phase. Salivary progesterone did not differ between HC phases, $t_{(18)} = 1.60$, $p = .13$ (mean ± SEM: hormone-present = 58.92 pg/mL ± 8.82, hormone-absent = 46.77 pg/mL ± 8.02). Salivary estradiol levels were higher during the hormone-present phase than during the hormone-absent phase, $t_{(16)} = 2.44$, $p = .03$ (mean ± SEM: hormone-present = 1.09 pg/mL ± 0.08, hormone-absent = 0.95 pg/mL ± 0.06).

### 3.2. Subjective feelings of stress and pain in response to the cold pressor task

Women experienced increases in subjective stress, $F_{(1,19)} = 91.35$, $p < .001$, $\eta_p^2 = 0.83$, and pain, $F_{(1,19)} = 205.62$, $p < .001$, $\eta_p^2 = 0.92$, after CPT exposure in both phases. There were no effects of HC phase on stress, $F_{(1,19)} = 0.13$, $p = .72$, $\eta_p^2 = 0.01$, or pain, $F_{(1,19)} = 2.86$, $p = .11$, $\eta_p^2 = 0.13$. Nor did the change in stress, $F_{(1,19)} = 0.12$, $p = .74$, $\eta_p^2 = 0.01$, or pain, $F_{(1,19)} = 0.09$, $p = .77$, $\eta_p^2 = 0.01$, ratings differ across HC phases. There were no differences in stress or pain ratings between session 1 and 2.

### 3.3. Salivary cortisol response to stress

CPT resulted in significant increases in salivary cortisol levels, $F_{(5,80)} = 7.92$, $p < .001$, $\eta_p^2 = 0.33$. There was no effect of HC phase, $F_{(1,16)} = 0.02$, $p = .90$, $\eta_p^2 = 0.001$, nor a time by HC phase interaction, $F_{(5,80)} = 0.84$, $p = .52$, $\eta_p^2 = 0.05$ (see Fig. 1). The salivary cortisol response to stress did not differ between session 1 and 2.

### 3.4. N-back: behavioral response

Stress exposure affected 0-back and 2-back performance (hit proportions) differently, $F_{(1,19)} = 8.93$, $p = .01$, $\eta_p^2 = 0.32$. Prior to stress exposure, women performed similarly on the 0-back and 2-back task, $p = .3$, 95% CI = −0.12, 0.04. After stress exposure, women performed better on the 0-back task than the 2-back task, $p = .01$, 95% CI = 0.04, 0.26, resulting from impaired 2-back performance after stress exposure, $p = .03$, 95% CI = −0.24, −0.01. The three-way HC phase x pre-to post-stress x n-back load interaction did not reach significance, $F_{(1,19)} = 3.57$, $p = .07$, $\eta_p^2 = 0.16$ (see Fig. 2A). There were no main effects of HC phase, $F_{(1,19)} = 0.20$, $p = .66$, $\eta_p^2 = 0.01$, stress, $F_{(1,19)} = 1.20$, $p = .29$, $\eta_p^2 = 0.06$, or load, $F_{(1,19)} = 2.66$, $p = .12$, $\eta_p^2 = 0.12$, on behavioral n-back performance. There also were no interactions between HC phase and stress, $F_{(1,19)} = 0.46$, $p = .51$, $\eta_p^2 = 0.02$, or HC phase and load, $F_{(1,19)} = 0.84$, $p = .37$, $\eta_p^2 = 0.04$. Behavioral n-back performance did not differ between session 1 and 2 pre- or post-stress.

### 3.5. N-back: reaction time

Women were significantly slower on the 2-back than the 0-back, $F_{(1,16)} = 55.52$, $p < .001$, $\eta_p^2 = 0.78$, however, there was no effect of stress, $F_{(1,16)} = 2.26$, $p = .15$, $\eta_p^2 = 0.12$, phase, $F_{(1,16)} = 0.08$, $p = .78$, $\eta_p^2 = 0.01$, nor a three-way interaction, $F_{(1,16)} = 2.62$, $p = .13$, $\eta_p^2 = 0.14$, on reaction time to hits during the n-back task (see Fig. 2B).

### 3.6. N-back: performance, salivary cortisol response, and hormonal contraceptive phase

Linear regression analyses revealed that increases in salivary cortisol levels predicted better performance on the 2-back during the hormone-present HC phase, accounting for 27% of the variance, $R^2 = 0.27$, $F_{(1,18)} = 6.25$, $p = .02$ (see Fig. 2C), but did not predict performance on the 2-back during the hormone-absent HC phase, accounting for only 6% of the variance, $R^2 = 0.06$, $F_{(1,16)} = 0.96$, $p = .34$ (see Fig. 2C).[1] These different patterns observed between phases for effects of cortisol change

[1] One woman was removed from the hormone-absent phase for regression analyses due to experiencing a markedly stronger cortisol response to the cold pressor stress (solid dark data point in Fig. 2C and D). When this participant was included in the analyses, change in cortisol negatively predicted performance on the 2-back during the hormone-absent HC phase, accounting for 35% of the variance, $R^2 = 0.35$, $F_{(1,17)} = 8.73$, $p = .01$, and making the relationships between cortisol and performance in the conditions even more different from each other.

