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Neural - Hormonal Responses to Negative Affective Stimuli: Impact of Dysphoric Mood and Sex

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Contributors

1. **K. Mareckova:** served as the primary data analyst and wrote the initial draft.
2. **L. Holsen:** was responsible for the collection, organization and analysis of fMRI data and was critical in rewriting and adding to the manuscript.
3. **R. Admon:** was responsible for some of cortisol data analysis.
4. **S. Whitfield-Gabrieli:** used her expertise in fMRI imaging to serve as a consultant for the study design and data analysis.
5. **L.J. Seidman:** was involved in the data collection for the psychosis sample and the editing of the manuscript.
6. **S.L. Buka:** oversaw the location and recruitment of study subjects and assisted in drafting the manuscript.
7. **A. Klibanski:** Oversaw the design and collection of all neuroendocrine data, served as an analysis consultant, and assisted in manuscript preparation.
8. **J.M. Goldstein:** was responsible for the overall oversight of the study, including the initial hypotheses, study design, analyses, quality control of the data, and the writing of the manuscript.

Abstract

Background—Maladaptive responses to negative affective stimuli are pervasive, including clinically ill and healthy people, and men and women respond differently at neural and hormonal levels. Inspired by the Research Domain Criteria initiative, we used a transdiagnostic approach to investigate the impact of sex and dysphoric mood on neural-hormonal responses to negative affective stimuli.

Methods—Participants included 99 individuals with major depressive disorder, psychosis and healthy controls. Functional magnetic resonance imaging (fMRI) was complemented with realtime acquisition of hypothalamo-pituitary-adrenal (HPA) and -gonadal (HPG) hormones. fMRI data were analyzed in SPM8 and task-related connectivity was assessed using generalized psychophysiological interaction.

Results—Across all participants, elevated cortisol response predicted lower brain activity in orbitofrontal cortex and hypothalamus-amygdala connectivity. In those with worse dysphoric mood, elevated cortisol response predicted lower activity in hypothalamus and hippocampus. In women, elevated cortisol response was associated with lower activity in medial prefrontal cortex and low hypothalamo-hippocampal connectivity. In women with high dysphoric mood, elevated cortisol response was associated with low hypothalamo-hippocampal connectivity. There were no interactions with diagnosis or medication.

Limitations—There was limited power to correct for multiple comparisons across total number of ROIs and connectivity targets; cortisol responses were relatively low.

Conclusions—We conclude that the pathophysiology in neural-hormonal responses to negative affective stimuli is shared across healthy and clinical populations and varies as a function of sex and dysphoric mood. Our findings may contribute to the development of hormonal adjunctive therapeutics that are sex-dependent, underscoring the importance of one's sex to precision medicine.

Keywords

negative affect; dysphoric mood; RDoC; fMRI; sex differences; cortisol response

INTRODUCTION

Maladaptive responses to negative affective stimuli are pervasive across healthy and ill populations, and men and women respond differently at the neural and hormonal levels. This is not surprising given that brain regions that regulate responses to negative affective stimuli, subsequently referred to as “stress response”, are highly sexually dimorphic (Tobet et al., 1993; Filipek et al., 1994; Giedd et al., 1996; Goldstein et al., 2001; Tobet et al., 2009), regulate the hypothalamic pituitary adrenal and gonadal (HPA and HPG) axes (Keverne, 1988; Tobet & Hanna, 1997; Ostlund et al., 2003; Swaab, 2004; Bao et al., 2005), arousal (Keverne, 1988; McEwen & Magarinos, 1997; Price, 1999) and mood (Mareckova et al., 2016). Brain regions include: periaqueductal gray (PAG), hypothalamus (HYPO), amygdala (AMYG), hippocampus (HIPPO), and medial and orbital prefrontal cortices (mPFC, OFC)

(see Mareckova et al., 2016), areas of the brain that have been found to be abnormal structurally and functionally across a number of psychiatric disorders.

