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Sex, Sleep Deprivation, and the Anxious Brain

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Abstract

Insufficient sleep is a known trigger of anxiety. Nevertheless, not everyone experiences these effects to the same extent. One determining factor is sex, wherein women experience a greater anxiogenic impact in response to sleep loss than men. However, the underlying brain mechanism(s) governing this sleep-loss-induced anxiety increase, including the markedly different reaction in women and men, is unclear. Here, we tested the hypothesis that structural brain morphology in a discrete network of emotion-relevant regions represents one such explanatory factor. Healthy participants were assessed across sleep-rested and sleep-deprived conditions, with brain structure quantified using gray matter volume measures. Sleep loss triggered greater levels of anxiety in women compared with men. Reduced gray matter volume in the anterior insula and lateral orbitofrontal cortex predicted the anxiogenic impact of sleep loss in women, yet predicted resilience in men, and did so with high discrimination accuracy. In contrast, gray matter volume in ventromedial prefrontal cortex predicted the anxiogenic impact of sleep loss in both men and women. Structural human brain morphology therefore appears to represent one mechanistic pathway (and possible biomarker) determining anxiety vulnerability to sleep loss—a discovery that may help explain the higher prevalence of sleep disruption and anxiety in women.

INTRODUCTION

A lack of sleep amplifies anxiety and subjective negative emotional responses in otherwise healthy individuals and does so in a dose–response manner with increasing time awake (Babson, Trainor, Feldner, & Blumenthal, 2010; Sagaspe et al., 2006). Clinical evidence supports this outcome, wherein sleep disturbance is recognized as a common symptom of anxiety disorders and, reciprocally, a strong factor in the development and progression of anxiety disorders (Neckelmann, Mykletun, & Dahl, 2007; Breslau, Roth, Rosenthal, & Andreski, 1996; Ford & Kamerow, 1989). Importantly, the anxiogenic impact of sleep loss is not common across all individuals. Evidence indicates that women are more susceptible to

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the maladaptive emotional consequences of sleep deprivation than men (van der Helm, Gujar, & Walker, 2010; Birchler-Pedross et al., 2009). In addition, the prevalence rates for both insomnia (Zhang & Wing, 2006) and anxiety disorders (Kessler et al., 1994) are higher in women relative to men. Women also demonstrate a stronger comorbidity of sleep disturbance and anxiety disorders than men (Johnson, Roth, & Breslau, 2006), suggesting a potential causal interaction between sleep disturbance and anxiety in women.

Although such evidence establishes an anxiogenic impact of insufficient sleep and that the extent of this impact may be dependent on sex, the neurobiological factors that confer anxiogenic vulnerability (and conversely, resilience) to sleep loss remain largely unknown. Furthermore, whether these factors help explain the anxiogenic susceptibility to sleep deprivation in women relative to men remains similarly unexplored.

One candidate explanatory factor is brain structure, which is known to predict interindividual variability in a broad array of behavioral and subjective outcome measures (Kanai & Rees, 2011). In the current study, we tested the hypothesis that structural brain morphology in a specific set of affect-relevant regions represents a vulnerability biomarker for the anxiogenic impact of sleep deprivation in a sex-dependent manner. Four independent but converging lines of evidence motivated our a priori morphology-based hypothesis and our selected regions of the amygdala, insula, lateral OFC (lOFC), ACC, and ventromedial pFC (vmPFC) as ROIs.

First, structural differences in the emotion processing and viscerosensory integration regions of the amygdala, anterior insula, OFC, ACC, and vmPFC have been associated with anxiety disorder predisposition and symptomology (Elsenbruch et al., 2014; Perrin et al., 2014; Alemany et al., 2013; Liao et al., 2011; Schienle, Ebner, & Schäfer, 2011; Asami et al., 2009; Spampinato, Wood, De Simone, & Grafman, 2009; Welborn et al., 2009; Yamasue et al., 2008; Milham et al., 2005).

Second, sleep supports the homeostatic regulation of affective brain functions through a recalibration of these same limbic-related cortical regions (together with the subcortical amygdala), including a potentially palliative influence on anxiety (Goldstein & Walker, 2014). Conversely, sleep deprivation has been shown to reliably alter activity within these specific regions (Goldstein & Walker, 2014).

Third, and related, this same network of brain regions is known to express sex-specific responses and reactivity profiles to emotional stimuli and anxiety states under sleep-rested conditions (Schwabe, Höffken, Tegenthoff, & Wolf, 2013; Cosgrove, Mazure, & Staley, 2007; Goldstein et al., 2001).

Fourth, gray matter volume in the cortical regions of the vmPFC, OFC, ACC and insula have been linked to inter-individual differences in sleep, including homeostatic-related NREM sleep oscillations (Saletin et al., 2016; Saletin, van der Helm, & Walker, 2013), daytime sleepiness (Killgore, Schwab, Kipman, DelDonno, & Weber, 2012), and the frequency of early morning awakenings (Stoffers et al., 2012).

Therefore, these frontal–cortical regions, together with the amygdala, converge to represent a network with overlapping sensitivity to sex-dependent anxiety vulnerability and physiological sleep need, offering a set of a priori ROIs. Building on this evidence, we tested the hypothesis that gray matter volume in these select affective brain regions represents a vulnerability factor determining the anxiogenic impact of sleep loss and furthermore that relationships within these brain regions would be moderated by sex.

METHODS

Participants

Forty-four healthy adults, aged 18–25 years (mean = 20.02 years, *SD* = 1.78 years, 23 women) completed a repeated-measures crossover design (described below). Exclusion criteria were assessed using a prescreening questionnaire and conducted by trained research personnel. Specifically, participants were asked whether they had a history of sleep disorders, neurological disorders, open and closed head injury, history of drug abuse, and current use of antidepressant, psychostimulant, or hypnotic medication. They were additionally asked whether they had a prior Axis I psychiatric disorder diagnosis according to the DSM-IV-TR criteria (encompassing mental disorders including depression, anxiety disorders, bipolar disorder, attention deficit disorder, and schizophrenia). Consistent with this interview participants endorsed relatively low anxiety (mean) and depression symptoms with relatively little variation on the STAI-Trait (mean = 33.14 ± 8.60; minimum possible score = 20, maximum possible score = 80) and QIDS (mean = 3.30 ± 3.38; minimum possible score = 0, maximum possible score = 48) questionnaires. We did not find associations between STAI-Trait (all $|t|$s < 0.40, all ps > .690) or QIDS (all $|t|$s < 0.12, all ps > .20) and gray matter volume in any of our ROIs. Participant who reported drinking three or more caffeine-containing beverages a day such as caffeinated coffee, tea, or soft drinks, were excluded. Participants abstained from caffeine and alcohol for the 72 hr before and during the entire course of the study and kept a normal sleep–wake rhythm (7–9 hr of sleep per night with sleep onset before 2:00 hr in the morning and rise time no later than 9:00 hr) for the 2 nights before the study participation, as verified by sleep logs (see below). The study was approved by the institutional review board at the University of California, Berkeley (Committee for Protection of Human Subjects). All study methods and protocols were completed in accordance with these guidelines and regulations, and all participants provided written informed consent.

Experimental Design

Following screening, participants entered a repeated-measures crossover design with two experimental conditions in which they stayed in the laboratory: rested sleep (control) and sleep deprivation (24 hr of sleep deprivation). The STAI-State anxiety questionnaire was used to assess state-dependent changes in anxiety levels (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) on the evening of and morning after each experimental condition (sleep, sleep deprivation). STAI-State Anxiety Assessments were obtained at the same circadian time points, administered at 10:50 p.m. (±30 min, just before lights off in the sleep condition), and again the following morning at 8:10 a.m. (±60 min) in both sleep-rested and

sleep-deprived conditions (Figure 1). Participants completed a structural MRI scan on the morning of the sleep-rested condition.

In the sleep deprivation session, participants arrived at the laboratory at 9:00 p.m. and were continuously monitored throughout the enforced waking period by trained research assistants. Activities during the sleep deprivation period were limited to use of Internet, e-mail, short walks, reading, and movies of low emotionality, thereby providing a standardized regiment of waking activity without undue stress. The two experimental conditions were separated by at least 6 days (mean = 11.43 days, *SD* = 5.3 days), with the order of the sleep-rested and sleep-deprived sessions counterbalanced across participants.