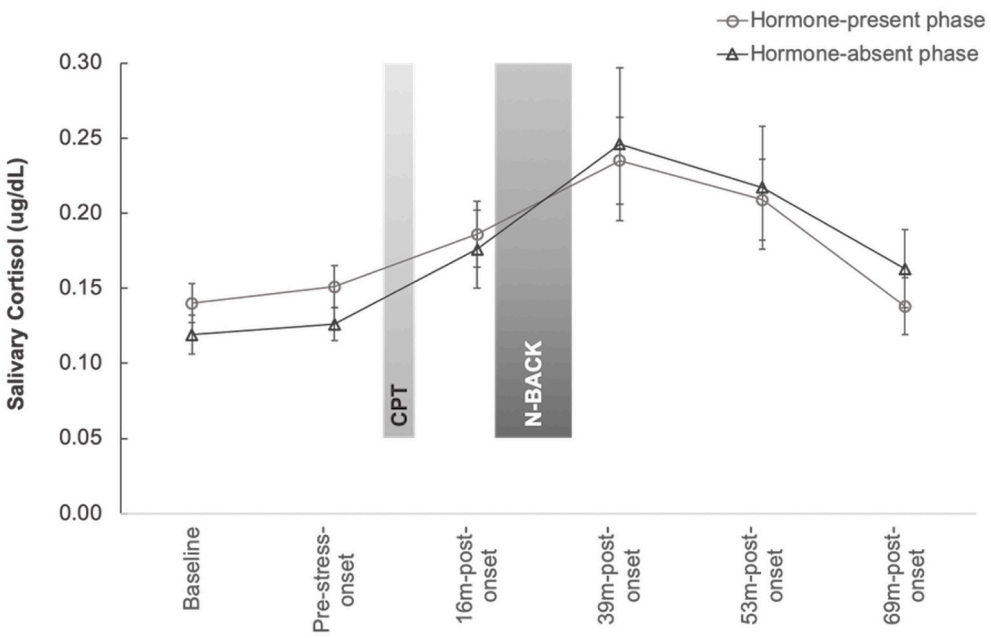



**Fig. 1.** Effects of stress exposure on salivary cortisol levels across the hormonal contraceptive cycle. The main effect of stress was significant ($p < .001$). Salivary cortisol levels increased to a similar magnitude and across a similar time course in both the hormone-present and hormone-absent phase.

on 2-back performance were significantly different from one another (z-score = −2.76, p = .006; Lee and Preacher, 2013). Regression analyses revealed no systematic relationship between magnitude of salivary cortisol change on 0-back performance in either the hormone-present, $R^2 = 0.001$, $F_{(1,18)} = 0.02$, $p = .89$, or hormone-absent, $R^2 = 0.07$, $F_{(1,16)} = 1.12$, $p = .31$, phase (see Fig. 2D).

### 3.7. Whole-brain activation during the 0-back and 2-back

High working memory load led to greater deactivation of default mode network regions during both HC phases (see Table 3 and Fig. 3A). Women in the hormone-present phase showed greater load-related deactivation in clusters centered in frontal pole, posterior cingulate cortex, and middle temporal gyrus, all regions associated with default mode network, than did women in the hormone-absent phase (see Table 3and Fig. 3A).

By contrast, higher working memory load led to greater activation in frontal regions in both phases, and cerebellar regions during the hormone-present phase (see Fig. 3B). However, HC phase did not significantly affect the degree of load-related increases in activation, though women showed less deactivation of regions associated with default mode network during the hormone-absent phase (see Fig. 3B).

### 3.8. ROIs: N-back performance and PFC and PCC activation

Region of interest analysis revealed significant activation of the dlPFC and deactivation of the PCC, $F_{(1,19)} = 136.32$, $p < .001$, $\eta_p^2 = 0.88$. Significant interactions between load and ROI, $F_{(1,19)} = 73.37$, $p < .001$, $\eta_p^2 = 0.79$, and between all three factors, $F_{(1,19)} = 6.60$, $p = .02$, $\eta_p^2 = 0.26$, were also found (see Fig. 4). Bonferroni-corrected pairwise comparisons showed that though dlPFC and PCC activation did not differ during the 0-back, $p = .71$; 95% CI = −0.07, 0.05, activation in the regions significantly differed during the 2-back, $p < .001$; 95% CI = 0.37, 0.53, resulting from significantly increased activation in dlPFC, $p = .001$; 95% CI = 0.10, 0.31, and significant deactivation in PCC, $p < .001$; 95% CI = −0.32, −0.19, during the 2-back. The deactivation of PCC during the 2-back was greater when women were in the hormone-present phase than during the hormone-absent phase, $p = .02$; 95% CI = −0.22, −0.03.

