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Disinhibition of right inferior frontal gyrus underlies alpha asymmetry in women with low testosterone

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ABSTRACT

Asymmetrical expression of alpha oscillations in the frontal cortex, increased left relative to right, is a phenotype associated with increased behavioral inhibition and mood-related psychiatric illnesses. However, investigations of frontal alpha asymmetry in mood-disorders have yielded inconsistent findings. A better understanding of factors that contribute to individual differences is required to establish a useful biomarker for the diagnosis and treatment of mood and stress related disorders. A novel factor is hormone concentration, as steroid hormones play a prominent role in regulating mood and stress. To investigate this question, concentrations of testosterone and estradiol were sampled. Multiple linear regression revealed that low levels of testosterone correlated with greater frontal alpha asymmetry in women. Source localization found that frontal asymmetry was driven by decreased alpha power in right inferior frontal gyrus that correlated with increased behavioral inhibition in women. Together, these findings might explain inconsistencies in previous investigation on frontal alpha asymmetry.

1. Introduction

Asymmetrical cortical activity in prefrontal cortex is proposed as a potential biomarker for the presence and severity of depression as well as treatment response (Arns et al., 2016; Coan & Allen, 2004; Smith, Cavanagh, & Allen, 2018; Stewart, Bismark, Towers, Coan, & Allen, 2010; Stewart, Coan, Towers, & Allen, 2014; Thibodeau, Jorgensen, & Sangmoon, 2006). Research into frontal asymmetry has found that elevated cortical activity in right frontal cortex is associated with behavioral withdrawal and initiation of the stress response (Quaedflieg, Meyer, Smulders, & Smeets, 2015; Wittling & Pflüger, 1990), and reduced cortical activity in left frontal cortex is associated with decreased motivation to seek rewards and reduced ability to down-regulate the stress response (Berkman & Lieberman, 2010; Coan & Allen, 2003; De Pascalis, Cozzuto, Caprara, & Alessandri, 2013; Harmon-Jones, 2003; Jesulola, Sharpley, Bitsika, Agnew, & Wilson, 2015). Since neuronal oscillations in the alpha frequency band (8−12 Hz) inversely index cortical excitability (Goldman, Stern, Engel, & Cohen, 2002; Laufs et al., 2003; Moosmann et al., 2003), asymmetrical cortical excitability in frontal cortex can be quantified using a brief resting-state recording of electroencephalography (EEG) and simple signal processing to calculate the relative difference in amplitude of alpha oscillations in electrodes over left and right frontal cortex (Smith, Reznik, Stewart, & Allen, 2017). With limited tools for objectively diagnosing depression based on

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neural activity, frontal alpha asymmetry has potential to be translated into the clinical setting. Despite a variety of positive findings, meta-analyses of the efficacy of frontal alpha asymmetry to predict depression presence or severity have yielded inconsistent results (Gold, Fachner, & Erkkilä, 2013; Jesulola et al., 2015; Kaiser, Gnjezda, Knasmüller, & Aichhorn, 2018; Van Der Vinne, Vollebregt, Van Putten, & Arns, 2017). Thus, discovering additional factors that may contribute to individual differences in frontal asymmetry may resolve inconsistencies in the literature.

One relatively unexplored domain of individual differences that may relate to frontal alpha asymmetry is steroid hormone concentration. Reproductive steroid hormones profoundly influence mood and cognition (Celec, Ostatníková, & Hodosy, 2015; Sherwin & Henry, 2008). Animal experiments have repeatedly found that treatment with testosterone or estradiol produces anxiolytic effects (Carrier et al., 2015; Filova et al., 2015). In humans, fluctuations in steroid hormone concentrations impact the development and severity of symptoms of depression (Gordon et al., 2015), and withdrawal from androgens and estradiol (E2) increases risk for developing depressive symptoms (Kanayama et al., 2015; Schmidt et al., 2015). Despite the use of E2 (Gordon et al., 2018; Rubinow, Johnson, Schmidt, Girdler, & Gaynes, 2015), testosterone (Aydogan et al., 2012; Seidman & Rabkin, 1998; Wang et al., 1996) and other hormones (Meltzer-Brody et al., 2018) in the treatment of reproductive mood disorders, there has yet to be an investigation into a potential relationship between these steroid hormones and frontal asymmetry.

Top-down signals from prefrontal cortex are theorized to converge with signals generated by reproductive steroid hormones in the medial temporal lobe. The amygdala, hippocampus, and bed nucleus of the stria terminalis within the medial temporal lobe include a large population of neurons with estrogen and androgen receptors (Handa & Weiser, 2014; Milner et al., 2001). The medial temporal lobe projects to and regulates the periventricular nucleus (PVN) of the hypothalamus, which is responsible for initiating the stress response (see (Handa & Weiser, 2014) for review). Thus, reproductive steroid hormones are theorized to exercise their anxiolytic effects via the medial temporal lobe. Similarly, connectivity between the prefrontal cortex and the medial temporal lobe has been identified in emotion regulation and is decreased in anxiety and depression (Banks, Eddy, Angstadt, Nathan, & Luan Phan, 2007; Kim, Gee, Loucks, Caroline Davis, & Whalen, 2011; Moses-Kolko et al., 2010; Yoo, Gujar, Hu, Jolesz, & Walker, 2007). Activation of the HPA-axis via a stressful task or administration of cortisol both increases frontal alpha asymmetry (Hewig et al., 2008; Tops et al., 2005) and inhibits the production of anxiolytic reproductive steroid hormones in the gonads (Toufexis, Rivarola, Lara, & Viau., 2014). Therefore, previous research implicates a potential relationship between frontal asymmetry and reproductive steroid hormone concentration via convergent signaling in the medial temporal lobe.

Previous investigation into the processes modulated by frontal asymmetry has found that motivational systems that correspond to approach and withdrawal behavior were related to frontal asymmetry. Extensive research has found that elevated activity in the right frontal cortex promotes the behavioral inhibition system (BIS); whereas, left frontal cortex activity promotes the behavioral approach system (BAS) (Berkman & Lieberman, 2010; Coan & Allen, 2003; De Pascalis et al., 2013; Harmon-Jones, 2003; Jesulola et al., 2015). Anhedonia, a core symptom present in many diagnoses of depression, has been shown through neuroimaging and behavioral assessment to correlate with deficits in motivation, suggesting that dysfunction of motivational systems maybe underlie symptoms of anhedonia (Nusslock & Alloy, 2017). Consistent with this framework, frontal asymmetry is related to symptoms of anhedonia and decreased approach motivation (Nusslock, Walden, & Harmon-Jones, 2015). Furthermore, a recent experiment found that depression and anxiety onset in the future was predicted by elevated behavioral withdrawal (BIS) in a longitudinal study of sub-clinical participants (Struijs et al., 2018).