In the sleep-rested session, participants arrived at the lab at 8:00 p.m. and were prepared for an ~8-hr (11:00 p.m. to 8:00 a.m. ± 60 min; details below) night of sleep monitored in the laboratory by polysomnography (PSG). To assess the degree of difference between the structured sleep schedule of the experiment and each participant's unrestricted sleep schedule, participants completed the Pittsburgh Sleep Quality Index upon study entry. This instrument contains questions relating to the bed time, rise time, and duration of sleep episodes across the past month (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). In addition, to better characterize recent sleep status, participants further completed sleep logs 5 days before each experimental session. The sleep log questionnaires quantified when participants went to bed, what time they began trying to fall asleep, how long it took for them to fall asleep, the number of night-time awakenings, and what time they woke up. Participants conformed to the structured sleep schedule during the month before the experiment, including across the 5 days before the experimental session. Specifically, in the 5 days leading up to each experimental visit, participants reported average bed times before 2:00 a.m. (mean: 12:21 a.m., *SD* = 120 min), rise times before 10:00 a.m. (mean: 8:06 a.m., *SD* = 120 min), and sleep duration lengths between 7 and 9 hr (mean = 7.60 hr, *SD* = 0.77 hr) as measured by sleep logs. Although it is important to note the inherent limitations of self-report measures, these findings support the likelihood that participants were entering the study in a rested state and that their normative schedules were congruent with the study requirements. Sleep statistics for the night of PSG recorded sleep in the sleep-rested session are provided in Tables 1 and 2 and conform to population norms for this age range (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004).

Sleep Recordings

Sleep on the sleep-rested night was monitored in the laboratory with PSG sleep (11:00 p.m. to 8:00 a.m. ± 60 min) using a Grass Technologies Comet XL system (Astro-Med, Inc., West Warwick, RI). EEG was recorded at 19 standard locations conforming to the International 10–20 System (Jasper, 1958; FP1, FP2, F7, F3, FZ, F4, F8, T3, C3, CZ, C4, T4, T5, P3, PZ, P4, T6, O1, O2). Electrooculography was recorded at the right and left outer canthi (right superior, left inferior). Electromyography was recorded via three electrodes (one mental, two submental). Reference electrodes were recorded at both the left and right mastoid (A1, A2). All data were stored at a 400 Hz sampling rate.

Sleep Scoring

Sleep architecture was visually staged from the C3–A2 derivation in 20-sec epochs according to standardized procedures (Rechtschaffen & Kales, 1968). Sleep architecture values are reported in Tables 1 and 2, consistent with previous cross-sectional normative values for this age group (Ohayon et al., 2004).

Structural MRI Acquisition and Analysis

A high-resolution T1 weighted structural scan was acquired the morning of the sleep-rested condition using a Siemens (Erlangen, Germany) 3-T MRI scanner with a 12-channel head coil at the end of the sleep-rested session (256 × 256 matrix, repetition time = 1900, echo time = 2.52, flip angle = 9°, field of view = 256 mm, 1 × 1 × 1 mm).

Individual estimates of gray matter volume, a stable measure previously demonstrated to offer sensitivity to inter-individual variability in brain morphology (Thompson et al., 2001), was calculated using the validated voxel-based morphometry (VBM) approach (Ashburner & Friston, 2000). VBM analysis quantified the signal intensity of each voxel in the brain for a gray matter segmentation image, given the differential signal intensity yielded by magnetic resonance properties of gray and white matter, respectively. Image processing used Statistical Parametric Mapping (www.fil.ion.ucl.ac.uk/spm) in conjunction with the VBM 8 Toolbox for SPM8 (dbm.neuro.uni-jena.de/vbm/) using the default settings. In short, high-resolution T1 images were DARTEL normalized to MNI space and segmented into gray matter, white matter, and CSF. Modulated gray matter maps were then smoothed using an 8-mm Gaussian kernel to reduce signal-to-noise ratio for statistical analysis. Measures of total intracranial volume for each participant were estimated from the sum of gray matter, white matter, and CSF segmentation from each participant's native space using VBM8. This measure of total intracranial volume was verified against an independent analysis using the reconstruction scheme in the software package FreeSurfer (Fischl & Dale, 2000), which derives intracranial volumes from cortical surface reconstruction, with the two methods resulting in similar estimates (correlations between the values from each method: $r = 0.80$, $p < .00001$).

Given our hypothesis to test whether interindividual differences in gray matter volume would interact with sex to predict vulnerability/resilience to anxiety, we focused on cortical and subcortical brain regions described in the introduction that not only have consistently been associated with anxiety and emotion generation (Alemany et al., 2013; Carlson et al., 2012; Liao et al., 2011; Schienle et al., 2011; Spampinato et al., 2009; Yamasue et al., 2008; Milham et al., 2005) but have also demonstrated alterations in emotion processing after sleep deprivation (Goldstein-Piekarski, Greer, Saletin, & Walker, 2015; Goldstein et al., 2013; Greer, Goldstein, & Walker, 2013; Yoo, Gujar, Hu, Jolesz, & Walker, 2007) and sex differences in either brain function and/or structure (Schwabe et al., 2013; Sokolowski & Corbin, 2012; Biswal et al., 2010; Goldstein, Jerram, Abbs, Whitfield-Gabrieli, & Makris, 2010; Cosgrove et al., 2007; Goldstein et al., 2001).

We performed an ROI-based analysis in R (www.R-project.org) using cortical ROI volumes defined as a 14-mm sphere, with the smaller subcortical amygdala ROI volume defined as a

10-mm sphere. Each ROI was centered on a coordinate set defined by previously published neuroimaging studies investigating anxiety- and sex-dependent differences in a discreet frontal-limbic network (Xu et al., 2013; Goldstein et al., 2010; Bishop, Duncan, & Lawrence, 2004): MNI coordinates [*x, y, z*]: left amygdala [−22, −2, −12], right amygdala [22, −2, −12], left insula/inferior frontal gyrus (IFG) [−30, 28, −12], right insula/IFG [30, 28, −12], left lOFC [−34, 58, −4], right lOFC [34, 58, −4], ACC [10, 12, 42], vmPFC [−4, 42, −20]. Mean normalized gray matter volume across bilateral ROIs were extracted and used in the interaction analyses, as described below. In addition, all statistical regression analyses controlled for individual measures of total intracranial volume for each participant, included as a nuisance regressor in each model.

Statistical Analysis

A three-way, full-factorial repeated-measures ANOVA with type III Satterthwaite approximation for degrees of freedom conducted in R (using the *ezANOVA* function from the *ez* package, https://cran.r-project.org/web/packages/ez/), with Condition (sleep-rested vs. sleep-deprived) and Time (evening vs. morning) as within-subject factors and Sex (male vs. female) as a between-subject factor and their interactions, was used to determine whether the anxiogenic effects of sleep deprivation were moderated by sex. Specifically, given the hypothesis that the effects of sleep deprivation should be dependent on sex, we were particularly interested in the full three-way interaction, simple interaction effects (i.e., Condition × Time) at level of Sex and simple effects of Time at each level of Condition and Sex.

One participant was excluded from this portion of the analysis as an outlier (values were greater than 3 *SD* above the mean for anxiety scores). Another participant did not receive the anxiety questionnaire on the sleep-rested evening and was further excluded from the analyses. To test the hypothesis that interindividual differences in gray matter volume in the above frontal-limbic ROIs sensitive to sleep (Saletin et al., 2013, 2016; Spiegelhalder, Regen, Baglioni, Riemann, & Winkelman, 2013; Weber et al., 2013; Killgore et al., 2012; Stoffers et al., 2012), sex (Schwabe et al., 2013; Sokolowski & Corbin, 2012; Biswal et al., 2010; Goldstein et al., 2001, 2010; Cosgrove et al., 2007), and anxiety (Alemany et al., 2013; Carlson et al., 2012; Liao et al., 2011; Schienle et al., 2011; Spampinato et al., 2009; Yamasue et al., 2008; Milham et al., 2005) moderate the sex-specific anxiogenic impact of sleep deprivation, robust linear regression analyses were performed in R (*rlm* function from *MASS* package; Huber, 1981) for each of the five bilateral ROIs (amygdala, insula/IFG, lOFC, vmPFC, and ACC) independently (i.e., five regression models).

Robust regression techniques weight each observation by the distribution of the data and are thus more conservative and less biased by outliers than ordinary least squares regression (Huber, 1973) and are recommended for use in neuroimaging data (Wager, Keller, Lacey, & Jonides, 2005). Here, for each regression model, the overnight change in anxiety in the sleep-deprived versus sleep-rested conditions ($\text{Sleep-deprived}_{[\text{Morning} - \text{Evening}]} - \text{Sleep-rested}_{[\text{Morning} - \text{Evening}]}$) was entered as the dependent variable with greater numbers indicative of larger evening to morning increase in anxiety in the sleep-deprived condition relative to the sleep-rested condition. The mean-centered gray matter volume in the ROI, the

categorical variable of sex (male/female) using dummy coding with women coded as the reference group and their interactions entered as predictors (Anxiety Sleep-deprived$_{[Morning - Evening]}$ − Sleep-rested$_{[Morning - Evening]}$ ~ Sex × Gray matter volume). Given that gray matter volume in these ROIs have previously been associated with anxiety and/or sleep need, we additionally tested for relationships between gray matter volume and anxiety that were independent of sex (Sleep-deprived$_{[Morning - Evening]}$ − Sleep-rested$_{[Morning - Evening]}$ Anxiety ~ Sex + Gray matter volume). All models included the mean-centered total intracranial volume as a covariate, thus adjusting regional volumes for global differences in brain size. For completeness, we also include the model estimates using standard, nonrobust regression also completed in R.