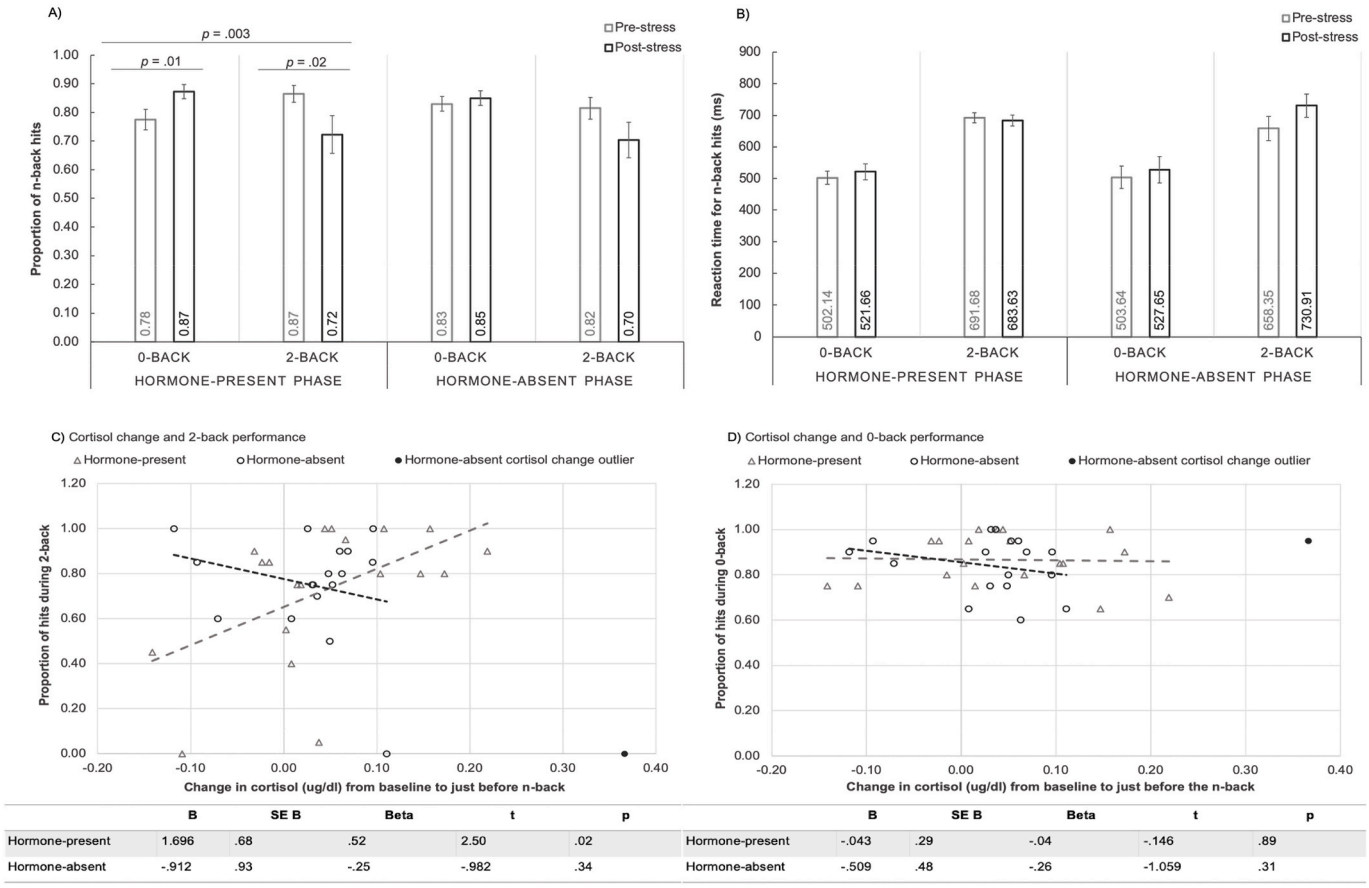
## 4. Discussion

A previous study found that stress exposure inhibits dlPFC activation and PCC deactivation in women taking HCs (Qin et al., 2009). In the current study, we examined whether these patterns indicating impaired cognition under stress differ across the phases of the HC cycle, when synthetic hormones are present and when they are absent. Given that other work shows that brain activation (Petersen et al., 2014) and some cognitive processes (Mordecai et al., 2008) differ across phases of the HC cycle, we investigated whether stress exposure differentially affected working memory processes across the HC cycle within individual women. We found that women showed more effective DMN deactivation during high-demand working memory loads during the hormone-present phase versus the hormone-absent phase of the HC cycle. The relationship between the magnitude of the salivary cortisol response to CPT stress and working memory performance also differed across the HC cycle, with larger cortisol responses predicting better performance during the hormone-present phase only.