Convergent evidence suggests that frontal alpha asymmetry in a subclinical population might be predictive of future pathology, but additional constructs are required to increase the translational utility of frontal alpha asymmetry. We hypothesized that low levels of hormone concentration (testosterone or E2) and elevated BIS, or decreased BAS, were related to greater pathological frontal activity, left greater than right. We recorded high-density electroencephalography (EEG) during the eyes-open resting state, collected saliva samples for enzyme immunoassay, and queried motivational systems of behavioral inhibition and activation (BIS/BAS). The relative amplitude of alpha oscillations, an inverse index of cortical activity, over left and right frontal cortex was calculated as an estimate of frontal asymmetry. Frontal alpha asymmetry was the primary dependent variable for our investigation, and the independent variables under investigation were the concentrations of steroid hormones, testosterone and E2, and self-reported motivational systems, BIS and BAS. Steroid hormone concentrations were novel explanatory variables, whereas BIS and BAS have previously been found to correlate with left and right frontal alpha activity. Our primary investigation consisted of a single statistical model for frontal alpha asymmetry using steroid hormone concentrations (Methods 2.2) as independent variables. On the basis of previous research, the amplitudes of alpha oscillations in the left and right frontal cortex were hypothesized to be related to motivational systems. As a confirmatory analysis, we investigated whether left frontal alpha related to the behavioral activation system (BAS) and right frontal alpha related to the behavioral inhibition system (BIS) (Methods 2.3). By replicating the known relationship between BIS/BAS and alpha asymmetry, we ensure that alpha asymmetry measured here corresponds to the one reported in previous literature. Next, we investigated the spatial origin of frontal alpha asymmetry using spectral source localization to define regions of interest in left and right prefrontal cortex, and ran two statistical models for source-space alpha amplitude using steroid hormone concentration and motivational systems as independent variables (Methods 2.4). The inclusion of known explanatory variables, motivational systems, in our experiment provided a means of validating that our sample replicated previous findings, served as a baseline for understanding the added explanatory power from the inclusion of a novel variable (hormone concentration), and increased the translational relevance of our findings. Finally, increased understanding of factors that contribute to individual differences in the asymmetrical expression of alpha oscillations in frontal cortex is of high translational relevance since there are several brain stimulation treatments either approved (George et al., 1995; Leuchter, Cook, Jin, & Phillips, 2013) or under development (Alexander et al., 2019; Fröhlich, 2015) to modulate frontal alpha oscillations for the treatment of mood disorders.

2. Materials and methods

Data from fifty-five participants (23 women, ages 18–59, and 32 men, ages 18–70) were pooled across three experiments (Table 1): 22 from National Clinical Trial 03244501 (Sheffield, Ahn, Alagapan, & Fröhlich., 2019), 14 from NCT03243084 (Ahn, Prim, Alexander, McCulloch, & Fröhlich, 2019), and 19 from NCT03178344. For all experiments, eyes-open resting-state electrical brain activity was recorded with high-density EEG to estimate frontal alpha asymmetry, saliva samples were collected and pooled for each subject to estimate hormone concentration, testosterone and E2, and motivational systems of behavioral inhibition and behavioral activation (BIS/BAS) were collected via self-report assessment (Carver & White, 1994) (Table 2). All data were collected at the Carolina Center for Neurostimulation with identical EEG, hormone sampling, and clinical assessment methodology. Participants provided written consent and all experiments were approved by the institutional review board at the University of North Carolina at Chapel Hill. Participants were screened by phone prior to enrollment with the following inclusion criteria: reported no personal or immediate family history of neurological or psychiatric illness, no

Table 1
Summary of National Clinical Trials from which data was pooled for analysis.

CLINICAL TRIAL	N	Women (Men)	Population	Primary Outcome	Completion Date	Citation
NCT03244501	24	9 (13)	Healthy controls	Effect of tSMS on neural oscillations in EEG	Sept. 15, 2017	Sheffield et al., *EJN* 2019
NCT03243084	20	9 (5)	Chronic low back pain, healthy otherwise	Effect of alpha-tACS on heart rate variability and frontal alpha oscillations	Nov. 4, 2017	Ahn, Prim, et al. *J. Pain*, 2019
NCT03178344	20	5 (14)	Healthy controls	Effect of alpha-tACS on alpha amylase and cortisol levels	Dec. 12, 2018	Unpublished

Complete description of experimental design and results can be found on ClinicalTrials.gov. Participants with all three metrics of interest were included from each study: eyes-open resting-state EEG, saliva samples, and BIS/BAS assessment. Total number of participants from the original data (*N*). Some women and men were missing hormone samples in the original datasets. tSMS = transcranial static magnetic stimulation. tACS = transcranial alternating current stimulation.

Table 2
Descriptive statistics of frontal alpha asymmetry, steroid hormone concentration, age, and behavioral inhibition/activation.

MEAN (SD)	Women (N = 23)	Men (N = 32)	Sex Difference (w - m)	
			t(53)	d
Alpha Asymmetry [dB]	−0.10 (0.37)	−0.05 (0.30)	−0.57	0.15
Testosterone [pg/mL]	20.2 (8.51)	94.3 (38.5)	−13.4*	3.62
Estradiol (E2) [pg/mL]	1.70 (0.87)	1.67 (0.76)	−0.05	0.01
Age [years]	31.1 (13.7)	27.2 (12.3)	1.10	0.30
Behavioral Inhibition [7–28]	21.8 (2.81)	17.9 (3.96)	3.99*	1.14
Behavioral Activation [13–52]	39.5 (4.29)	39.44 (4.86)	0.032	0.01

Women exhibited greater behavioral inhibition than men. Men had higher concentration of testosterone relative to women. * $p < .001$, else $p > .1$. Hormone data were log-normalized prior to *t*-test.

ongoing psychotherapy or use of medication to treat a neurological or psychiatric illness, no major head injury or brain surgery, no brain implants (including cochlear implants), no history of cardiovascular disease. All participants were required to pass a drug test and women took a pregnancy test. Participants were asked to maintain a regular sleep schedule over the course of their participation, as well as abstain from alcohol and caffeine for the 24 hours prior to the visit.

2.1. Investigating asymmetry in the frontal cortex

Asymmetry of frontal cortical activity was estimated using electroencephalography (EEG) during the eyes-open resting-state, and individual differences analysis was run using multiple linear regression. To calculate frontal asymmetry, the ratio between the amplitude of alpha oscillations in electrodes over left and right frontal cortex was calculated as a proxy for asymmetry in cortical excitability, as alpha oscillations are inversely related to cortical excitability (Goldman et al., 2002). The methods for calculating frontal alpha asymmetry are as follows. EEG data were collected with a high-density 128-channel electrode net at 1000 Hz (HydroCel Geodesic Sensor Net) and the EGI system (NetAmps 410, Electrical Geodesics Inc., OR, USA). The impedance of each electrode was ensured to be below 50 kΩ at the start of each session. The first two minutes of eyes-open resting-state EEG that was collected were analyzed. Eyes-open resting-state was used for analysis, as eyes-closed resting-state is typically dominated by alpha oscillations in parietal-occipital cortex. The resting-state EEG data were preprocessed using custom scripts in MatLab (MathWorks Inc., Natick, MA, USA) and the EEGLAB toolbox (Delorme & Makeig, 2004). Resting-state EEG data were epoched into two second windows. Each epoch was visually inspected in time domain, and epochs with abnormal signal transients were rejected from analysis (range 0%–5%, average of 0.1 %). Abnormal signal transients are defined as excessive noise (on the order of 10 standard deviations from the mean) and arise from unusual and rare events. For example, the participant physically moved the EEG cap with their hand while scratching their scalp, or stretched their neck and shoulders in the middle of a session resulting in exaggerated movements. The signal artifacts caused by such events cannot be removed with standard procedures, and so the corresponding data was not included in the subsequent analyses. All EEG data were downsampled to 250 Hz with anti-aliasing filtering and band-pass filtered from 1 to 50 Hz. The data were then preprocessed using an artifact subspace reconstruction algorithm to remove high-variance data and reconstruct missing data, default parameters of the "clean_rawdata" function in EEGLAB (Mullen et al., 2013). Channels that were found to contain above threshold noise levels from artifact subspace reconstruction were interpolated using a spherical interpolation (range 0–24, average of 8.35). Global average rereferencing was applied, which is an approximate solution for the spherical electrical field assumption that was uniquely enabled by use of a 128-channel system. An infomax independent component analysis (ICA) was performed to separate plausible neural activity from eye blinks, eye movement, muscle activity, heartbeats, and channel noise. The number of ICA components was equal to the total number of channels (128) minus the number of channels interpolated, minus one for rereferencing. All ICA components were visually inspected and components corresponding to noise were manually rejected (range 19–43, average 34.65).