To test the regional specificity of our effects, we completed a supplementary analysis for both the interaction and main effects using total gray matter volume as the predictor. To unpack the model interactions for men and women separately, sex was recoded with men as the reference group and rerun. Supplemental analyses were also conducted including age as a covariate of noninterest. Alpha level was considered at the two-sided, .05 level, given a priori hypotheses. Moreover, to account for multiple statistical tests across our five ROIs, we also report significance for our brain morphology hypotheses at the more conservative Bonferroni- corrected level: alpha = .05/5, that is, alpha = .010.

Prediction Accuracy, Sensitivity, and Specificity Using Median Split

To further aid in the interpretation of these results and to test the generalizability of these findings, we undertook secondary analyses using hierarchical logistic regression and receiver operating characteristic (ROC) analyses implemented in R. These analyses tested whether the interaction of sex and structural brain morphology in the regions that survived multiple correction above would accurately discriminate between those individuals who experienced an increase in anxiety following sleep loss from those that did not using a median split. Specifically, hierarchical regression analysis examined relative improvements in predictive performance when including additive and interactive effects of sex and gray matter volume of each of the ROIs that survived multiple correction as follows:

Step 1: Anxiety Status ~ Sex (sex only model)

Step 2: Anxiety Status ~ Sex + gray matter volume (additive model)

Step 3: Anxiety Status ~ Sex × gray matter volume (interactive model)

ROC analyses were used to determine how to increase the generalizability of the model predictions and reduce the bias caused by model fitting (Tibshirani & Efron, 2002); predictive performance of the ROC analyses was also examined using leave-one-out cross-validation. ROC curves were drawn using the *Epi* package of R (Carstensen, Plummer, Laara, & Hills, 2013).

RESULTS

Sleep Deprivation Effect on Anxiety

Consistent with prior reports (Goldstein et al., 2013; Babson, Feldner, Trainor, & Smith, 2009; Sagaspe et al., 2006), the three-way ANOVA revealed significant main effects of Condition (sleep-deprived, sleep-rested, $F(1, 40) = 14.01$, $p = .001$) and Assessment Time (morning, evening, $F(1, 40) = 53.07$, $p < .0001$), as well as a significant Assessment Time × Condition interaction, $F(1, 40) = 15.06$, $p < .001$ (Figure 2A). Specifically, increases in anxiety from evening to morning were greater in the sleep-deprived condition, relative to the sleep-rested condition (Sleep-deprived$_{[\text{Morning} - \text{Evening}]}$ − Sleep-rested$_{[\text{Morning} - \text{Evening}]}$: Sleep-deprived$_{[\text{Morning} - \text{Evening}]}$ − Sleep-rested$_{[\text{Morning} - \text{Evening}]}$, $F(1, 40) = 14.30$, $p < .001$; Figure 2B). Thus, a full night of sleep minimized the otherwise progressive increase in anxiety that occurred across accruing time awake throughout the sleep deprivation night.

Targeting our hypothesis, the three-way ANOVA revealed a significant Time × Sex, $F(1, 40) = 5.50$, $p = .02$, and, most critically, a significant Condition × Time × Sex interaction, $F(1, 40) = 5.87$, $p = .02$. Expanding this interaction revealed that women demonstrated a significant Time × Condition interaction, $F(1, 21) = 17.88$, $p = .0003$, expressing a near fourfold increase in anxiety from evening to morning on the sleep deprivation night (Sleep-deprived$_{[\text{Morning} - \text{Evening}]}$: 10.77 ± 1.94 [mean ± *SEM*]; Figure 3) relative to the full night of sleep (Sleep-rested $_{[\text{Morning} - \text{Evening}]}$: 2.77 ± 0.88; Figure 3). In contrast, there was no Time × Condition interaction for men, $F(1, 19) = 1.24$, $p = .28$: The increase in anxiety from evening to morning on the sleep deprived night (Sleep-deprived $_{[\text{Morning} - \text{Evening}]}$: 4.4 ± 1.09; Figure 3) did not differ from that of the sleep-rested night (Sleep-rested$_{[\text{Morning} - \text{Evening}]}$: 2.55 ± 1.09; Figure 3).

Although the difference between overnight anxiety increases in men as a whole did not differ across the sleep-deprived and sleep-rested conditions, there was still variation in individual responses (Sleep-deprived$_{[\text{Morning} - \text{Evening}]}$ − Sleep-rested$_{[\text{Morning} - \text{Evening}]}$: 1.85 ± 1.67; min = −12, max = 20), indicating that some men did show an anxiogenic consequence of sleep loss. We test whether these interindividual differences in anxiety in response to sleep deprivation are associated with gray matter volume in the next section. It is also important to note that there was a trending yet nonsignificant main effect of sex on anxiety across all time points within the three-way ANOVA, $F(1, 40) = 3.14$, $p = .08$, and no observable sex differences in the change of anxiety across the sleep-rested night, $F(1, 40) = 0.01$, $p = .90$, suggesting that the anxiogenic vulnerability to sleep deprivation in women was not likely due to general differences in subjective anxiety between sexes.

Brain Structure, Sex, and Sleep Deprivation-induced Anxiety

Next, we sought to test the prediction that gray matter volume in a priori frontal cortex and amygdala regions would significantly account for interindividual differences in the anxiogenic impact of sleep deprivation and in a sex-dependent manner. These findings are summarized in Table 3. Consistent with this hypothesis, robust regression analyses revealed a significant interaction between sex and gray matter volume in both the insula/IFG ($b_{\text{sex*insula volume}} = 158.94$, $t = 3.77$, $df = 1$, $p < .001$; Figure 4A) and lOFC ($b_{\text{sex*ofc volume}} =$

212.00, $t = 3.32$, $df = 1$, $p < .005$; Figure 4B) on sleep deprivation-induced anxiety. Both interaction effects survived Bonferroni correction ($p < .010$) and remained unchanged when including age as a covariate. Both interactions also remained significant when using a nonrobust, linear regression approach ($b_{\text{sex*insula volume}} = 174.95$, $t = 2.47$, $df = 1$, $p = .018$ and $b_{\text{sex*ofc volume}} = 265.26$, $t = 3.11$, $df = 1$, $p < .005$). Expanding the robust regression results, women demonstrated a significant negative association between anxiety and gray matter volume in the emotion generation and integration region of the insula/IFG ($b_{\text{insula volume}} = -88.21$, $t = -3.18$, $df = 1$, $p < .005$, surviving Bonferroni correction) and a marginally significant negative association in the lOFC ($b_{\text{ofc volume}} = -105.81$, $t = -2.41$, $df = 1$, $p = .017$).

Men on the other hand demonstrated marginally significant positive associations in the insula/IFG ($b_{\text{insula volume}} = 70.72$, $t = 2.23$, $df = 1$, $p = .028$) and lOFC ($b_{\text{ofc volume}} = 106.20$, $t = 2.29$, $df = 1$, $p = .025$). Thus, although men as a whole did not demonstrate a significant increase in anxiety due to sleep deprivation, the variance of anxiogenic response in men, described above, was related to variation in gray matter volume of these regions.

A Sex × Gray matter volume interaction was similarly observed in the emotion regulation region of the ACC ($b_{\text{ACC volume}} = 142.22$, $t = 2.14$, $df = 1$, $p = .037$; Figure 4C); however, this did not survive for multiple comparisons. This result remained nonsignificant at the corrected level when including age as a covariate ($p = .044$). Of note, it was men who uniquely demonstrated positive predictive relationships between gray matter volume and anxiety in the ACC (women: $b_{\text{ACC volume}} = -53.13$, $t = -1.05$, $df = 1$, $p = .30$; men: $b_{\text{ACC volume}} = 89.09$, $t = 2.11$, $df = 1$, $p = .041$).

Contrary to the original hypothesis, no significant sex-dependent relationships were identified between the interaction of gray matter volume and sleep loss-induced anxiety in the amygdala ($b_{\text{sex*Amygdala volume}} = 57.29$, $t = 0.69$, $df = 1$, $p = .486$) or the vmPFC ($b_{\text{sex*vmPFC volume}} = 98.38$, $t = 1.56$, $df = 1$, $p = .147$; Figure 4D). These results remained unchanged and nonsignificant when including age as a covariate and when using nonrobust linear regression. In addition, as expected no interaction effect was present when examining total gray matter volume ($b_{\text{sex*gray matter volume}} = 0.058$, $t = 1.29$, $df = 1$, $p = .202$).