Understanding the physiology and the impact of sex on the regulation of responses to negative affective stimuli may provide clues to the development of therapeutics that may be shared across disorders. In fact, the NIMH Research Domain Criteria (RDoC; Insel et al., 2010) initiative argues that it is critical to identify neurobiologic signatures at the level of genes, brain circuits, and physiology associated with behavioral traits that are shared across broadly defined diagnostic categories (Van Os & Kapur, 2009). Consistent with this approach, we aimed to identify neural and hormonal responses to negative affective stimuli across individuals with varying levels of dysphoric mood, regardless of their diagnosis (major depressive disorder, bipolar disorder, schizophrenia), and the sex differences therein (Pariante & Lightman, 2008; Holsen et al., 2013; Dedovic & Ngiam, 2015; Colich et al., 2015; Mocking et al., 2015; Pruessner et al., 2013; Goldstein et al., 2010b and 2015; Belvederi Murri et al., 2016).

Neural responses to stress are defined by their associated physiology. That is, stress triggers secretion of corticotropin-releasing hormone (CRH) by the hypothalamic paraventricular nucleus (PVN), which in turn triggers release of adrenocorticotropin hormone (ACTH) from the anterior pituitary, and ultimately release of cortisol from the adrenal cortex (Tsigos & Chrousos, 2002; Goldstein et al., 2014). The PVN, located in anterior hypothalamus, is considered the key relay center for HPA axis function (Herman et al., 1996; Goldstein et al., 2007; Tobet et al., 2013; Evanson & Herman, 2015; Laryea et al., 2015). It receives negative feedback from stress circuitry regions with high densities of glucocorticoid receptors, particularly from hippocampus, and, to a lesser extent, from amygdala and medial and orbital prefrontal cortices (mPFC, OFC) (De Kloet et al., 1998; Myers et al., 2012). Cortisol is the most direct measure of “stress hormone physiology” related to HPA axis functioning (Dickerson & Kemeny, 2004; Bozovic et al., 2013), and it provides a stimulus for negative feedback that attenuates activity of the PVN through limbic (hippocampal) and mPFC areas (Herman et al., 2005). Behavioral studies reported a positive relationship between negative affect trait and cortisol response (Mendoca-De-Souza et al., 2007; Quiring et al., 2009). Functional MRI (fMRI) studies showed that increased cortisol response predicted blood oxygen level dependent (BOLD) signal activity in limbic and frontal regions (Thomason et al., 2011; Collip & Nicolson, 2011; Holsen et al., 2013; Goldstein et al., 2014), modulating neural circuits related to affect and cognition (Henckens et al., 2010; Holsen et al., 2011).

The current study investigated the coupling between neural and hormonal physiology during a mild visual stress task with negative valence/high arousal and neutral valence/low arousal images adapted from the International Affective Picture System (IAPS; Lang et al., 2008) for this fMRI paradigm (Goldstein et al., 2005). The ability of this task to evoke BOLD responses in the neural circuitry associated with arousal and negative affect has been demonstrated in multiple studies of ours over the last 11 years in healthy populations and distinct psychiatric disorders (i.e., Goldstein et al., 2005; and 2010; Holsen et al., 2011; 2012; 2013; Jacobs et al., 2014), and recently, we demonstrated shared brain activity responses in the regions listed above across healthy and ill populations in the same sample as

reported here (Mareckova et al., 2016). The ability of this task to elicit HPA response has been demonstrated by Admon et al (2015) and Holsen et al (2013).

Brain activity in response to negative affective stimuli in these regions has also been reported by others using passive viewing of negative valence/high arousal stimuli (van Stegeren et al., 2007; Cunningham-Bussel et al., 2009; Root et al., 2009) or other stress-related paradigms, including psychosocial stressors such as mental arithmetic task with continuous negative feedback on task performance (Wang et al., 2007; Pruessner et al., 2008). Activity in these regions has been associated with physiologic responses, including cortisol response (e.g. Urry et al., 2006; Liberzon et al., 2007; Kern et al., 2008; Cunningham-Bussel et al., 2009; Veer et al., 2012; Holsen et al., 2013) and loss of parasympathetic cardiac tone (Holsen et al., 2012). This is consistent with other studies linking hypercortisolemia (Collip & Nicolson, 2011) and autonomic arousal (Wik et al, 1991; Lane & Wager, 2009) with response to negative affective stimuli. Further, exogenous administration of cortisol has been significantly associated with decreased activation of the AMYG and HIPPO during rest (Lovallo et al., 2010).