Analysis was restricted to the 90 channels on the scalp. The fast Fourier transform was applied to each two-second window with 0% overlap between windows and the average spectral power was calculated within the canonical alpha frequency band from 8 to 12 Hz. Alpha asymmetry was calculated as the log-normalized ratio between right frontal (electrode F4) and left frontal (electrode F3) alpha amplitude with the unit of decibels (Formula (1)), which is a standardized formula (Gheza, Bakic, Baeken, De Raedt, & Pourtois., 2019). These frontal alpha asymmetry values were submitted as the dependent variable to individual differences statistical analyses that are described below for steroid hormone concentration (Methods 2.2) and motivational systems (Methods 2.3).

$$10 * \log_{10}\left(\frac{F4}{F3}\right) \quad (1)$$

2.2. Role of steroid hormone concentration in frontal alpha asymmetry

Our statistical analysis was designed to investigate whether individual differences in steroid hormone concentration of testosterone or E2 were related to individual differences in frontal alpha asymmetry. First, we describe our method for assessing steroid hormone concentration, then we describe our statistical approach. Steroid hormone concentrations were assessed via sample kits from Doctor's Data (St. Charles, IL, USA) using four saliva samples taken throughout a single day: before breakfast, before lunch, before dinner and before bed. Participants were instructed not to eat for an hour before providing each sample. In every experiment, participants were provided with the sample kit in their first session (during which EEG and BIS/BAS were acquired) and returned the kit in their second session, which was

scheduled for a week later. The same saliva sampling method was used for all experiments. While there were multiple EEG recordings per experiment, EEG data used for this analysis was a single resting-state recording from the first session of each experiment prior to any experimental manipulation. The saliva samples were collected within a week following the EEG data used for analysis. The limitations from this sampling methods are surveyed in the discussion section. Samples were sent to Doctor's Data (St. Charles, IL, USA) for assay. The assays were batched in two groups with 41 participants in the first batch (17 women, 41%) and 14 in the second batch (5 women, 36%) that were processed by Doctor's Data. When the reagent lot used by the enzyme immunoassay kit is depleted and swapped, variability in signal can be introduced. Thus, the use of only two batches with comparable sex ratios reduces the variability that is inherent to the method. Doctor's Data combined the four samples for each participant into a single mixed sample estimate and conducted enzyme immunoassays for testosterone and E2 on that mixed sample. Thus, analyses were run using a single hormone concentration estimate per participant.

The testosterone enzyme immunoassay kit was the Pantex Salivary Direct Testosterone EIA Kit by PANTEX Division of Bio-Analysis (Santa Monica, CA, USA) with a standard curve ranged from 10.0−2400 pg/mL and limit of detection of 2.1 pg/mL; intra-assay precision for a high concentration (mean: 318.7 pg/mL) sample is 13.52 pg/mL SD and coefficient of variation is 4.2% and for a low concentration (mean: 21.0 pg/mL) sample is 1.06 pg/mL SD and coefficient of variation is 5.1%; inter-assay precision for a high concentration (mean: 285.5 pg/mL) sample is 8.85 pg/mL SD and coefficient of variation is 3.1% and for a low concentration (mean: 19.1 pg/mL) sample is 1.23 pg/mL SD and coefficient of variation is 6.4%. One to two percent of testosterone is free in plasma, and this free fraction is highly correlated with testosterone in saliva. The kit measures only biologically active (i.e., non-protein bound) testosterone, although testosterone bound to albumin is also bioactive and not measured by this test. Cross-reactivity was greatest for 5 α-dihydro-testosterone (5.47%), 5 α-androstane-3 β, 17 β-diol (2.75%), methyl testosterone (1.60%), and 11 α-OH testosterone (1.03%). There was low cross-reactivity with 17β-estradiol (0.173%), progesterone (P4) (0.28%), DHEA (0.003%), and cortisol (0.002%) (Pantex Salivary Direct Testosterone EIA Kit Catalog No. 635).

The estradiol concentrations were calculated using the high sensitivity salivary 17β-estradiol enzyme immunoassay kit by Salimetrics Inc (State College, PA, USA) with a standard curve ranged from 1 to 32 pg/mL and limit of detection is 0.1 pg/mL; intra-assay precision (mean: 3.81 pg/mL) is 0.31 pg/mL SD, and coefficient of variation is 7.0%, inter-assay precision (mean: 4.76 pg/mL) is 0.42 pg/mL SD and coefficient of variation is 8.9%. Salivary E2 levels are strongly correlated with serum estradiol ($r(9) = .80$). The kit measured E2 in the saliva that is not bound to serum proteins and reflects free E2, which is considered biologically active. Cross-reactivity was greatest for estrone (1.276%), estriol (0.234 %), ethynyl estradiol (0.189%), and prednisone (0.016%). There was no detectable (<0.004%) cross-reactivity with testosterone, DHEA, aldosterone, cortisol, and P4 (Salimetrics Salivary 17β-estradiol Enzyme Immunoassay Kit Item No. 1-4702).

The hormone concentrations from the immunoassays have an exponential distribution. Therefore, raw values were log-transformed prior to statistical analysis. To investigate individual differences that contribute to frontal alpha asymmetry (dependent variable), a multiple linear regression analysis was run with hormone concentrations of testosterone and E2 as independent variables. Previous research has found sex differences between testosterone and E2 concentrations and a decrease in hormone concentration with age (Ukkola et al., 2001). Thus, age and sex were included as independent variables. Multiple linear regression analysis was run to reduce multiple comparisons by including all factors within a single model. After finding a significant relationship with an independent variable at alpha of .05, post-hoc correlation analysis was run to understand the specificity of the effect. All post-hoc tests were two-tailed with a standard significance threshold of $p < .05$. Normality testing using the one-sample Kolmogorov-Smirnov test determined if a parametric (Pearson, $p > .05$) or non-parametric (Spearman, $p < .05$) test was performed. Age has previously been shown to negatively correlate with hormone concentration (Ukkola et al., 2001). Thus, all post-hoc individual difference analyses that included hormone concentration used a partial correlation that accounted for variance explained by age. If sex was determined to be significant in the multiple linear regression in addition to a steroid hormone concentration, then Fisher's Z was used to test for a significant difference between women and men for the correlation of steroid hormone concentration and frontal alpha asymmetry. With a multicollinearity between steroid hormone concentrations, post-hoc partial correlation was used to determine unique contributions of testosterone or E2 accounting for the variance explained by the other hormone. Altogether, this analysis runs a single statistical model for all independent variables relevant to steroid hormone concentration that might contribute to frontal alpha asymmetry, then systematically establishes the specificity of any discovered relationship. Statistical analyses were run in R (R Foundation for Statistical Computing, Vienna, Austria) and MatLab.