Taken together the lack of interaction effects in the amygdala, vmPFC and total gray matter volume suggest that the Sex × Gray Matter Volume interaction may be specific to a select group of cortical affective regions and is not likely to be a result of a more global relationship. Moreover, supplementary robust regression analyses with gray matter volume as the dependent variable and sex as the predictor revealed that gray matter volume itself in each of the a priori ROIs did not differ by sex even when correcting for total intercranial volume (all $|t|$s < 1.65, $p > .11$). This finding indicates that sex (male/female) influences the relationship between gray matter volume and sleep loss-induced anxiety and is not simply the expression of sex differences in gray matter volume mediating differences in anxiety.

Sex-independent Associations between Brain Structure and Sleep Deprivation-induced Anxiety

Although the above analyses expressly examined sex-dependent effects, we also examined the relationships between gray matter volume and sleep loss-induced anxiety that were sex-independent, conducted using the same robust regression analysis approach, but without the interaction term. A full summary of these findings is presented in Table 3. Gray matter volume in the emotion regulation/evaluation region of the vmPFC significantly and positively predicted the degree of sleep deprivation-induced anxiety across both sexes ($b_{\text{vmPFC volume}} = 73.86$, $t = 2.32$, $df = 1$, $p = .033$; controlling for sex; Figure 5). However, the robust regression results did not survive Bonferroni correction. The effect did remain when using nonrobust linear regression ($b_{\text{vmPFC volume}} = 104.81$, $t = 3.07$, $df = 1$, $p < .005$). No such common, sex-independent associations were found between gray matter volume and the anxiogenic impact of sleep deprivation in any of the other a priori ROIs (amygdala, insula/IFG, ACC, or lOFC; Table 3) nor for total gray matter volume ($t = 0.01$, $df = 1$, $p = .690$). Similar effects were observed for all ROIs when including age as a covariate.

Therefore, increasing gray matter volume in the vmPFC conferred a shared vulnerability to the anxiety promoting effects of insufficient sleep across men and women alike, though this relationship failed to meet conservative statistic threshold correction.

Prediction Accuracy, Sensitivity, and Specificity Using Median Split

Only the insula/IFG and lOFC regions survived multiple correction and thus were included in this portion of the analysis. Using a median split of the increase in anxiety after sleep loss (median = 4.0), 74% (14 of 19) of those that demonstrated an increase in anxiety following a single night of sleep deprivation were women.

Conversely, men made up of 67% (16/24) of the non-anxious subset. Yielding an odds ratio of 5.6 (95% CI [1.56, 22.91]). Said another way, sleep deprivation triggered an anxiogenic response in 64% (14 of 22) of women as compared with only 24% (5 of 21) of men. To serve as a basis of comparison for more complex models, a regression model consisting solely of sex was used to classify those individuals who experienced anxiety after sleep loss. Sex alone was a significant predictor of sleep deprivation-induced anxiety status ($\chi^2 = 51.89$, $df = 1$, $p = .008$; Table 4). ROC analyses revealed an accuracy of 0.70, with 74% sensitivity and 67% specificity.

Consistent with the linear regression models described above, including the gray matter volume of either insula/IFG or lOFC alone did not significantly increase the logistic regression model performance (insula/IFG model: $\Delta\chi^2 = 1.54$, $df = 1$, $p = .214$; lOFC model: $\Delta\chi^2 = 0.09$, $df = 1$, $p = .769$; Table 4). Critically, including the interaction term between sex and the gray matter volume of both the insula/IFG and lOFC significantly increased the logistic regression model performance above and beyond that of sex and gray matter volume alone for both the insula/IFG and the lOFC (IFG × Sex model: $\Delta\chi^2 = 11.51$, $df = 1$, $p < .001$; lOFC × Sex model: $\Delta\chi^2 = 16.08$, $df = 1$, $p < .001$).

ROC analyses demonstrated that the interaction model for the insula/IFG classified sleep deprivation-induced anxiety status with a leave-one-out cross-validated AUC of 0.77,

sensitivity of 68.4%, and specificity of 79.2%, suggesting relatively good generalizability (Figure 6). Similarly, the interaction model for the lOFC yielded strong cross-validated accuracy, sensitivity, and specificity (0.82, 100%, and 58.3% respectively; Figure 6). Thus, knowing only the sex of the individual and the volume of the lOFC and insula/IFG, we could predict which individuals would show an increase in anxiety beyond median levels with 77% and 82% accuracy, respectively, indicating generalizability promise.

DISCUSSION

Taken together, these data establish that morphology within select emotion-related regions of the human brain represents a significant predictor of vulnerability to the anxiogenic impact of sleep deprivation. Specifically, gray matter volume in the anterior insula, anterior cingulate, lateral regions of the orbitofrontal cortex, and ventromedial prefrontal cortex regions accounted for interindividual differences in sleep deprivation-associated anxiety, the former three doing so in a sex-dependent manner. Of importance, the latter relationship suggests that there is also a shared brain loci of association linking sleep loss and anxiety that is sex independent, common in both women and men alike.

Sex-dependent associations:

Sleep loss induced the predicted increase in anxiety in women, relative to men, resulting in a 3.8-fold average escalation in anxiety compared with a full night of sleep. This female-specific increase in anxiety is consistent with other experimental findings demonstrating that women are more susceptible to the emotional consequences of sleep deprivation than men (van der Helm et al., 2010; Birchler-Pedross et al., 2009), and with the strong comorbidity of anxiety disorders and sleep disruption in women more so than men (Johnson et al., 2006). Our structural brain analyses offer neural insights underpinning this association. A female-specific relationship was identified between sleep loss, anxiety, and brain structure in affective brain regions involved in viscerosensory signal integration (e.g., autonomic activity, endocrine factors, immunological factors, cognitive context, etc.), namely the anterior insula and lOFC, and not regions associated with emotion regulation, such as the dACC (dorsal ACC). Specifically, less gray matter volume in the anterior insula and lOFC in women was related to more severe increases in anxiety following sleep deprivation.

The anterior insula and lOFC regions are believed to unite primary visceral and affective signals arising from subcortical systems such as the amygdala and brainstem, together with sensory and cognitive information. The insula and OFC then assimilate such information into an in-the-moment second-order map of the internal state of the organism (Craig, 2010; Critchley, 2005, 2009). These second-order maps consequently facilitate descending efferent autonomic and motor output, ultimately guiding behavior, closing the loop between emotional perception, comprehension, and subsequent (re)action (Critchley & Harrison, 2013; Critchley, 2005). It has been argued that only through such holistic mapping of the current body state can the brain accurately process and discriminate between affective signals that promote adaptive decisions and actions in the moment (Critchley & Harrison, 2013; Critchley, 2005). Of key relevance to the current results, anxiety states are believed to arise as a consequence of improper integration and use of viscerosensory information to

predict aversive body states, particularly at the level of these integrative cortices, leading to anxiety (Paulus & Stein, 2006).

Consistent with this notion and our findings, reduced gray matter volume in these cortical integration regions is associated with higher anxiety status (Goodkind et al., 2015; Umeda, Harrison, Gray, Mathias, & Critchley, 2015). Furthermore, sleep deprivation has been shown to compromise affective signal integration processes within the anterior insula and lOFC (Goldstein-Piekarski et al., 2015; Goldstein & Walker, 2014). Men and women also differentially recruit the anterior insula and lOFC across a variety of emotional evaluation tasks (Schwabe et al., 2013; Sokolowski & Corbin, 2012; Cosgrove et al., 2007; Goldstein et al., 2001). Specifically, women demonstrate greater reactivity in emotion generation and integration cortical regions, as well as stronger associations between brain activation in these regions and subjective emotional states, than men.

Our findings suggest that sex differences in anxiety following sleep deprivation are not the direct result of sexual dimorphisms of brain structure, but rather, an interaction between brain structure and sex-specific recruitment of these regions. While remaining speculative, we provide one testable framework that may account for these sex-specific differences in anxiety in Figure 7.

Amygdala:

Counter to our predictions, we did not find associations between amygdala volume and sleep deprivation-induced anxiety. The lack of a structural association with amygdala volume may indicate that sleep deprivation increases anxiety more powerfully through a failure of cortical top–down control of the amygdala or disrupted affective signal integration in higher cortical areas, rather than the amygdala in isolation. Alternatively, the functional activity of the amygdala, rather than its structural features, may determine sex-dependent difference in the anxiogenic impact of sleep deprivation. Studies that examine functional brain activity in the context of anxiety induction, in combination with detailed structural brain measures, will be needed to dissociate these alternative possibilities. Nevertheless, the absence of interaction effects in the vmPFC and lack of effects found using total gray matter volume highlight an anatomically specific and restricted subset of regions (insula/IFG, lOFC, and dACC) governing the sex-dependent impact of insufficient sleep on anxiety.

Sex-independent associations:

In contrast to the sex-*dependent* associations described above, a sex-independent association was identified between the anxiogenic impact of sleep deprivation and gray matter volume in the vmPFC, common across both women and men.