Substantial data support sex differences in neural and physiologic responses to stress in healthy (Kudielka & Kirschbaum, 2005; Goldstein et al., 2010a) and clinical populations with psychiatric disorders associated with this circuitry, such as major depressive disorder (MDD) and schizophrenia. Women are at significantly higher risk for mood disorders (Kendler, 2006; Kessler, 2003; Goldstein et al., 2014; Seney & Sibille, 2014) and demonstrate greater arousal to aversive cues (Lane et al., 1997; Lang et al., 1998; Bradley et al., 2001; Caseras et al., 2007) than men (although this varies in healthy women by timing of menstrual cycle). Sex differences may be related to the fact that the brain regions implicated in stress response and HPA axis function include some of the most sexually dimorphic nuclei in the brain (Tobet et al., 1993; Filipek et al., 1994; Giedd et al., 1996; Goldstein et al., 2001; Tobet et al., 2009). While women have significantly larger hippocampal volumes, relative to cerebrum size (Filipek, 1994; Giedd, 1996; Goldstein, 2001), men have relatively larger volumes of the amygdala (Giedd, 1996; Goldstein, 2001), many of the hypothalamic nuclei (Swaab & Fliers, 1985), paracingulate gyrus (Goldstein, 2001; Paus, 1996), and mPFC (Goldstein, 2001). These regions are also dense in sex steroid receptors (McEwen, 1981; Keverne, 1988; Ostlund et al., 2003; Bao et al., 2005).

Sex differences in stress response and the prevalence of psychiatric disorders have also been linked to dysregulation of HPG function. Human studies have found deficits in gonadal function in MDD (Goldstein, et al, 2014; Rubinow & Schmidt, 1996; Harlow et al, 2003; Jacobs et al, 2014), including androgens (Rubinow & Schmidt, 1996; Schweiger et al, 1999; Seidman et al, 2001; Weiner et al., 2004), estradiol (Young et al., 2000; Holsen et al, 2011; Jacobs et al, 2014; Harlow et al, 2003), and progesterone (Schiller et al., 2014). HPA (e.g., hypercortisolemia) and HPG (hypogonadal) deficits have also characterized people with psychotic disorders, such as schizophrenia and bipolar disorder (Collip et al., 2011; Walker & Diforio, 1997; Walder et al., 2000; Seeman & Lang et al., 1990; Canuso et al., 2000; Kulkarni et al., 2001).

This study builds on the fMRI findings of Mareckova et al (2016) and extends them by studying the coupled hormonal responses acquired *in real time* during the functional imaging. Based on the substantial body of functional imaging work relating endocrine function with mood regulation (e.g. Goldstein et al., 2010a; Root et al., 2009; Pruessner et al., 2008; Wang et al., 2007; Stark et al., 2006; Goldstein et al., 2005; Andreano & Cahill, 2010; Protopopescu et al., 2005; Amin et al., 2006) and reports on abnormal HPA axis functioning (Carroll et al., 1976 a, b) and response to stress (Burke et al., 2005), we hypothesized that sex and dysphoric mood symptomatology would be associated with deficits in neural-hormonal response to negative affect. We predicted that women would demonstrate stronger coupling between neural and cortisol responses to negative affective stimuli than men and that these sex differences would be further modulated by the levels of androgens in men and estradiol in women. We also predicted that the coupling of neural and cortisol responses would increase as a function of worsening mood. These hypotheses were explored initially in the context of BOLD response and additionally in the context of connectivity between hypothalamus, the key relay station for HPA axis function, and the other ROIs defined in Mareckova et al (2016). Findings will contribute to elucidating the neural and physiologic mechanisms regulating stress and mood responses and the impact of sex.