2.3. Role of motivational systems in frontal alpha asymmetry

Our statistical analysis for motivational systems mirrored that of steroid hormone concentration in that we investigated whether individual differences in approach motivation, behavioral activation system (BAS), or withdrawal, behavioral inhibition (BIS), were related to individual differences in frontal alpha asymmetry. First, we describe the BIS/BAS assessment, then we describe our statistical approach. Participants completed the behavioral inhibition system (BIS) and behavior activation system (BAS) self-report assessment (Carver and White, 1994) on the first day of each experiment just prior to the EEG recording that was used for analysis. The survey is 24 questions on a 4-point Likert scale. All items on the BIS/BAS are reverse scored except for items 2 and 22. The BIS subscale is the sum of seven of these questions (2, 8, 13, 16, 19, 22, 24) for a range of 7–28. BIS quantifies inhibition of movement towards goals and increased sensitivity to punishment (Carver and White, 1994). The BAS subscale is the sum of 13 questions (3, 4, 5, 7, 9, 10, 12, 14, 15, 18, 20, 21, 23) for a range of 13–52. BAS quantifies sensitivity to rewards and propensity to move towards goals (Carver and White, 1994). Composite reliability was assessed using Cronbach's alpha and was considered high for BIS ($\alpha = 0.821$) and BAS ($\alpha = 0.903$).

Previous research found that BIS positively correlated with neural activity (negatively with alpha oscillations) in right prefrontal electrodes and BAS positively correlated with neural activity (negatively with alpha oscillations) in left prefrontal electrodes (Amodio, Master, Yee, & Taylor., 2008; Berkman & Lieberman, 2010), and the difference between BAS and BIS was positively correlated with frontal alpha asymmetry (Sutton & Davidson, 1997). To analyze alpha amplitude in left frontal and right frontal individually, the amplitude values needed to be normalized such that overall alpha amplitude across the scalp did not bias the effect. Note that the frontal alpha asymmetry metric removed this potentially confounding effect by calculating the difference between left and right frontal regions. Thus, alpha amplitude was normalized within each participant by applying the z-transformation across all data channels. As a confirmatory analysis, we tested for the hypothesized pattern of individual differences relationships: BIS was negatively correlated with right frontal alpha and BAS was negatively correlated with left frontal alpha. In order to test for this pattern of hypothesized relationships, BIS was sign flipped and the difference between correlations was calculated using Pearson and Filon's z (Pearson & Filon, 1898) by the "cocor" toolbox in R-Statistics (Formula 2) (Diedenhofen & Musch, 2015).

$$z = \frac{\sqrt{n}*(r_{jk} - r_{hm})}{\sqrt{(1 - r_{jk}^2)^2 + (1 - r_{hm}^2)^2 - k}}, \quad (2)$$

where $k = (r_{jh} - r_{jk}\, r_{kh})(r_{km} - r_{kh}\, r_{km}) + (r_{jm} - r_{jh}\, r_{hm})(r_{kh} - r_{jk}\, r_{jh}) + (r_{jh} - r_{jm}\, r_{hm})(r_{km} - r_{jk}\, r_{km}) + (r_{jm} - r_{jk}\, r_{km})(r_{kh} - r_{km}\, r_{hm})$, with variables j = left frontal alpha amplitude, k = BAS, h = right frontal alpha amplitude, m = - BIS

BIS was sign flipped for this difference in correlation test because we hypothesized two negative relationships, and the pattern of relationships was investigated with a single contrast. This statistical approach reduces multiple comparisons by running a single test with alpha threshold set at 0.05. Next, post-hoc correlation analysis was run for each hypothesized relationship: left frontal alpha to BAS and right frontal alpha to BIS. As a control analysis, we tested for a relationship with the opposite pairing: left frontal alpha to BIS and right frontal alpha to BAS. Finally, we ran an individual differences multiple linear regression analysis with dependent variable, frontal alpha asymmetry, and independent variables, BIS, BAS, age, and sex. This analysis was used to evaluate the explanatory power of BIS/BAS to individual differences in frontal alpha asymmetry.

2.4. Source localization of frontal alpha asymmetry

In order to understand the unique variance explained by the different brain regions that contribute to frontal alpha asymmetry, source localization analysis was run on alpha oscillations and the individual differences analyses were repeated on regions of interest in source space. Source localization was a follow-up analysis to our primary multiple linear regression on frontal alpha asymmetry that used steroid hormone concentration as independent variables. Thus, if a relationship was found between steroid hormone concentration and a single sex, then the source space analyses were run for that sex. In addition, a topographic correlation analysis between the significant independent variable(s) from multiple linear regression and alpha amplitude was run for each data channel. This analysis provides a sensor-space analysis to complement the source-space analysis. First, we describe the method for source localization. Second, we describe region of interest definition. Finally, we describe the individual differences multiple linear regression analysis run on those regions.

The use of a 128-channel EEG system provided a methodological foundation for source localization analysis, as electrical sources can more effectively be localized with nearly spherical coverage. Source localization was run using the Dynamical Imaging of Coherent Sources (DICS) beamformer algorithm implemented in the FieldTrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). For each two second epoch of resting-state data, the spectral amplitude via 5-cycle Morlet wavelet convolution at 10 Hertz was calculated for each channel. In addition, a cross-spectral density matrix was quantified that estimated the phase difference and shared amplitude of each pair of channels for use in source localization. The beamformer algorithm was run using a lead field calculated in the Montreal Neurological Institute (MNI) space using standard skull, skin, and brain tissue estimates provided in the FieldTrip toolbox. Alpha amplitude was source localized into MNI space for each two second epoch and then averaged across epochs. The alpha amplitude estimates from source localization were not normally distributed. Therefore, the data were log-transformed. The data were spatially normalized using the z-transformation for all voxels within a grey-matter mask in MNI space that was derived from tissue segmentation estimates provided by the SPM12 toolbox (Penny, Friston, Ashburner, Kiebel, & Nichols, 2011).

To determine significant regions of interest, we performed a median split based on frontal alpha asymmetry. The source localized alpha amplitude for the high frontal alpha asymmetry group was compared to the low alpha asymmetry group. An independent *t*-test between these groups was run on alpha-amplitude for every voxel in source-space. Significant clusters were defined as $p < .05$ and with a minimum contiguous cluster size of 250 voxels. Regions of interest were defined as five-millimeter radius spheres (56 voxels, standard size for common atlases, e.g. (Power et al., 2011)) at the peak voxel in the center of mass of each significant cluster. Source localization was hypothesized to reveal distinct clusters of increased alpha amplitude in left lateral prefrontal cortex and decreased alpha amplitude in right lateral prefrontal cortex. Alpha amplitude was extracted for each region of interest in the prefrontal cortex and individual difference analyses were run using multiple linear regression.

Two multiple linear regression analyses were run to estimate factors that contributed to alpha amplitude in the region of interest. The first model investigated the contribution of steroid hormone concentrations, testosterone and E2, and the second model investigated contribution from motivational systems, BIS and BAS. These secondary analyses were constrained to the sex that displayed a significant relationship in the initial multiple linear regression analyses. Age was included as an additional independent variable. Post-hoc correlations were run in the same manner as previously described (Methods 2.2). In addition, post-hoc correlations were run using the independent variables that showed a significant effect with frontal alpha asymmetry.