The affective functional role of this region and its sex-common sensitivity to insufficient sleep offers a potential explanation for this result. First, the vmPFC plays an important role in the subjective experience of sleep need (Killgore et al., 2012). For example, greater vmPFC volume is associated with reduced levels of daytime sleepiness in healthy individuals, potentially indicating that individuals with greater vmPFC volume receive a greater restorative benefit of sleep and thus would experience a greater impact of sleep deprivation (Killgore et al., 2012). In addition, human neuroimaging studies indicate that the

vmPFC demonstrates a high degree of sensitivity to insufficient sleep: Both vmPFC activity and its functional coupling with the amygdala are degraded by sleep loss (Chuah et al., 2010; Yoo et al., 2007). This association is conversely restored by a full night of sleep (van der Helm et al., 2011). Second, the vmPFC contributes to the top–down regulation of subcortical limbic brain reactivity, including the experiences of anxiety, and importantly serves this function in a sex-independent manner, common across men and women (Phelps, Delgado, Nearing, & LeDoux, 2004; Rosenkranz, Moore, & Grace, 2003; Davidson, 2002).

It is therefore possible that greater vmPFC volume contributes to individuals being more vulnerable to consequences of sleep loss as a function of decreased top–down regulatory control of subcortical limbic regions that contribute to anxiety states (Kim et al., 2011). Reflecting a sex common vulnerability factor, those individuals with larger vmPFC gray matter volume would experience the greatest sleep deprivation-induced diminution of vmPFC function and thus greater anxiogenic impact. Within this context, it is interesting to note that prior experimental findings have shown that greater gray matter volume in the vmPFC may confer resilience, rather than vulnerability, to anxiety and depression symptomology (van Tol et al., 2010) under normative sleep conditions. The current findings in no way challenge these established relationships. Rather, our complementary findings suggest that the relationship between vmPFC structure and anxiety is not stable but varies as a function of brain state, here the functional presence or absence of sleep.

Study considerations:

These findings should be appreciated within the context of certain limitations. First, study participants were young healthy adults by design, as this was the first characterization of the interaction between brain structure, sex, and sleep deprivation-induced anxiety. Nevertheless, that similar relationships would be observed in samples with clinically diagnosed anxiety remains speculative as a consequence (though clear testable predictions can be made on the basis of these first studies). Second, it is possible that a third unexplored variable may be responsible for producing both interindividual differences in gray matter volume and anxiety increases following sleep loss. Combining structural and functional measures of brain activity with other candidate factors associated with anxiety, such as peripheral body measures, will undoubtedly illuminate a more detailed set of interacting features that determine sex-specific and sex-independent relationships between sleep deprivation and anxiety.

In summary, these findings establish that the morphology of emotion-relevant regions represents both sex-common and sex-specific biomarkers, explaining greater vulnerability to the anxiogenic impact of insufficient sleep. Clinically, these data offer an emerging pathophysiological mechanism that may, at least in part, account for the long-recognized comorbidity of sleep disruption and anxiety disorders in men and women. Finally, such findings suggest that, for women especially, targeted sleep restoration may offer a novel, nonpharmacological therapeutic pathway for ameliorating anxiety, further advocating for greater public health awareness regarding the importance of insufficient sleep in those at greatest risk of anxiety disorders.

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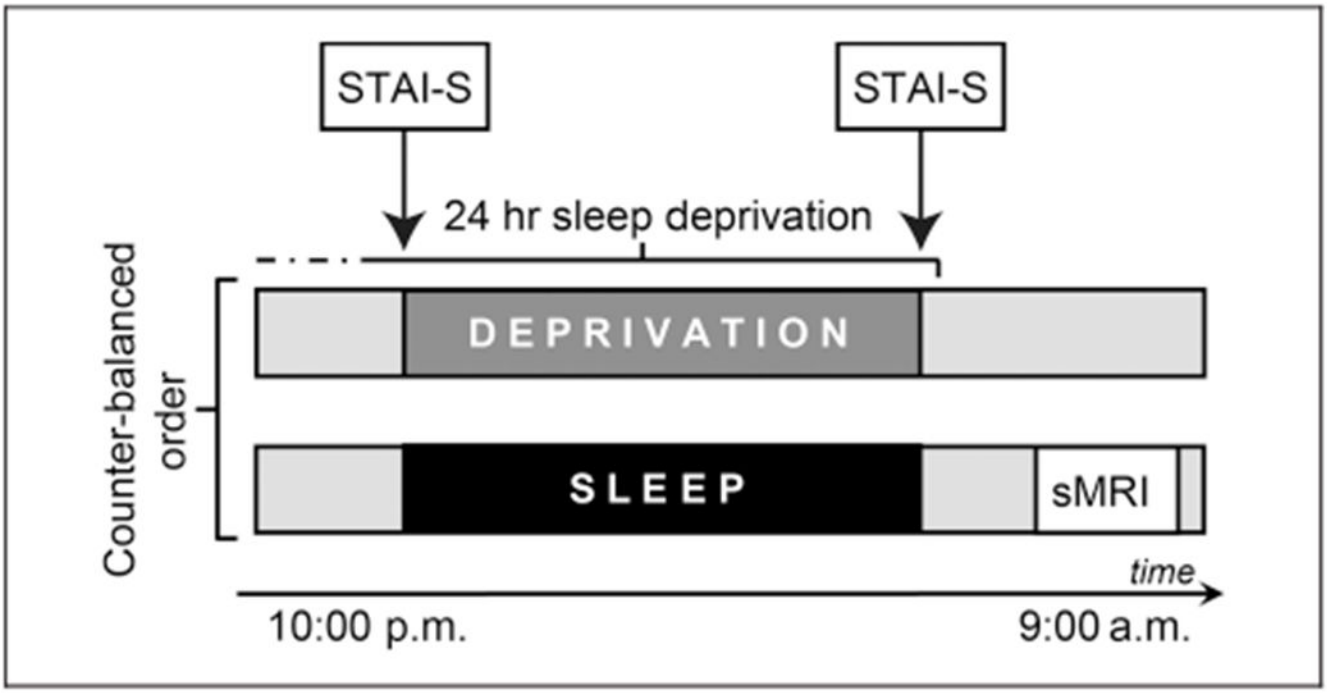

Figure 1.

Experimental design. Time course for the experimental protocol for both sleep-rested and sleep-deprived conditions. Following screening, participants entered a repeated-measures crossover design with two experimental conditions in which they stayed in the laboratory: rested sleep (control) and sleep deprivation (24 hr of sleep deprivation). The STAI-State anxiety questionnaire was used to assess state-dependent changes in anxiety levels (Spielberger et al., 1983) on the evening of and morning after each experimental condition (sleep, sleep deprivation). STAI-State Anxiety Assessments were obtained at the same circadian time points, administered at 10:50 p.m. (±30 min, just before lights off in the sleep condition) and again the following morning at 8:10 a.m. (±60 min) in both sleep-rested and sleep-deprived conditions. Participants completed a structural MRI scan on the morning of the sleep-rested condition. STAI = Spielberger State-Trait Anxiety Inventory; sMRI = structural magnetic resonance imaging scan.