METHODS

Participants

Participants included 99 adults (48 females, 51 males). The majority were offspring of women who took part in the large scale (n=17,741) Boston and Providence Collaborative Perinatal Project (CPP) and whom we have been following for about 20 years in the New England Family Studies (NEFS; Niswander & Gordon, 1972; Goldstein et al., 2014). Approximately 14% were non-NEFS subjects but recruited using the same criteria, from the same community catchment area and not different on any sociodemographic or clinical characteristic than the rest of the sample. Thirty-one were diagnosed with psychoses (PSY; 15 females, 16 males), 27 with recurrent major depressive disorder without psychosis and in remission (MDD; 14 females, 13 males), and 41 were healthy controls (CTRL; 19 females, 22 males). The PSY group consisted of 16 individuals with affective psychosis including bipolar disorder with psychosis and schizoaffective disorder-bipolar type (BP; 9 females, 7 males) and 15 individuals with non-affective psychosis including schizophrenia, schizoaffective-depressed type, and psychosis not otherwise specified (SCZ; 6 females, 9 males). These PSY classifications have a long history and validation in family studies and have been used in our population-level NEFS studies over many years (Goldstein et al., 2010b; Goldstein et al., 2015). Individuals with either MDD or SCZ were not currently in an episode (no clinically-significant depressive symptoms in the MDD group; no active psychosis in the SCZ group) and the healthy controls were not diagnosed with any DSM Axis I disorders. Further exclusion criteria included MRI exclusion criteria, the use of caffeine in the morning prior to the fasting blood sample, alcohol consumption 24 hours prior to the blood draw, and any current substance use disorders, as assessed by the Structured Clinical Interview for the DSM (SCID). For details of the demographics and clinical characteristics of the sample, see Mareckova et al 2016. In brief, there were no

significant differences between men and women in ethnicity, handedness, age, body mass index, parental SES, educational level, WAIS-R vocabulary scores, rate of DSM diagnoses, or the proportion taking psychotropic medication. Men scored higher on WAIS block design and had higher rate of substance abuse disorders than women. All participants provided written informed consent to a protocol approved by Harvard University, Brown University, Partners Healthcare system, and local psychiatric facilities.

Steroid Hormone Assessments

We acquired hormones (estradiol, testosterone, cortisol, sex hormone binding globulin [SHBG]) in response to the negative affect task *in real time* during functional imaging (Goldstein, et al, 2015; Holsen et al, 2013). An in-scanner baseline blood (Time 0) was acquired following five minutes of pre-scan protocols. Subsequent samples, timed to hormonal responses based on pituitary (15–30 minutes post-visual stress challenge) and steroid hormones (60–90 minutes post-stress challenge), were drawn at 15 and 30 minutes in-scanner and 60 and 90 minutes out-of-scanner in a quiet room (total of approximately 30cc of blood). See Supplementary Methods for further information on blood taking and hormone analysis.

Cortisol response was calculated consistent with Admon et al. (2015) as $(\text{Cortisol}_{\text{Peak}} - \text{Cortisol}_{\text{Time 0}}) / \text{Cortisol}_{\text{Time 0}}$, where $\text{Cortisol}_{\text{Time 0}}$ was measured from blood draw 5 minutes after the onset of scanning session, thus allowing us to control for the possibility that the cortisol response was due to MRI-related anxiety. Since timing of cortisol response was shown to be highly variable (see meta-analysis by Dickerson & Kemeny, 2004), $\text{Cortisol}_{\text{Peak}}$ was allowed to vary and calculated as the highest cortisol value reached during the subsequent blood draws (15, 30, 60 or 90 minutes after the onset of scanning). Means and standard deviations of free androgens, 17 β -estradiol, and cortisol response by sex are provided in Supplementary Table 1. Due to the non-normal distribution of these steroid hormones, all our analyses used natural log-transformed values of Cortisol response, Free Androgens and 17 β -estradiol.