3. Results

Our investigation was designed to uncover individual differences in hormone concentration that were related to variation in frontal alpha asymmetry in women and men (Results 3.1). As a validation of our techniques and to provide comparison to a known relationship, we performed a confirmatory analysis for the role of motivational systems, behavioral activation system (BAS) and behavioral inhibition system (BIS) in left and right frontal alpha amplitude (Results 3.2). Finally, source localization analysis was performed to better understand the neural origin of frontal alpha asymmetry and individual differences analyses were repeated for identified regions of interest (Results 3.3).

3.1. Role of steroid hormone concentration in frontal alpha asymmetry

Multiple linear regression analyses were run to investigate the contribution of steroid hormone concentration, testosterone and E2, to frontal alpha asymmetry. Multiple linear regression analysis found a significant relationship between frontal alpha asymmetry and both testosterone concentration and sex ($p < .05$) (Table 3). The multiple linear fit of testosterone and sex to frontal alpha asymmetry was calculated for display purposes in Fig. 1A. Post-hoc correlation analysis was run that accounted for the decrease in hormone concentration over the lifetime (Spearman testosterone to age: $r(54) = -.391$, $p = .003$). Testosterone concentration was negatively correlated with frontal alpha asymmetry in women (Spearman partial-age, $r(22) = .616$, $p = .002$) but not in men (Spearman partial-age, $r(31) = .019$, $p = .921$) (Fig. 1B). Furthermore, the correlation between testosterone and frontal alpha asymmetry was significantly greater in women than in men (Spearman partial-age, Fisher, $z(53) = 2.407$, $p = .016$). In addition, testosterone in women was positively correlated with E2 (Spearman partial-age, $r(22) = .624$, $p = .002$). To address this multicollinearity, post-hoc partial correlation analysis found that the relationship between testosterone and

Table 3
Multiple linear regression for frontal alpha asymmetry.

Independent Variable	β	Std. err	*t*	*p*
Testosterone [pg/mL]	−0.077	0.032	−2.435	*.01850**
Estradiol (E2) [pg/mL]	0.012	0.028	0.426	.67165
Age [y]	−0.005	0.004	−1.228	.22508
Sex [W0, M1]	0.452	0.225	2.009	*.04997**

Note. N = 55, * $p < .05$, $R^2 = 0.13$, $F(4,50) = 1.789$, $p = .146$. W = women, M = men.

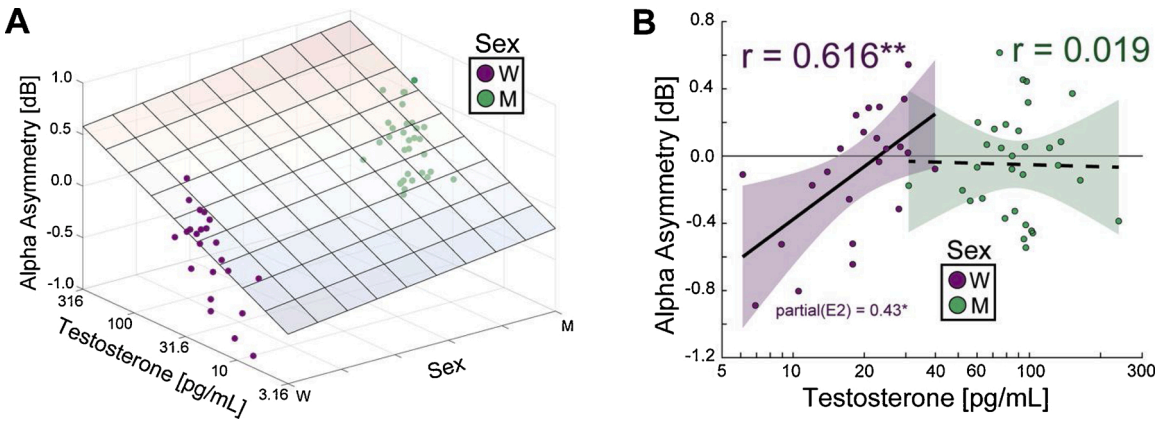

Fig. 1. Frontal alpha asymmetry negatively correlated with testosterone in women. Multiple linear regression of testosterone concentration, estradiol (E2) concentration, age, and sex yielded a significant relationship of testosterone concentration and sex with frontal alpha asymmetry. (A) Three-dimensional linear fit displays the relevant variables, testosterone concentration and sex in relation to frontal alpha asymmetry. Colormap on the plane redundantly depicts the dependent variable, frontal alpha asymmetry, on the Z-axis. (B) Post-hoc correlation analysis (Spearman partial-age) revealed that frontal alpha asymmetry showed a significant negative correlation with testosterone concentration in women (purple), but not in men (green). Partial correlation (Spearman) between testosterone concentration and frontal alpha asymmetry accounting for E2 concentration in women was also significant. * $p < .05$. ** $p < .005$. Shaded area is the 95 % confidence interval for each correlation. Dashed line depicts a relationship that is not statistically significant at $p < .05$. W = women, M = men.

frontal alpha asymmetry in women was significant when accounting for variance explained by E2 (partial-E2, $r(22) = .431$, $p = .045$). These findings suggest that testosterone, but not E2, concentration in women, but not men, accounts for variance in frontal alpha asymmetry.

3.2. Role of motivational systems in frontal alpha asymmetry

Previous research found that the amplitude of alpha oscillations in left frontal cortex has been shown to inversely relate to the behavioral activation systems (BAS) and alpha amplitude in the right frontal cortex has been shown to inversely relate to the behavioral inhibition system (BIS). Thus, we explicitly tested for the hypothesized pattern of individual difference relationships, defined as a significant difference between these correlations with BIS sign-flipped. This analysis revealed the expected difference (Pearson and Filon, $z(54) = 3.346$, $p = .0008$) (Fig. 2A). As a control analysis, the difference between the alternative pairing was tested, i.e. left frontal alpha with BIS and right frontal alpha with BAS, and was not significant (Pearson and Filon, $z(54) = 0.0861$, $p = .9314$) (Fig. 2B). For BAS, one participant was an outlier ($z = 2.71$, 0.33 percentile). After removal of this outlier, the hypothesized pattern of relationships was numerically stronger (Pearson and Filon, $z(53) = 3.8709$, $p = .0001$). Post-hoc correlation analysis revealed the expected negative relationships between left frontal alpha amplitude and BAS ($r(53) = -0.334$, $p = .014$) and between right frontal alpha amplitude and BIS ($r(54) = -.332$, $p = .013$).

In addition to the predicted relationships between left and right frontal alpha power, a multiple linear regression was run on frontal alpha asymmetry with four independent variables, BIS, BAS, age, and sex to test for relationship with frontal alpha asymmetry. This analysis did not yield any significant relationship between motivational systems and frontal alpha asymmetry (Table 4). The complete covariance matrix for all variables is reported in Fig. 3.