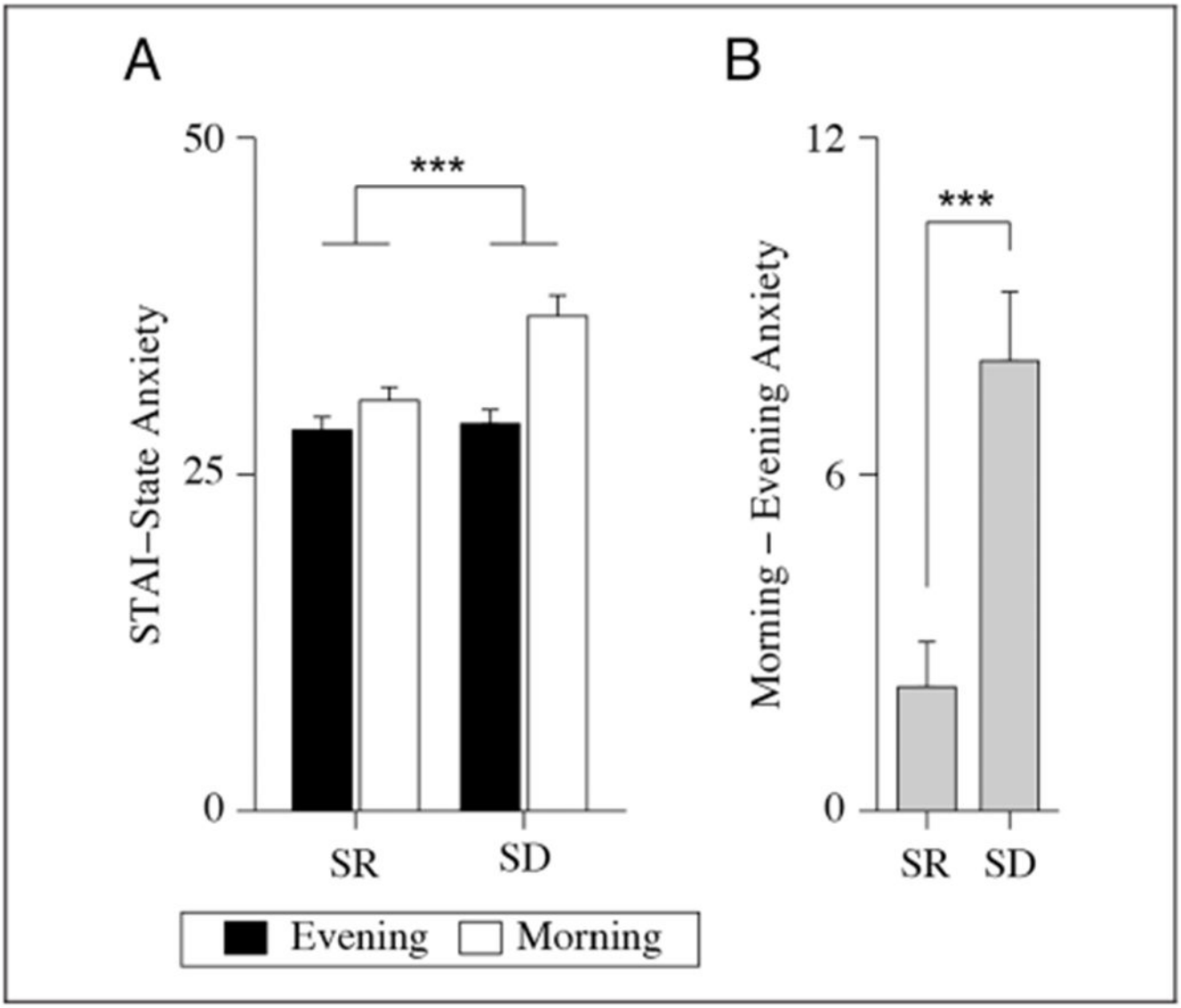

Figure 2.

(A) Average state anxiety scores for all participants across the sleep-rested (SR) and sleep-deprived (SD) conditions. (B) Corresponding overnight changes in anxiety for rested and deprived conditions, represented as the subtracted difference in the evening relative to the morning anxiety scores. Comparison reflects significance at $p < .001$ (***). Error bars represent *SEM*.

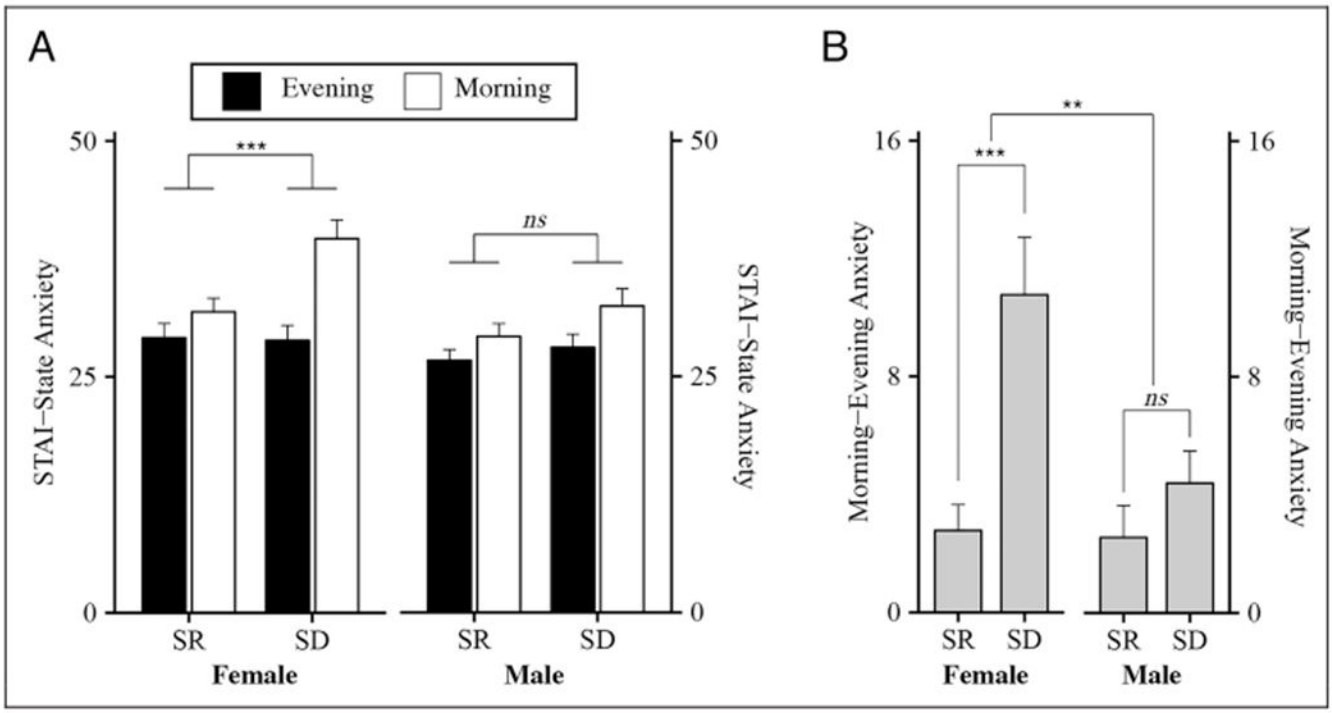

Figure 3.

(A) Average state anxiety scores for women (left) and men (right) across the sleep-rested (SR) and sleep-deprived (SD) conditions. (B) Corresponding overnight changes in anxiety for rested and deprived conditions for women and men separately, represented as the subtracted difference in the evening relative to the morning anxiety scores. Comparison reflects significance at $p < .01$ (**) and $p < .001$ (***). Error bars represent *SEM*.

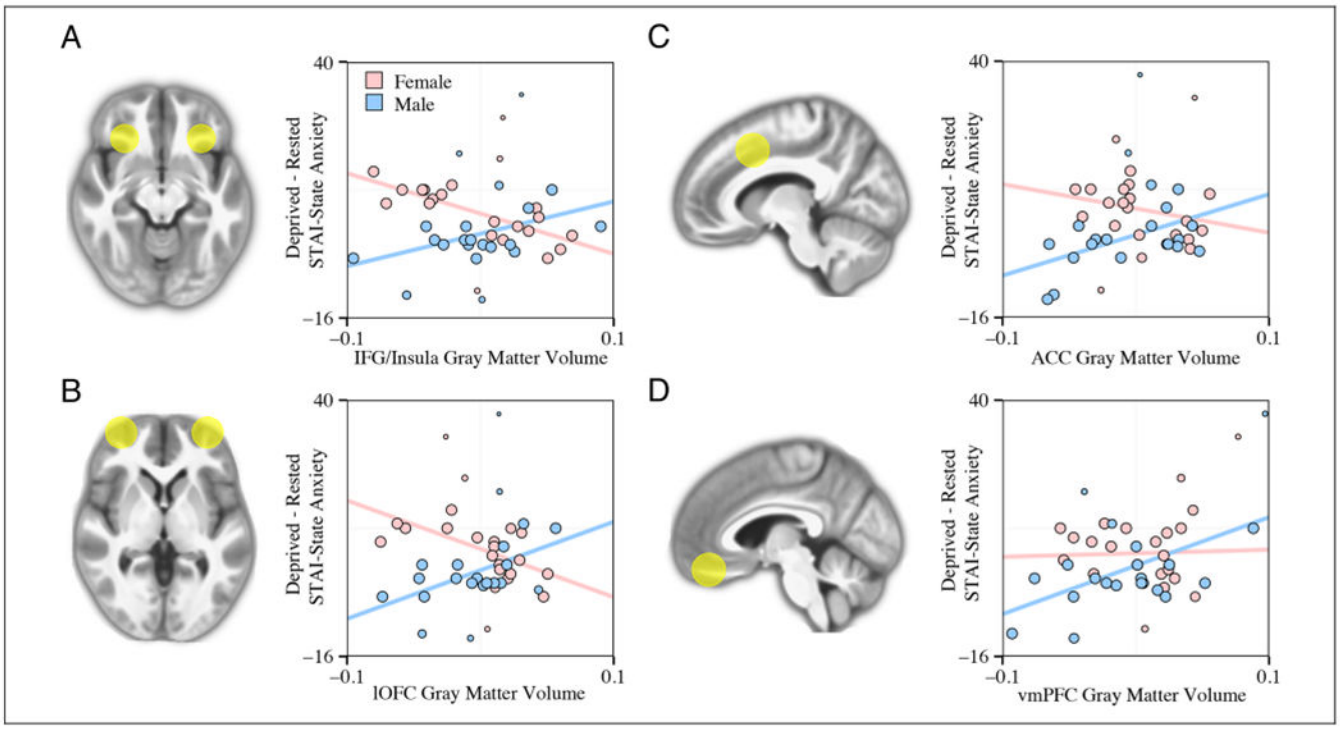

Figure 4.