Assessment of mood and anxiety

The Profile of Mood States (POMS; McNair et al., 1992) and State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983) were administered before the MRI scan to assess mood and anxiety. As reported in Mareckova et al (2016), a composite measure of the mood and anxiety state/trait was created using factor analysis of the five POMS subscales (TA, DD, AH, FI, VA, CB) and two STAI subscales (Anxiety State, Anxiety Trait) which identified Dysphoric Mood State as the primary factor explaining the highest portion of variance. Evidence for its clinical validity was demonstrated given significant relationship with the Hamilton Depression Scale (Mareckova, et al, 2016). While men scored higher on current dysphoric mood state than women, there was no effect of diagnosis on dysphoric mood state (see Mareckova et al, 2016)

Acquisition of fMRI data

fMRI data were acquired on a Siemens Tim Trio 3T MRI scanner with a 12-channel head coil. The fMRI task consisted of three runs (180 volumes each) acquired using a spin echo,

T2*-weighted sequence (TR=2000 ms, TE=40 ms, FOV=200×200 mm, matrix 64×64, in-plane resolution 3.125 mm, slice thickness 5 mm, 23 contiguous slices aligned to AC-PC plane). The fMRI task presented negative valence/high arousal (e.g. snake, car accident, burial, gun, bomb, tornado), neutral valence/low arousal (e.g. plant, umbrella, office, mushrooms, stool, bus), and fixation images adapted from the *International Affective Picture System* (IAPS; CSEA-NIMH, 1999), a well known set of images with demonstrated reliability and validity and known to invoke HPA-related hormonal and neural responses and behavior (Bradley et al., 1996; Lang et al., 1997; Goldstein et al, 2005 and 2010b; Holsen, et al, 2013). These specific IAPS images chosen to evoke negative affect, arousal and mild stress response in general are provided in Supplementary methods. The task lasted approximately 18 minutes (3 runs, 6 minutes each) and it was the only task in the scanning session. To ensure attention, participants pressed a button when each new image appeared.

fMRI Analyses

As reported in Mareckova et al (2016), data were preprocessed and analyzed using SPM8 (Wellcome Trust Centre for Neuroimaging, 2013). Non-linear volume-based normalization used the MNI152 brain template and spatial smoothing with 6 mm FWHM Gaussian filter, which was then re-sampled to 3 mm isotropic. Outliers in global mean image time series (threshold: 3.5 SDs from the mean) and movement (threshold: 0.7 mm, measured as scan-to-scan movement, separately for translation and rotation) were detected using an artifact detection toolbox (ART; Whitfield-Gabrieli, 2011) and entered as nuisance regressors in the first-level, single-subject general linear model (GLM). Masks excluding voxels outside the brain were applied to ensure that voxels in regions with high inter-participant variability in signal drop-out (e.g. OFC) were not arbitrarily excluded. Comparisons of interest (negative>neutral) from the first-level, single-subject analyses were tested using linear contrasts and SPM t-maps. Outputs from the first-level, single-subject analyses were submitted to second-level random effects analysis (see below).

BOLD-response analysis

A one-sample t-test examined the BOLD response to negative > neutral stimuli across the full sample (n=99), with a whole-brain, voxel-wise family-wise error (FWE)-corrected threshold of $p < 0.05$ (Mareckova, et al, 2016). Next, an intersection analysis, performed using MarsBaR (Brett et al., 2002), identified clusters (minimum cluster size 32 voxels) with voxel-wise FWE-corrected $p < 0.05$ threshold that were conjointly located within anatomic boundaries of PAG, HYPO, AMYG, HIPPO, OFC, ACC, and mPFC (anatomical masks defined using a manually segmented MNI-152 brain template). Given the small volumes and midline location of the PAG and HYPO, single ROIs combining right and left hemispheres were used for each of these two regions; remaining ROI masks were bilateral. The task elicited significant (FWE $p < 0.05$) BOLD responses in PAG, HYPO, AMYG, HIPPO, OFC, and mPFC (Mareckova et al, 2016). Average parameter estimates (percent signal change values) within each ROI were extracted for each participant using REX (Whitfield-Gabrieli, 2009) and exported into JMP (SAS Institute, Cary, NC, USA), which was used for all remaining BOLD response analyses. Stress circuitry activity, operationalized as parameter estimates identified and extracted as described above, was then examined as a function of cortisol response and sex using GLM. Significant interactions with sex were examined

further, separately in men and women, using GLM testing for interactions between cortisol response and free androgens (2 continuous variables) in men and cortisol response and 17 β -estradiol (2 continuous variables) in women. Finally, we assessed the modulatory role of dysphoric mood state using a GLM assessing the interactions between cortisol response and dysphoric mood on BOLD responses in stress circuitry. There were no outliers (score ± 3 SD from the mean) on any of the variables and thus our analyses did not use the Leave-One-Out Cross-Validation (LOOCV) approach.