3.3. Source localization of frontal alpha asymmetry

Frontal alpha asymmetry, associated with increased anhedonia and motivation deficits, arises from increased amplitude of left frontal alpha oscillations and concomitant decreased amplitude of right frontal alpha oscillations, but previous investigations occasionally found that one hemisphere demonstrated a more prominent effect (De Pascalis et al., 2013). Thus, a follow-up correlation analysis between testosterone concentration and alpha amplitude in women for all scalp electrodes was run to determine if left or right frontal cortex was the primary driver of alpha asymmetry. Correlation analysis revealed a significant positive correlation between testosterone concentration and right frontal alpha oscillations (F4 and many surrounding electrodes) (Fig. 4A) such that decreased alpha amplitude corresponded with a lower testosterone concentration in women. The left frontal electrodes displayed a negative relationship with testosterone that was consistent with the overall pattern of increased left frontal alpha amplitude with decreased testosterone concentration, but there was only a single left frontal electrode, F7, with $p < .05$. This analysis provided a sensor space complement to source localization analysis. To further investigate the neural origin of frontal alpha asymmetry in women in order to resolve the specific regions in prefrontal cortex that correlate with testosterone concentration, alpha amplitude was source localized into the Montreal Neurological Institute (MNI) standard space. Using a median split in sensor space, the difference in alpha amplitude for every voxel in MNI space was calculated between women with high versus low frontal alpha asymmetry. This analysis revealed a focal decrease in alpha power in right inferior frontal gyrus (IFG) and a distributed increase in alpha power across the left superior and inferior frontal gyri (Fig. 4B).

Source localization analysis revealed the primary anatomical sources of frontal alpha asymmetry, which were then investigated with multiple linear regression. Cluster analysis found a single region in the right frontal cortex with a significant decrease in alpha amplitude for women with greater frontal alpha asymmetry (Fig. 5A). Peak decrease in alpha amplitude in this cluster was centered on the right inferior frontal gyrus (IFG) at the MNI coordinate (44, 43, 3). Additionally, the left frontal cortex was hypothesized to show an increase in alpha amplitude in participants with greater alpha asymmetry. However, there were no significant clusters with increased alpha amplitude in the left frontal cortex. Thus, alpha asymmetry in our dataset was driven by a decrease in alpha amplitude in the right IFG. Cluster analysis also yielded a significant increase in alpha amplitude in the right superior intraparietal sulcus (sIPS) centered on (38, −57, 41). Analysis was restricted to frontal cortex a priori. A 5-millimeter sphere was drawn at the center of mass of the right IFG cluster, and alpha amplitude within this region of interest was submitted to multiple linear regression.

Hormone concentration was investigated for a potential relationship with alpha amplitude in the right IFG of women using multiple linear regression with a covariate for age. This analysis did not find a significant relationship between testosterone ($t(22) = 1.644$, $p = .117$) or E2 ($t(22) = -0.491$, $p = .629$) concentration with alpha amplitude in right

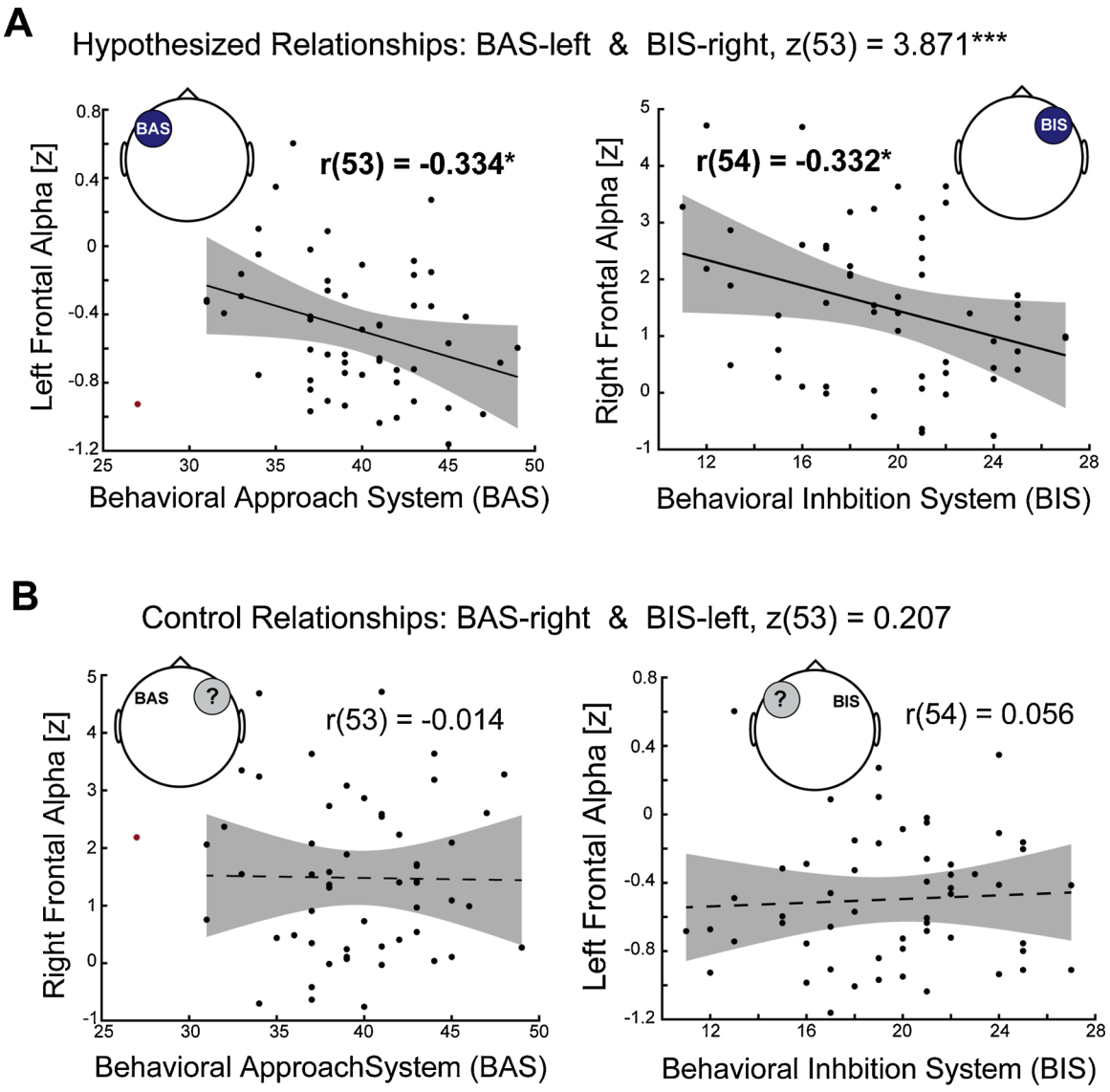

Fig. 2. Alpha amplitude in left and right frontal cortex relates to motivational systems. (A) The hypothesized pattern of relationships between left and right frontal alpha amplitude with BAS and BIS was significant (BIS was coded as negative for the contrast of interest). Post-hoc correlations revealed the hypothesized inverse relationships. Outlier for BAS in red was removed from analysis, z = −2.71. (B) Analysis of the control pattern of relationships with the opposite pairings revealed no significant effect. Post-hoc correlations revealed no relationship between the control pairings. * p < .05. *** p < .001. Shaded area is 95 % confidence interval. Dashed line depicts a non-significant correlation (p > .05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 4
Multiple linear regression for frontal alpha asymmetry.