### 4.1. Bioavailable cortisol response across the HC cycle

We replicated previous work in our lab (Herrera et al., 2019) showing no difference in free cortisol response to stress across the HC cycle. The importance of establishing how HC phase affects the cortisol response to stress is twofold. First, establishing this pattern can help elucidate possible mechanisms contributing the differences observed between HC-users and non-users. Second, establishing this pattern has implications for methodological considerations in stress studies including HC-users in the participant pool. Much of the stress research including HC-users does not explicitly state when in the HC cycle women were tested, as is routinely done for the menstrual cycle. Thus, determining whether or not the stress response remains stable across the HC cycle can help inform whether such considerations must be made in future research.

The similar free cortisol responses to CPT across the HC cycle suggests that the mechanism contributing to smaller cortisol responses in HC-users compared with non-users remains stable across the HC cycle. One possible consistent feature of the HC cycle is reduced ovarian output of estradiol and/or progesterone, which is low during the hormone-




| | B | SE B | Beta | t | p |
|---|---|---|---|---|---|
| Hormone-present | 1.696 | .68 | .52 | 2.50 | .02 |
| Hormone-absent | -.912 | .93 | -.25 | -.982 | .34 |

| | B | SE B | Beta | t | p |
|---|---|---|---|---|---|
| Hormone-present | -.043 | .29 | -.04 | -.146 | .89 |
| Hormone-absent | -.509 | .48 | -.26 | -1.059 | .31 |

**Fig. 2.** Effects of stress exposure on behavioral working memory performance across the hormonal contraceptive cycle. A) Proportion of hits during the 0-back and 2-back before and after stress in the hormone-present and hormone-absent phase. The interaction between load and time was significant ($p = .008$). Hits for the 0-back and 2-back were similar pre-stress but differed post-stress ($p = .01$), with 2-back hits declining post-stress ($p = .03$). During the hormone-present phase the interaction between load and time was significant ($p = .003$). 0-back hits increased after stress ($p = .01$), while 2-back hits decreased post-stress ($p = .02$). During the hormone-absent phase there were no significant changes in 0-back and 2-back hits pre- and post-stress. B) Reaction time to hits during the 0-back and 2-back in the hormone-present and hormone-absent phases before and after stress. The main effect of load was significant, with slower reaction times for 2-back hits than 0-back hits ($p < .001$). C) Relationship between change in free cortisol from baseline to just before the n-back task and 2-back hits in the hormone-present and hormone-absent phases. One cortisol change outlier was removed from the hormone-absent phase (solid black marker). Change in cortisol positively predicted 2-back hits in the hormone-present phase but had no relationship to 2-back hits in the hormone-absent phase. When the hormone-absent phase outlier is included, change in cortisol negatively predicted 2-back hits, accounting for 35% of the variance ($R^2 = 0.35$, $F_{(1,17)} = 8.73$, $p = .01$). D) Relationship between change in free cortisol from baseline to just before the n-back task and 0-back hits in the hormone-present and hormone-absent phases. One cortisol change outlier was removed from the hormone-absent phase (solid black marker). There was no relationship between change in cortisol and 0-back hits in either phase. The relationship during the hormone-absent phase did not change when the cortisol outlier was included.

present phase and remains low during the hormone-absent phase. This consistent pattern across the HC cycle also suggests that stress studies need not control for HC cycle phase in future work limited to the physiological stress response. However, we have previously shown that the synthetic progestins contained in combined hormonal contraception (i.e., contraception containing both an estradiol and progestin component) can affect the pattern of salivary cortisol response to stress observed across the HC cycle (Herrera et al., 2019). Thus, care should be taken to record the formulation used by participants.

### 4.2. Effects of post-stress changes in bioavailable cortisol on working memory performance

Although women performed similarly on the n-back task after stress in both HC phases, our findings suggest that the magnitude of cortisol increase differentially affects working memory performance in each phase. Specifically, it appears that larger changes in cortisol from before to after stress exposure predicts better working memory performance during the hormone-present phase but not the hormone-absent phase. The pattern suggests that future work examining the effects of stress on cognition in HC-users should control for HC cycle position. The pattern also suggests that the neurophysiological effects of HCs on cortisol activity or detection and cognition might change across the HC cycle.