Functional connectivity analysis

Similar to BOLD response analyses above, we assessed task-related connectivity using generalized psychophysiological interaction (gPPI; McLaren et al., 2012). HYPO (the cluster from the BOLD-anatomic ROI intersection analyses in MarsBaR described above) was used as a seed, given that it is the key relay station for HPA axis function. Time courses from this seed ROI were extracted and added to two additional PPI regressors (interaction of the seed time course with regressors for negative and neutral content) to individual subject-level GLMs. These interaction regressors were orthogonal to the task and seed regressors, ensuring that the seed ROI activation and PPI connectivity were independent (McLaren et al., 2012). Connectivity was measured at the single-subject level by estimating the interaction of the seed timecourse with the regressor for negative compared with neutral stimuli.

Results of the single-subject analysis were entered into second-level random effects analysis to test for group-level changes in connectivity during negative versus neutral conditions. Connectivity between HYPO and other regions was tested as a function of cortisol response and sex using GLM. Significant interactions with sex were then examined further testing for interactions between cortisol response and free androgens (2 continuous variables) in men and cortisol response and 17 β -estradiol (2 continuous variables) in women. Finally, we assessed the possible modulatory role of dysphoric mood state using GLM assessing the interactions between cortisol response and dysphoric mood (2 continuous variables) on HYPO connectivity. For these functional connectivity analyses, we used small volume correction (SVC) approach in SPM8, which limits voxel-wise analyses to voxels within *a priori* hypothesized ROIs. Target ROIs (PAG, AMYG, HIPPO, OFC, mPFC) were defined as anatomical masks (manually segmented from MNI-152 brain template, as described above) and implemented as overlays on the SPM8 canonical brain using the Wake Forest University Pick Atlas ROI toolbox (Maldjian et al., 2003). False positives were controlled using FWE-correction: within an anatomical ROI, significant results identified using SVC (initial voxel-wise threshold $p < 0.05$ uncorrected) were reported as significant if they additionally met the peak-level threshold of $p < 0.05$, FWE-corrected. For calculation of effect sizes and illustrative purposes in figures below, average connectivity values (beta weights of PPI regressors) in significant target clusters were extracted using REX (Whitfield-Gabrieli, 2009).

RESULTS

Steroid Hormone Responses to Negative Affective Stimuli

First, we assessed the overall impact of sex and sex steroid hormones on cortisol response to negative affective stimuli. Cortisol response was significantly higher in men than women ($t_{(90)}=1.97$, $p=0.049$; Figure 1). Within sex overall, there was little association between free androgens and cortisol response in men ($t_{(48)}=-0.35$, $p=0.73$) or 17β -estradiol and cortisol response in women ($t_{(40)}=-1.43$, $p=0.16$). The means and standard deviations for (non-log-transformed) free androgens, 17β estradiol, and cortisol response by sex are available in Supplementary Table 1. The change in cortisol over time in men and women is illustrated in Supplementary Figure 1

Next, we assessed the relationship between cortisol and BOLD response to negative vs. neutral affective stimuli and potential modulatory role of sex and gonadal hormones. Two-way ANOVA assessing the interaction between cortisol response and sex on BOLD signal in stress circuitry showed a significant main effect of cortisol response on BOLD in L OFC ($F_{(1,87)}=7.13$, $p=0.009$), with higher cortisol response related to lower BOLD in L OFC (Figure 2A, 2B), and significant cortisol response by sex interaction on BOLD in L mPFC ($F_{(1,88)}=6.56$, $p=0.01$; Figure 3A). Posthoc analyses revealed that higher cortisol response was related to lower BOLD in L mPFC in women ($t_{(42)}=-2.08$, $p=0.04$, $R^2=0.10$) but less so men ($t_{(48)}=1.50$, $p=0.14$; Figure 3B).