Independent Variable	β	Std. err	*t*	*p*
Behavioral Inhibition [7–28]	−0.004	0.013	−0.321	.749
Behavioral Activation [13–52]	−0.006	0.011	−0.608	.546
Age [y]	−0.000	0.004	−0.081	.936
Sex [W0, M1]	−0.070	0.107	−0.650	.518

Note. N = 55, * p < .05, R^2 = .02, F(4,50) = 0.202, p = .936. W = women, M = men.

IFG. Post-hoc correlation analysis revealed a significant positive correlation between testosterone and alpha amplitude in rIFG (Spearman partial-age, r(22) = .441, p = .040). Thus, testosterone concentration displayed the same relationship to alpha amplitude in rIFG, although numerically weaker, as it did to frontal alpha asymmetry. In addition, motivational systems of behavioral inhibition and activation were investigated using the same approach. This analysis found a significant relationship of BIS (t(22) = -3.897, p = .001), but not BAS (t(22) = 1.368, p = .187), to alpha amplitude in right IFG in women. Post-hoc correlation analysis revealed a significant negative correlation between BIS and alpha amplitude in rIFG in women (r(22) = -.624, p = .001) (Fig. 4B). This pattern of findings suggests that testosterone concentration in women shows a strong relationship to the overall balance between left and right frontal alpha amplitude and that these effects are partially determined by alpha amplitude in the right IFG, whereas BIS tracks most closely with alpha amplitude in the right IFG.

4. Discussion

Frontal cortical asymmetry, wherein right cortical activity is increased relative to left, correlates with symptoms of negative mood and is associated with a phenotype of decreased motivation and reward hyposensitivity (Hughes, Yates, Morton, & Smillie, 2014; Nusslock et al., 2015). Alpha oscillations serve as an inverse index of cortical excitability (Moosmann et al., 2003) that can be calculated from

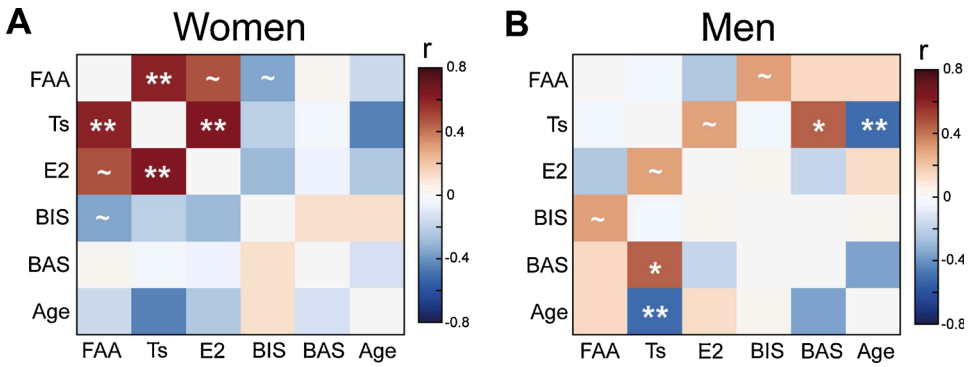

Fig. 3. Covariance matrix for women and men. Correlation of all pair-wise relationships in (A) women and (B) men. FAA = frontal alpha asymmetry. Ts = testosterone concentration. E2 = estradiol concentration. BIS = behavioral inhibition. BAS = behavioral activation. Diagonal is zero. Significance thresholds are not corrected for multiple comparisons as the primary analysis was multiple linear regression. ** $p < .01$, * $p < .05$, ~ $p < .1$.

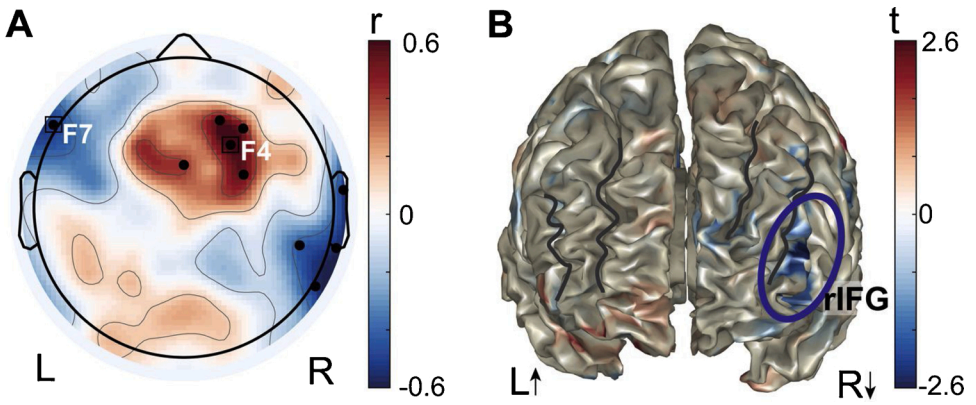

Fig. 4. Spatial distribution of alpha oscillations in women. (A) Sensor space correlation between alpha amplitude and testosterone concentration in women revealed the expected pattern of frontal alpha asymmetry. Low testosterone correlated with decreased right frontal alpha oscillations (electrode F4) and increased left frontal alpha oscillations (electrode F7). A black dot represents an electrode with a correlation of $p < .05$. (B) Using a median split of frontal alpha asymmetry in sensor space in women, source localization revealed decreased alpha amplitude in right inferior frontal gyrus (IFG) in source space from high versus low frontal alpha asymmetry. Coronal view of prefrontal cortex. Image was mirrored such that left prefrontal cortex is on the left. Dark grey lines depict the superior frontal sulci and inferior frontal sulci. Right IFG is labeled and highlighted with a blue oval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

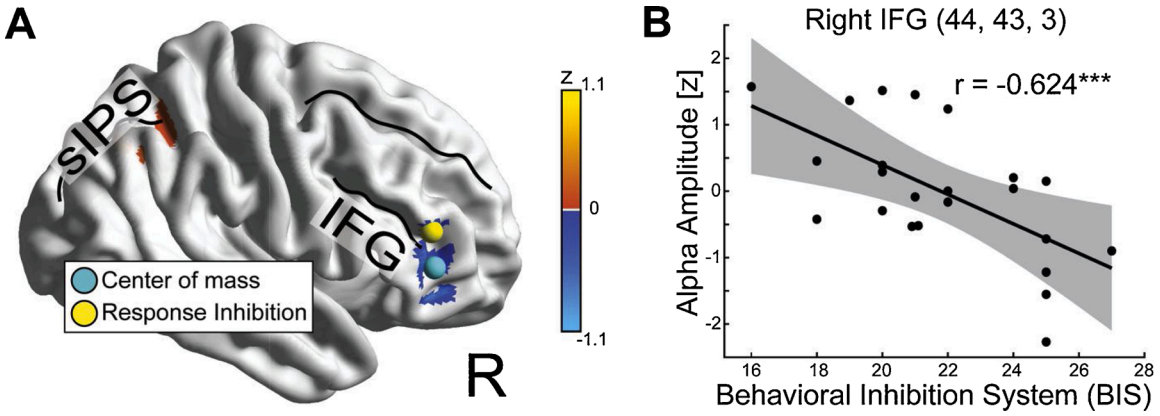

Fig. 5. Alpha oscillations in right inferior frontal gyrus negatively correlated with behavioral inhibition in women. (A) Source localization cluster analysis of frontal alpha asymmetry in women revealed two significant clusters in the right cerebral cortex, and none in the left cerebral cortex (not pictured). Alpha amplitude was decreased in the right inferior frontal gyrus (IFG) and increased in the right superior intraparietal sulcus (sIPS). The inferior frontal sulcus, superior frontal sulcus, and intraparietal sulcus are traced in black. A meta-analysis conducted in the NeuroSynth database (Yarkoni et al., 2011) of 218 fMRI studies using the word "response inhibition" found a cluster in the right IFG (peak activation in yellow). (B) Center of mass for the significant cluster in the right IFG at MNI coordinates (44, 43, 3) (cyan) showed significant negative correlation with behavioral inhibition (BIS) in women. *** $p < .001$. Shaded area is the 95 % confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