Finding a positive relationship between cortisol response and working memory performance during the hormone-present phase is surprising given that higher cortisol levels are typically associated with greater working memory impairment (Lupien et al., 1999; Oei et al., 2006; Taverniers et al., 2010). The effect of stress on cognitive performance is often described as following an inverted-U shaped pattern, with moderate levels of stress leading to enhanced performance and too little or too much stress leading to impaired performance (Yerkes and Dodson, 1908; Salehi et al., 2010; but see, Hancock and Ganey, 2003). Based on this inverted-U function of stress exposure on cognitive performance, our pattern of results suggests that, although women may not experience changes in cortisol reactivity to stressors across the cycle, women may experience shifts in the "optimal" level of stress that leads to enhanced performance. One possible mechanism for why cortisol would differentially affect cognition across the HC cycle could be modulation of type II glucocorticoid receptor concentration across the HC cycle. Cortisol binds to two types of glucocorticoid receptors, type I (MR) and

**Table 3**
Clusters of significant activation retuned in whole-brain voxel-wise analyses. Clusters are shown for 0-back minus 2-back and 2-back minus 0-back in the hormone-present and hormone-absent phases separately and for the same load contrasts in hormone-present minus hormone-absent.

| | Brain Region | H | MNI | | | Voxels | Z-max | P |
|---|---|---|---|---|---|---|---|---|
| Contrast | | | x | y | Z | | | |
| **Hormone-present phase** | | | | | | | | |
| **0-back > 2-back** | Superior Frontal Gyrus | L | −2 | 56 | 20 | 82,136 | 6.18 | <.00001 |
| **2-back > 0-back** | Lateral Occipital Cortex, superior division | L | −30 | −62 | 50 | 4851 | 5.04 | <.00001 |
| | Precentral Gyrus | L | −36 | −2 | 32 | 2911 | 4.65 | <.00001 |
| | Middle Frontal Gyrus | R | 36 | 6 | 66 | 1517 | 4.4 | <.00001 |
| | Supplementary Motor Cortex (juxtapositional lobule cortex) | L | −6 | 6 | 60 | 976 | 4.75 | <.0001 |
| | Frontal Pole | R | 40 | 40 | 32 | 767 | 3.88 | 0.00027 |
| | Frontal Pole | L | −46 | 56 | 8 | 719 | 4.22 | 0.00046 |
| | Cerebellar VI | R | 30 | −64 | −26 | 485 | 5.24 | 0.0080 |
| | Cerebellar VI | L | −26 | −62 | −28 | 419 | 4.33 | 0.019 |
| **Hormone-absent phase** | | | | | | | | |
| **0-back > 2-back** | Posterior Cingulate Gyrus | L | −6 | −38 | 40 | 8796 | 4.55 | <.00001 |
| | Frontal Pole | R | 14 | 50 | 46 | 4301 | 4.5 | <.00001 |
| | Parietal Operculum Cortex | R | 56 | −28 | 22 | 3397 | 5.23 | <.00001 |
| | Lingual Cortex | R | 28 | −46 | −8 | 1526 | 4.08 | <.00001 |
| | Angular Gyrus | L | −50 | −54 | 28 | 477 | 3.85 | 0.011 |
| **2-back > 0-back** | Lateral Occipital Cortex, superior division | L | −16 | −60 | 56 | 4126 | 4.06 | <.00001 |
| | Precentral Gyrus | L | −32 | 2 | 30 | 1051 | 3.64 | <.0001 |
| | Precentral Gyrus | L | −26 | −14 | 46 | 685 | 3.47 | 0.00095 |
| | Precentral Gyrus | R | 62 | 18 | 32 | 572 | 3.36 | 0.0035 |
| | Superior Frontal Gyrus | R | 20 | 8 | 46 | 551 | 3.96 | 0.0046 |
| | Paracingulate Gyrus | L | −12 | 18 | 40 | 428 | 3.39 | 0.021 |
| | Frontal Pole | L | −36 | 50 | 0 | 383 | 4.17 | 0.039 |
| **Hormone-present > Hormone-absent** | | | | | | | | |
| **0-back > 2-back** | Frontal Pole | L | −8 | 42 | 48 | 1778 | 3.87 | <.00001 |
| | Posterior Cingulate Cortex | L | −8 | −24 | 38 | 683 | 3.4 | <0.001 |
| | Middle Temporal Gyrus, Temporo-occipital cortex | R | 50 | −58 | 6 | 341 | 3.26 | 0.046 |
| **2-back > 0-back** | no significant results | | | | | | | |

type II (GR), with a greater affinity to MR. The greater affinity to MR results in cortisol saturating MR at basal levels and GR at stress levels (Reul and Kloet, 1985). Saturation of GR with stress hormone exposure negatively impacts cognitive performance and is proposed to account for the inverted-U function of stress on cognitive performance (Conrad et al., 1999). GR mRNA levels have been reported to change across the natural menstrual cycle (Altemus et al., 1997), as has the relationship between cortisol levels and performance (Andreano et al., 2008). These findings suggest it is possible that the cyclic presence and absence of synthetic hormones across the HC cycle might also affect GR concentration, which could influence learning and memory processes (Lupien and McEwen, 1997; Conrad et al., 1999). Specifically, in the case of HC use, GRs might be upregulated during the hormone-present phase of the HC cycle. This upregulation of GR would result in a smaller proportion of occupied GR under stressful conditions during the hormone-present HC phase, despite experiencing the same levels of bioavailable cortisol across the HC cycle. This reduced occupation of GR would prevent stress-induced impairment in performance by limiting the proportion of occupied receptors, effectively shifting the inverted-U and leading to maintained cognitive function in the face of comparable stress levels.