Sex moderates the relationship between gray matter volume and sleep-deprived increases in anxiety. Brain map displaying ROI mask and robust regression between gray matter volume and the sleep-deprived overnight increase in anxiety for the (A) insula/IFG (female: $b = -88.21$, $t = -3.18$, $p = .002$; male: $b = 70.72$, $t = 2.23$, $p = .028$), (B) lOFC (female: $b = -105.81$, $t = -2.41$, $p = .017$; male: $b = 106.20$, $t = 2.29$, $p = .025$), (C) ACC (female: $b = -53.13$, $t = -1.05$, $p = .305$; male: $b = 89.09$, $t = 2.11$, $p = .035$), and (D) vmPFC (female: $b = 7.79$, $t = 0.16$, $p = .879$; male: $b = 106.16$, $t = 2.73$, $p = .014$) when controlling for sex and total intracranial volume. The relative size of circles represents the individual weight of each point in the robust regression analysis.

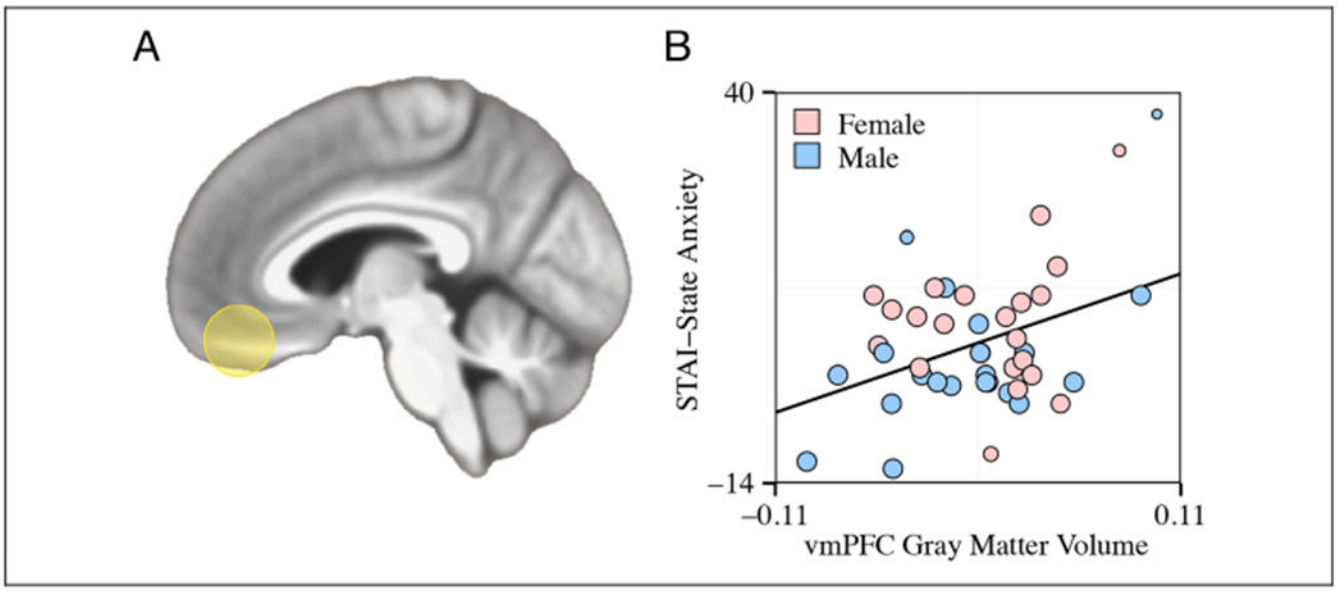

Figure 5.

Gray matter in vmPFC predicts sleep-deprived increases in anxiety for women and men together. (A) Brain map displaying vmPFC ROI mask. (B) Robust regression between vmPFC gray matter volume and the sleep-deprived overnight increase in anxiety when controlling for sex and total intracranial volume ($b = 73.86$, $t = 2.32$, $p = .033$). The relative size of the circles represents the individual weight of each point in the regression analysis.

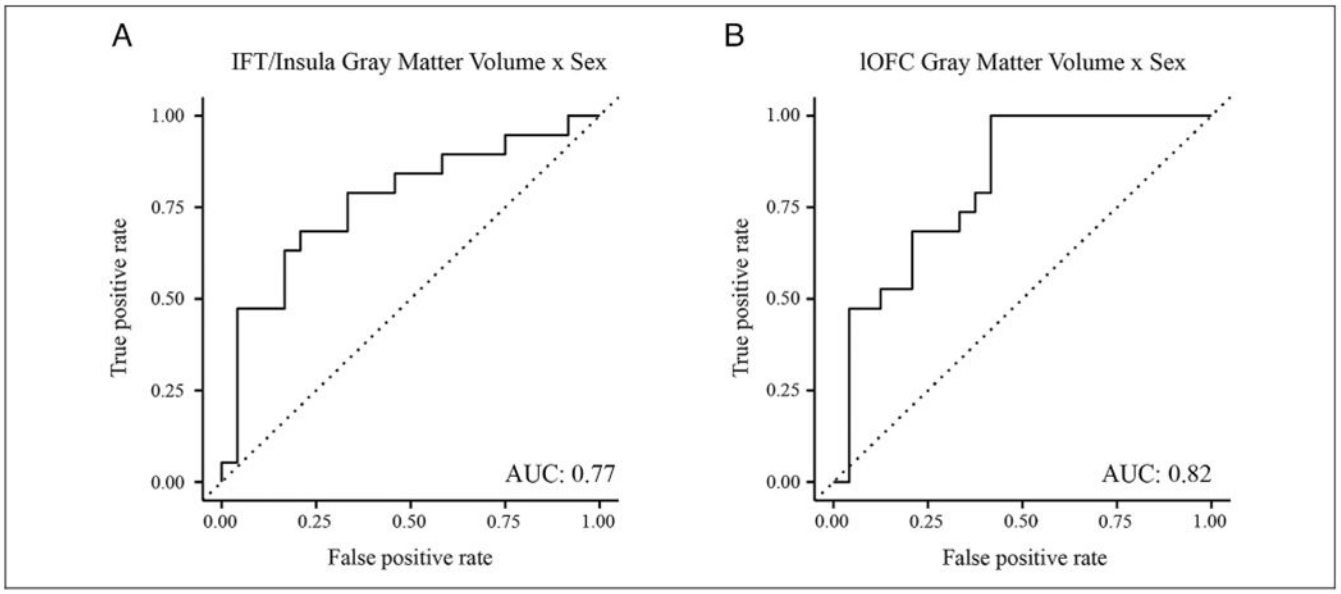

Figure 6.

ROC curves showing the leave-one-out, cross-validated performance of the candidate regression models in predicting anxiety increases following sleep loss. ROC curves model performance in predicting anxiety for 43 individuals using the interaction between sex and gray matter volume in the (A) IFG/insula and (B) lOFC.