electroencephalography (EEG) of a brief resting-state. In this experiment, novel factors, concentration of testosterone and its metabolite estradiol (E2), that may contribute to individual differences in frontal alpha asymmetry were investigated. Variance explained by hormone concentration was compared with motivational systems of behavioral approach and withdrawal that have been previously found to show a relationship with frontal alpha oscillations (Coan & Allen, 2003; Sutton & Davidson, 1997). Individual differences analysis revealed a significant

relationship between testosterone concentration and frontal alpha asymmetry in women, such that women with lower testosterone concentration displayed the greatest asymmetry towards heighted right frontal cortical activity. This relationship explained more variance in frontal alpha asymmetry than motivational systems of approach and withdrawal, although our data replicated previous findings for an association between BAS with left frontal activity and BIS with right frontal activity. Source localization analysis found that frontal alpha asymmetry was primarily driven by increased cortical activity, indexed by decreased alpha amplitude, in the right inferior frontal gyrus (IFG). Increased activity in right IFG showed a negative correlation with testosterone concentration and a positive correlation with behavioral inhibition (BIS). These findings suggest that testosterone concentration estimated from saliva sample may be a relatively inexpensive and novel explanatory factor for interpreting frontal alpha asymmetry in women with translational relevance to behavioral withdrawal.

As in any scientific study, this experiment has limitations. First, information on hormonal birth control use, menstrual phase, and menopausal status for women was not collected since this was not the focus of the trials in which we collected the saliva samples analyzed here. This limits the ability to interpret the role that menopause and menstrual phase may play in mediating the effect of testosterone or E2 concentration on frontal alpha asymmetry. In particular, E2 and frontal alpha asymmetry displayed a trend-level negative correlation in women ($r(22) = .387$) that was not-significant when accounting for the variance explained by testosterone ($r(22) = .167$). Thus, the interpretation of a null finding for the weak relationship between E2 and frontal alpha asymmetry should be taken with caution. The variable time delay between saliva sample and EEG (one to seven days) means that the EEG was not guaranteed to be taken during the same phase of the menstrual cycle as the saliva sample. The weaker correlation may have been driven by a subset of participants in which the EEG and saliva sample were collected at different phases of the menstrual cycle. Despite the use of a single timepoint, individual differences in overall testosterone concentration were a robust signal that correlated with frontal alpha asymmetry, perhaps because testosterone does not change much over the course of the menstrual cycle.

Emerging research suggests that the change in hormone concentration is most predictive of fluctuations in mood and stress (Gordon, Rubinow, Eisenlohr-Moul, Leserman, & Girdler, 2016; Schmidt et al., 2017). Therefore, future research on testosterone concentration should use repeated measures across the menstrual phase in naturally cycling women to determine if frontal alpha asymmetry fluctuates with hormone dynamics or reflects overall concentration. As testosterone increases during ovulation, an acute reduction in frontal alpha asymmetry may also occur during ovulation. Furthermore, some women have been found to be more sensitive to fluctuations in hormone concentration leading to reproductive mood disorders such as premenstrual dysphoric disorder (PMDD) (Schmidt et al., 2017). Consistent with our findings, a series of previous studies found that frontal alpha asymmetry was increased during the luteal phase of the menstrual cycle in women with PMDD (Accortt & Allen, 2006; Baehr, Rosenfeld, Miller, & Baehr, 2004). While speculative, future studies could investigate a possible failure for testosterone concentration increase during ovulation to reduce frontal alpha asymmetry in women with PMDD.

In addition, salivary samples of testosterone and E2 are only reflective of steroid concentrations in the periphery, so the direct effect of testosterone and E2 on the central nervous system was not investigated. However, testosterone and E2 readily pass through the blood brain barrier (Pardridge, Mietus, Frumar, Davidson, & Judd, 1980). While our data does not support E2 as a mechanism by which testosterone impacts brain activity, we cannot rule out local conversion of testosterone to E2 in the brain. Another potential pathway for testosterone to impact brain activity is via a different metabolite, androsterone. Androsterone is an anxiolytic and allosteric modulator of the $GABA_A$ receptor, which is upregulated during stress (Servatius et al., 2016). Future studies could differentiate the particular endocrine pathway by which testosterone concentration might impact frontal alpha asymmetry in women.

Source localization of frontal alpha asymmetry revealed decreased alpha amplitude in the right IFG. The functional role of right IFG may provide an additional lens by which to interpret these data. The right IFG is considered the primary "stop-signal" in the brain based on experiments using the go/no-go task paradigm (see (Aron, Robbins, & Poldrack, 2014) for review). In the go/no-go task, a stimulus is presented frequently that is associated with a button press, and another stimulus is presented infrequently in which the participant must withhold a response. During the trials where a response must be withheld, the right IFG increases in activity presumably to facilitate response inhibition. A meta-analysis of 218 studies using the NeuroSynth database (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011) for the term "response inhibition" shows the strongest activation in the right IFG and right anterior insula. Peak activity in right IFG from this meta-analysis was centered on coordinate (44, 42, 16) in the Montreal Neurological Institute (MNI) space and is depicted in yellow in Fig. 5A. The anatomical source of the canonical stop-signal of the brain is spatially consistent with the decrease in alpha amplitude in the right IFG that was found in women with the greatest frontal alpha asymmetry. Although response inhibition was not directly measured, frontal alpha asymmetry might arise from a disinhibition of the right IFG and, therefore, an upregulation of response inhibition. Furthermore, the negative correlation between alpha amplitude in right IFG and BIS score suggests that women with increased traits of behavioral withdrawal might inhibit goal-directed behavior via activation of the right IFG.

In conclusion, these data provide a novel factor that explains individual differences in frontal alpha asymmetry: decreased testosterone concentration in women. A recent meta-analysis on frontal alpha asymmetry found divergent patterns between the sexes as a function of depression status (Van Der Vinne et al., 2017). While the current study did not investigate depression, testosterone concentration in our dataset captured a divergent association between women and men. Furthermore, our frontal alpha asymmetry effects were primarily driven by the right frontal cortex, which also related to increased behavioral withdrawal, a known vulnerability for depression and anxiety (Struijs et al., 2018). Future investigation could use sex, age, behavioral inhibition, and testosterone concentration as covariates to improve interpretation of the relationship between frontal alpha asymmetry and depression.

Declaration of Competing Interest

FF is the lead inventor of IP filed by UNC. FF is founder, shareholder, and chief science officer of Pulvinar Neuro, which did not play any role in the writing of this article. FF has received honoraria from the following entities in the last twelve months: Sage Therapeutics, Academic Press, Insel Spital, and Strategic Innovation. DR consults to and is a stockholder in Sage Therapeutics.

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