We further showed that, in men, L mPFC BOLD was modulated by an interaction between cortisol response and free androgens ($F_{(1,45)}=5.94$, $p=0.02$). Posthoc analyses used a median split of the free androgens variable and revealed that, while men with high free androgens showed no relationship between cortisol response and BOLD in L mPFC ($t_{(23)}=-0.48$, $p=0.64$), men with low free androgens showed a significant and positive relationship ($t_{(24)}=2.29$, $p=0.03$, $R^2=0.19$; Figure 3C). In women, there was no cortisol by 17β -estradiol interaction ($F_{(1,37)}=0.95$, $p=0.34$) modulating BOLD in L mPFC.

Further analyses assessing the possible interaction between cortisol response and dysphoric mood state on BOLD activity in stress circuitry showed a significant interaction in HYPO ($F_{(1,64)}=4.74$, $p=0.03$), L HIPPI ($F_{(1,63)}=6.05$, $p=0.02$) and L mPFC ($F_{(1,64)}=9.98$, $p=0.002$). All three interactions were independent of sex and thus remained significant after controlling for sex (HYPO: $F_{(1,63)}=4.27$, $p=0.04$; L HIPPI: $F_{(1,62)}=6.03$, $p=0.02$; L mPFC: $F_{(1,63)}=9.04$, $p=0.004$). Posthoc analyses used a median split in dysphoric mood state and revealed that in individuals with higher levels of dysphoric mood, increased cortisol response was associated with lower BOLD in HYPO ($t_{(34)}=-2.61$, $p=0.01$, $R^2=0.17$; Figure 4A) and L HIPPI ($t_{(34)}=-3.31$, $p=0.002$, $R^2=0.25$; Figure 4B), with no significant effect for L mPFC ($t_{(34)}=1.77$, $p=0.09$). In contrast, in individuals with lower levels of dysphoric mood, increased cortisol response was associated with decreased BOLD in L mPFC ($t_{(32)}=-2.73$, $p=0.01$, $R^2=0.19$; Figure 4C), and unrelated to BOLD in HYPO ($t_{(32)}=-0.05$, $p=0.96$) and L HIPPI ($t_{(31)}=0.45$, $p=0.65$).

Brain Connectivity and Steroid Hormone Responses

Next, we assessed the relationships of HYPO connectivity and cortisol response to negative vs. neutral affective stimuli, and the potential modulatory role of sex and sex steroid hormones. Task-related connectivity analyses using HYPO as the seed showed a main negative effect of cortisol response on connectivity between HYPO-L AMYG ($z=3.36$, FWE $p=0.01$; cluster with a peak at $X=-27$, $Y=-13$, $Z=-14$; Figure 5A, 5B) and cortisol response by sex interaction on connectivity between HYPO-L HIPPI ($z=4.84$, FWE $p=0.006$; cluster with a peak at $X=-24$, $Y=-25$, $Z=-14$; Figure 5C, 5D). Posthoc analyses revealed that higher cortisol response was associated with lower HYPO-L HIPPI connectivity in women ($t_{(41)}=-2.38$, $p=0.02$, $R^2=0.12$) but not men ($p=0.14$). Further, there were no significant interactions between cortisol response and free androgen index in men ($F_{(1,45)}=1.71$, $p=0.20$) or any interaction between cortisol response and 17β -estradiol in women ($F_{(1,36)}=0.00$, $p=0.98$).

Further analyses assessing the interaction between cortisol response and dysphoric mood state on connectivity revealed a significant interaction on HYPO-L HIPPI connectivity in women ($F_{(1,31)}=3.95$, $p=0.05$). Posthoc analyses used a median split in dysphoric mood state and revealed that, in women with worse dysphoric mood, increased cortisol response was related to decreased HYPO-L HIPPI connectivity ($t_{(15)}=-2.27$, $p=0.04$, $R^2=0.26$), but not in women with low levels of dysphoric mood ($t_{(17)}=-0.05$, $p=0.96$).