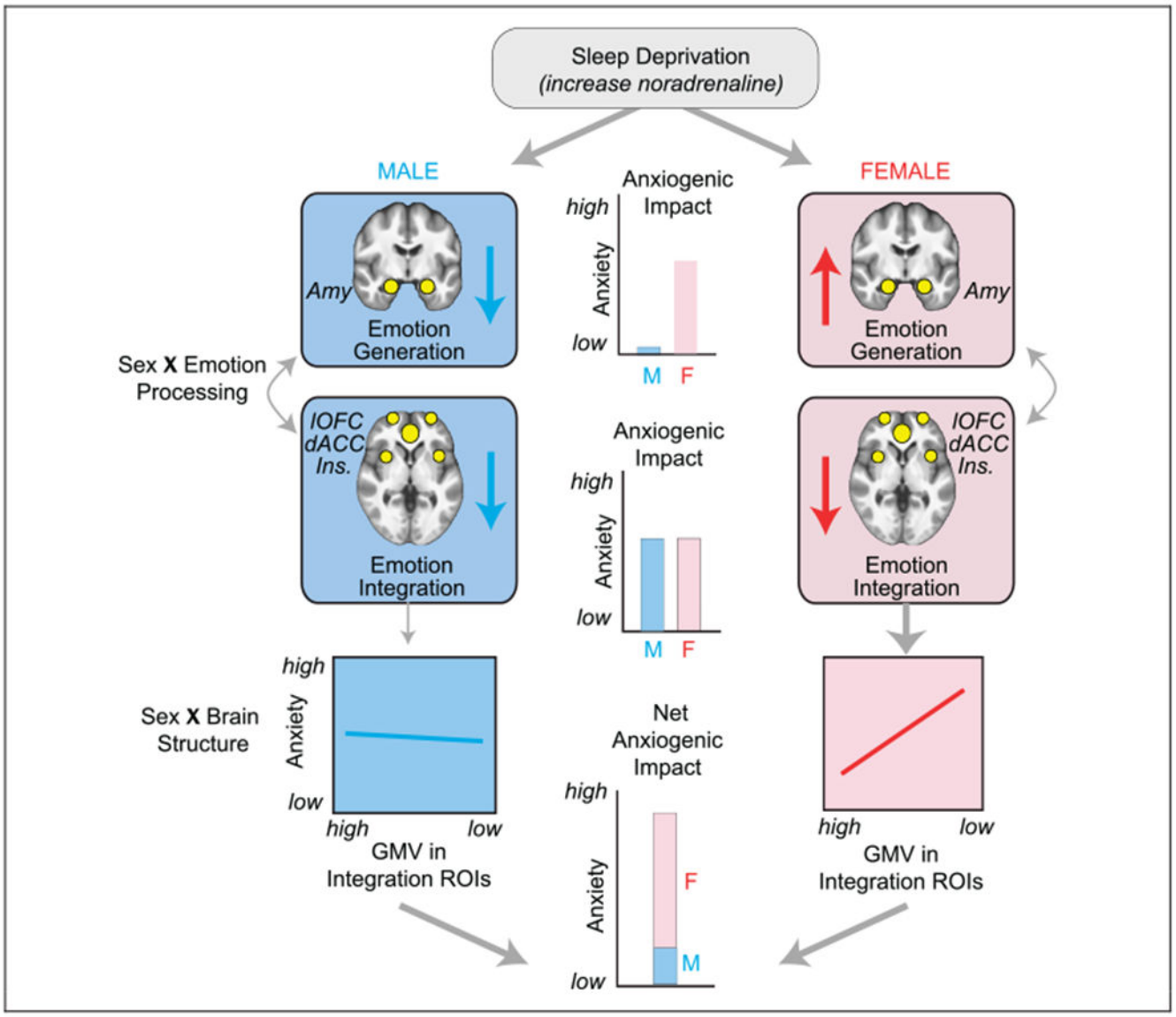

Figure 7.

Proposed model explaining sex differences in the anxiety promoting effects of sleep loss. Sleep deprivation increases noradrenaline levels beyond baseline in men and women (Mallick & Singh, 2011; Siegel & Rogawski, 1988). Top row: The increased noradrenaline levels are hypothesized to induce sex-specific alterations in emotional reactivity, with women showing increases in amygdala activity while men showing decreases in amygdala activity (Schwabe et al., 2013). These alterations in emotion generation regions are though to contribute to increasing anxiety-related behaviors in women, while conversely reducing anxiety in men. The bar graph demonstrates the predicted anxiogenic impact of sleep loss for men and women as a result of altered amygdala activation. Middle row: Sleep deprivation additionally induces sex-independent functional changes in emotional integration regions of the lOFC, dACC, and insula/IFG, potentially causing increases in anxiety-related behavior for both men and women (Goldstein-Piekarski et al., 2015;

Goldstein & Walker, 2014). The bar graph depicts the predicted anxiety common across men and women as a result of sleep impairments in integration regions. Bottom row: The magnitude of these effects may be further modulated by the sex-specific contribution of emotion integration and generation regions in the generation of emotional states (Schwabe et al., 2013; Sokolowski & Corbin, 2012; Cosgrove et al., 2007; Goldstein et al., 2001). Because the subjective emotional state of women is more tightly linked to functioning of emotion integration and generation regions, the sleep-deprived noradrenaline impacts on these regions maintains/amplifies anxiety (as by the thicker solid arrow). In contrast, because the subjective emotional state is less dependent on emotion integration and generation regions for men, the magnitude of the sleep-deprived impact of noradrenaline on anxiety is reduced (as indicated by the thinner solid arrow). The proposed sex-specific sleep-deprived effects in emotional reactivity are further exacerbated in those with less gray matter volume in emotion integration regions (Goodkind et al., 2015; Umeda et al., 2015). Together these factors contribute to sex differences in the sleep-deprived increase in anxiety (bar graph) as well as sex-specific relationships between the anxiogenic influence of sleep loss and gray matter volume of integration regions (line plots). Amy = amygdala; Ins = insula/IFG, GMV = gray matter volume.

Table 1.

PSG Sleep Stage Values for the Sleep-rested Night (Mean ± SD)

	Time (min)	*% Total Sleep Time*	*Obtained by % of Participants*
Time in bed	531.64 ± 40.83		
Sleep latency	12.01 ± 13.68		
Total sleep time	482.62 ± 45.51		
WASO	32.52 ± 29.17		
NREM Stage 1	38.01 ± 19.60	7.89 ± 4.05	100%
NREM Stage 2	249.52 ± 37.41	51.64 ± 5.55	100%
NREM SWS	89.48 ± 29.82	18.77 ± 6.71	100%
REM	105.61 ± 26.70	21.71 ± 4.23	100%

Sleep efficiency (total sleep time divided by time in bed) was within normal levels (90.84% ± 5.92%). WASO = wake after sleep onset; NREM = nonrapid eye movement sleep; SWS = slow-wave sleep (SWS; NREM Stages 3 and 4); REM = rapid eye movement sleep.

Table 2.

PSG Sleep Stage Values in Minutes for the Sleep-rested Night Split by Sex (Mean ± SD)

	Female (n = 23)	*Male (n =21)*	*t(42)*	*p*	*Cohen's d*
Time in bed	531.73 ± 40.55	531.54 ± 42.13	0.02	.987	0.00
Sleep latency	8.55 ± 7.15	15.81 ± 17.81	−1.80	.079	0.54
Total sleep time	491.38 ± 47.34	473.03 ± 42.46	1.35	.185	−0.41
WASO	27.68 ± 22.64	37.82 ± 34.77	−1.16	.254	0.35
NREM Stage 1	34.39 ± 13.69	41.97 ± 24.25	1.29	.204	0.39
NREM Stage 2	259.90 ± 28.30	238.16 ± 43.23	−1.99	.053	−0.60
NREM SWS	85.48 ± 24.17	93.87 ± 35.07	0.93	.357	0.28
REM	111.60 ± 28.66	99.03 ± 23.28	−1.59	.120	−0.48

Men and women did not significantly differ on any of the above sleep variables (all *t* tests, $p > .05$). WASO = wake after sleep onset; NREM = nonrapid eye movement sleep; SWS = slow-wave sleep (SWS; NREM Stages 3 and 4); REM = rapid eye movement sleep.

Table 3.

Summary of Robust Regression Results (All *dfs* = 1)

	Female			*Male*			*Interaction (All)*			*Main Effect (All)*		
Region	b_{volume}	***t***	***p***	b_{volume}	***t***	***p***	$b_{sex*volume}$	***t***	***p***	b_{volume}	***t***	***p***
Insula/IFG	**−88.21**	**−3.18**	**.002** *	70.72	2.23	.028	**158.94**	**3.77**	**<.001** *	−29.79	−1.19	.228
lOFC	−105.81	−2.41	.017	106.20	2.29	.025	**212.01**	**3.32**	**.002** *	−14.62	−0.40	.686
ACC	−53.13	−1.05	.305	89.10	2.11	.035	142.22	2.14	.037	31.97	0.83	.406
vmPFC	7.79	0.16	.880	106.16	2.73	.014	98.37	1.56	.147	73.86	2.31	.033
Amygdala	−21.78	−0.54	.576	35.51	0.49	.622	−57.29	0.69	.486	−7.50	−0.212	.829

* **Boldface** highlights significance at Bonferroni-corrected value ($p < .010$).

Table 4.

Hierarchical Regression Estimating Sleep Deprivation-induced Anxiety

			Model Fit		*Model Gain per Step*	
Model Predictors	***B (95% CI)***	***p***	χ^2 ***(df)***	***p***	$\Delta\chi^2$ ***(Δdf)***	***p***
Insula/IFG Gray Matter Volume and Sex Model						
Step 1						
Intercept	0.56 (−0.29–1.48)	.207	51.89 (1)	.008	7.13 (1)	.008
Sex	**−1.72 (−3.13–−0.45)**	**.011**				
Step 2						
Insula/IFG volume	−10.44 (−28.52–5.90)	.225	50.35 (2)	.013	1.54 (1)	.214
Step 3						
Insula/IFG volume × Sex	**72.82 (27.64–135.51)**	**.006**	38.84 (3)	<.001	11.51 (1)	<.001
lOFC Gray Matter Volume and Sex Model						
Step 1						
Intercept	0.56 (−0.29–1.48)	.207	51.89 (1)	.008	7.13 (1)	.008
Sex	**−1.72 (−3.13–−0.45)**	**.011**				
Step 2						
lOFC volume	−3.06 (−24.39–17.45)	.769	51.81 (2)	.027	0.86 (1)	.769
Step 3						
lOFC volume × Sex	**138.86 (57.86–265.44)**	**.007**	35.73 (3)	<.001	16.08 (1)	<.001

Boldface highlights significance ($p < 0.05$). B = unstandardized beta coefficient; IFG = inferior frontal gyrus disorder.