### 4.3. Brain activation during the working memory task

Post-stress brain activation differed between HC phases during the working memory task. Brain regions associated with the DMN, including the PCC, were less deactivated under high working memory load in the hormone-absent phase than in the hormone-present phase.

Typically, the DMN disengages during working memory performance as dlPFC is engaged (Hampson et al., 2006; Esposito et al., 2006), a pattern disrupted by acute stress exposure (Qin et al., 2009). Our findings suggest that post-stress disruption of this pattern is stronger during the hormone-absent phase than the hormone-present phase. Particularly, that women are better able to disengage DMN as task demands increase under stressful conditions during the hormone-present phase than during the hormone-absent phase, despite having similar levels of bioavailable cortisol in both phases, suggests that presence of the synthetic hormones contained in HC modulates how cortisol acts on the brain to perform working memory tasks. Importantly, however, additional research including a no-stress control condition is necessary to determine whether the difference between HC phases is attributable to protection against stress and cortisol effects on brain activation during the hormone-present phase or whether this difference exists regardless of stress exposure.

Again, the implications of these findings are two-fold. First, that stress exposure leads to different patterns of brain activation across the HC cycle suggests that future studies including HC-users should control for HC cycle position during testing. Second, these findings further confirm mechanistic differences between phases on the neurophysiological effects of stress-induced cortisol increases across the HC cycle. For instance, if HC use can affect peripheral type II glucocorticoid receptor concentration, as observed across the menstrual cycle (Altemus et al., 1997), then it may be possible that HC use also affects receptor levels in the brain. If so, then the impact of cortisol on brain function would also be influenced by HC cycle phase.

### 4.4. Limitations and caveats

Our study has some limitations that should be addressed in future research. First, although our within-subject design helps improve power to detect HC cycle effects, we had a relatively small sample size. Thus, more extensive research should be completed to see if these effects replicate. Second, we did not use a between-session no-stress comparison condition in this study, because our primary question concerned whether stress effects differed across the HC phases. Also, as briefly

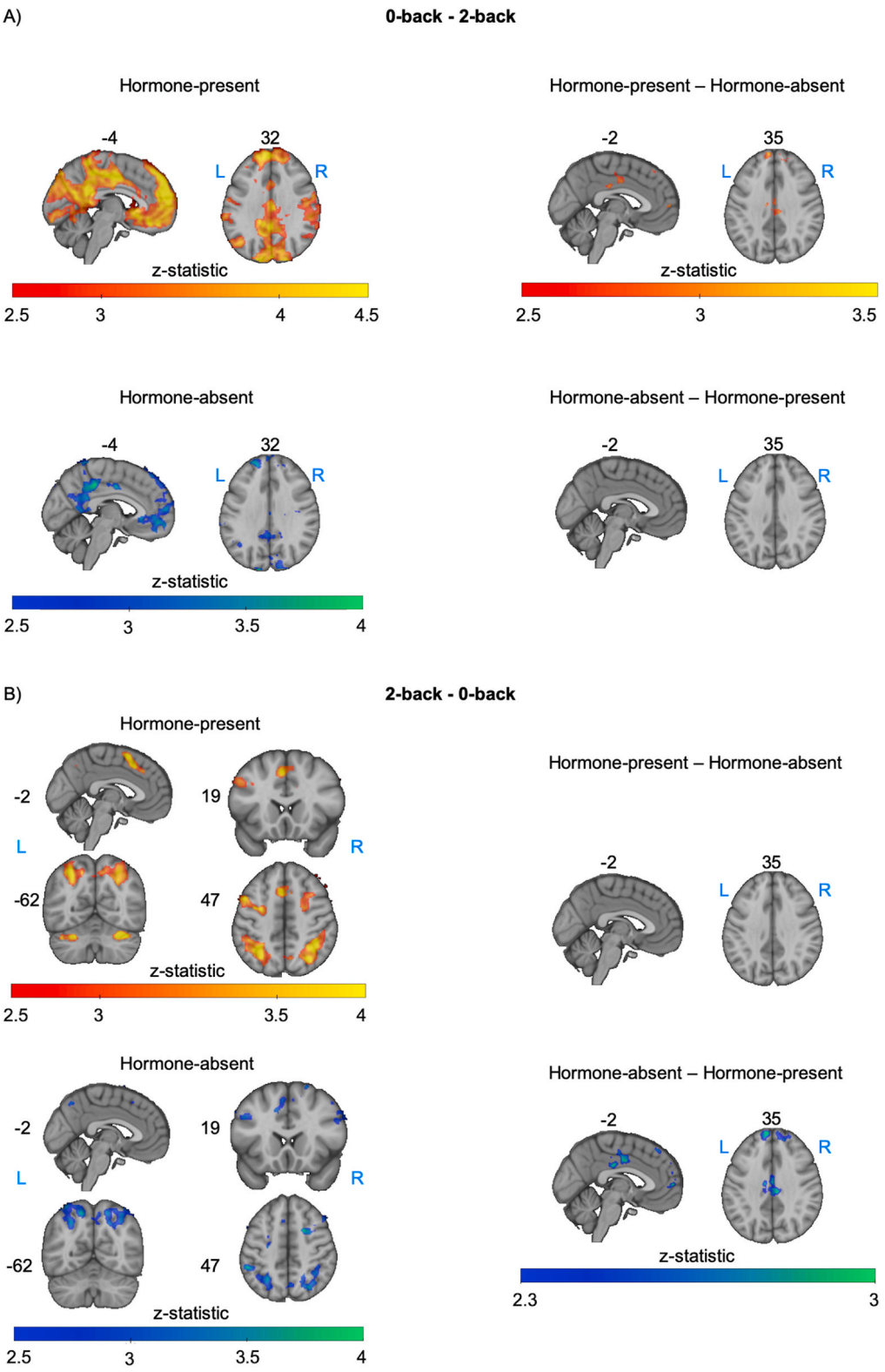



**Fig. 3.** Brain activation during a working memory task across the hormonal contraceptive cycle. A) Brain activation during the n-back task in the hormone-present and hormone-absent phases. Contrasts show activation for 0-back minus 2-back in each phase separately and activation for the same load contrast in hormone-present minus hormone-absent and hormone-absent minus hormone-present. The pattern suggests that default mode network deactivation was greater during the 2-back in both phases, with the greatest deactivation observed in the hormone-present phase. B) Brain activation during the n-back task in the hormone-present and hormone-absent phases. Contrasts show activation for 2-back minus 0-back in each phase separately and activation for the same load contrast in hormone-present minus hormone-absent and hormone-absent minus hormone-present. 2-back performance led to increased activation in frontal regions in both phases. There were no significant load-related increases in activation between phases. However, women showed less deactivation of regions associated with default mode network during the 2-back in the hormone-absent phase.

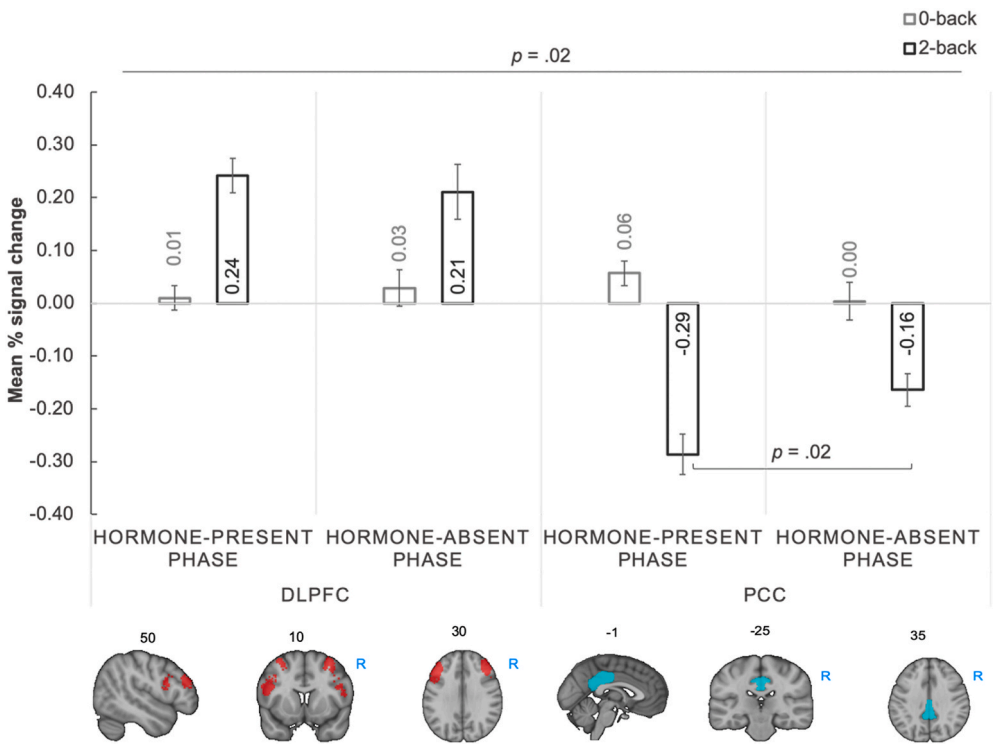



**Fig. 4.** Mean percent signal change in PCC and dlPFC during the 0-back and 2-back after stress and dorsolateral prefrontal cortex (dlPFC) and posterior cingulate cortex (PCC) masks used for region of interest (ROI) analyses. The three-way interaction between load, ROI, and phase was significant ($p = .02$) and women showed greater deactivation of the PCC during 2-back trials when in the hormone-present phase.

discussed above, by excluding a no-stress control session, we are unable to see how working memory performance or brain activation would have changed across the session in the absence of stress. This does limit conclusions that can be made regarding the direction of the brain activation effects observed in our study and influences how the post-stress change in working memory performance can be interpreted (e.g., the change in performance from before to after stress exposure could be from stress or fatigue) as well as the brain activation results (e.g., women may recruit fewer neural resources to complete tasks during the hormone-present phase regardless of stress exposure).

Including more working memory loads and/or utilizing a different stressor also would have benefited the study. Additional working memory loads (e.g., including 1-back and 3-back loads) would allow for more refined testing of whether optimal levels of stress on performance shift across the HC cycle. For instance, if the inverted-U function does shift across the HC cycle, changing the "optimal" amount of cortisol for peak performance, we might find that women show less deactivation of the PCC after stress exposure during a 3-back task during the hormone-present phase, but more effective disengagement of the PCC during a 1-back task during the hormone-absent phase. Alternatively, stressors eliciting larger free cortisol responses, such as the socially evaluated cold pressor (Schwabe et al., 2008) or the Trier Social Stress Test (Kirschbaum et al., 1993), could help determine the boundaries of "optimal" cortisol reactivity on cognition in a manner similar to including additional working memory loads.

Also unclear from this study is the potential role or influence of sex hormones. Previous work from our lab, in post-menopausal women, suggests that the addition of estradiol should help protect cognitive performance against the effects of stress, however, post-treatment levels of estradiol in post-menopausal women are substantially higher than the levels observed in the current study. Thus, although our participants had significantly higher estradiol levels during the hormone-present phase, the levels observed were still below the levels observed in the natural menstrual cycle, making it unlikely that the difference in estradiol levels between HC phases would be enough to account for the observed effects.

While differences in estradiol levels may not be responsible for the effects observed herein, it is likely that the synthetic sex hormones contained in HC exert influence on cognitive performance and brain activation. However, since the current study used combined HC formulations and did not have a naturally cycling (i.e., no-synthetic hormone) group, the current study cannot directly address the potential role of synthetic sex hormones. Future work can begin to address the influence of ethinyl estradiol and progestins by testing women before and after random assignment to HC or the individual hormone components. Alternatively, a design similar to the current study could be employed but add a no-stress control group and a group of naturally cycling women during the low-hormone follicular phase. Inclusion of these groups would allow for better investigation of the synthetic hormones in HC by removing the influence of stress and allowing for comparison to other low-hormone states not induced by synthetic hormone application. Work of this nature has been completed for other cognitive domains, such as emotional memory (Nielsen et al., 2014), and response inhibition (Gingnell et al., 2016), but limited work has investigated working memory. Nonetheless, existing work suggests that HCs alone, in the absence of stress, likely affect these processes.

## 5. Conclusions

Our results have important implications for methodology and mechanisms of HC action with regard to stress and cognition. First, the pattern of results indicate that HC cycle position should be controlled for in stress studies including HC-users, a practice currently only rarely utilized (Mordecai et al., 2008; Petersen et al., 2014; Mordecai et al., 2017; Herrera et al., 2019). HC cycle position should also be controlled for in brain imaging studies not utilizing stress until it is determined that the differences observed between HC phases in this study are limited to post-stress timepoints. Second, our findings may help elucidate the mechanisms by which HCs affect the stress response and modulate the

effects of stress on brain function. Showing that cortisol response remains stable across the HC cycle indicates that direct and short-acting effects of the synthetic estradiol and progestins, such as upregulation of corticosteroid binding globulin (Wiegratz et al., 2003), are not the only contributing factors to the smaller free cortisol responses to stress observed in HC users versus non-users. More research will be required to parse out the mechanisms supporting this effect. Additionally, despite comparable free cortisol responses to stress across the HC cycle, the post-stress task-related brain activation differed across the cycle, with women in the hormone-present phase being better able to flexibly suppress DMN-related activity after stress exposure than during the hormone-absent phase. This suggests that there is a direct, short-acting, effect of the synthetic estradiol and/or progestins that may diminish negative effects of stress on brain activation patterns underlying tasks of executive function. Again, the mechanisms driving this effect need to be studied, such as whether or not type II glucocorticoid receptor mRNA expression changes across the HC cycle.

## CRediT authorship contribution statement

**Alexandra Ycaza Herrera:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Ricardo Velasco:** Software, Formal analysis, Data curation. **Sophia Faude:** Investigation, Formal analysis, Data curation. **Jessica D. White:** Investigation, Formal analysis, Data curation. **Philipp C. Opitz:** Software. **Ringo Huang:** Software. **Kristie Tu:** Investigation. **Mara Mather:** Conceptualization, Methodology, Formal analysis, Resources, Writing - review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

None.

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