All primary findings were independent of diagnosis (no significant interactions between cortisol response and diagnosis on BOLD or connectivity) and medication (no significant interactions between cortisol response and medication on BOLD or connectivity). Three-way interactions were underpowered, but associations did not change when controlling for diagnosis or medication. The FWE related to the number of target ROIs was 0.23.

DISCUSSION

This study examined the coupling between neural and steroid hormonal responses to negative affective stimuli and the impact of sex and dysphoric mood in a sample of individuals with major depression, bipolar disorder, schizophrenia, and healthy controls. Our findings demonstrated that across all participants, higher cortisol response was associated with lower BOLD activity in orbitofrontal cortex and lower hypothalamic-amygdala connectivity during observation of negative vs. neutral affective stimuli. In women, higher cortisol response was also associated with deficits in the engagement of medial prefrontal cortex, a primary cortical inhibitory region in response to stress (Ferenczi et al, 2016), and lower hypothalamic-hippocampal connectivity. The lack of these associations in men suggests lower sensitivity of the negative affect circuitry in the male brain to cortisol. This is striking given that men in our sample, as in other population-level studies, had higher levels of cortisol than women.

Our findings of decreased BOLD responses in OFC and mPFC in people exhibiting a high cortisol response to negative affect are consistent with previous research demonstrating decreased BOLD in OFC (Pruessner et al. 2008) and mPFC (Kern et al, 2008) in response to psychosocial stress. OFC and mPFC are important in the regulation of emotion (Amodio &

Frith, 2006; Banks et al., 2007; Rive et al., 2013; Eden et al., 2015). They have a relatively high density of mineralocorticoid and glucocorticoid receptors, and are thus involved in stress-mediated HPA axis activity and potential neurotoxicity (De Kloet et al., 1998). Bipolar and schizophrenia patients have lower expression of glucocorticoid receptor mRNA in OFC (Sinclair et al., 2012) and lower expression of mineralocorticoid receptor mRNA in PFC (Xing et al., 2004) than controls. There is a substantial history of clinical and preclinical work demonstrating OFC and mPFC deficits in both schizophrenia and affective disorders, thus it is not surprising that we found a relationship between prefrontal activity deficits and an inability to regulate a stress response in these diagnostic groups.

Our findings of lower connectivity between hypothalamic nuclei and amygdala and hippocampus in subjects exhibiting high cortisol responses to negative affective stimuli is also consistent with previous studies. The paraventricular nucleus of the anterior hypothalamus is the key relay station for the regulation of the HPA axis with direct connections to amygdala and hippocampus (Herman et al., 2003). The critical role of hippocampus in providing negative feedback to the hypothalamus and thus in regulating HPA axis and stress response is well described (Dedovic et al., 2009; Hennerici & Szabo, 2014; Zschucke et al., 2015). Hippocampal dependent functions have been found to be impaired in numerous stress-related disorders (Lupien & McEwen, 1997; Herman et al., 2005; Goldstein et al., 2015) and developmental hippocampal abnormalities have been associated with risk for stress-related disorders (Seidman et al., 2002; Gilbertson et al., 2002; Meisenzahl et al., 2010; Anand & Dhikav, 2012; Levone et al., 2015; Pruessner et al., 2015). In fact, altered connectivity within this circuitry has been linked further to anxiety (Holmes et al., 2012), fear (Lebron-Milad et al., 2012) and reward (Admon et al., 2015).

Findings in our study also demonstrated that, overall, cortisol response in women was lower than in men. This is in agreement with population studies and a number of clinical studies showing that men experienced greater HPA-axis activity than women in response to various laboratory stressors, including examination stress, pain, cognitive tasks, and public speaking (Stephens et al., 2016; Kirschbaum et al., 1992; Zimmer et al., 2003; Kirschbaum et al., 1999; Kudielka et al., 1998; Seeman et al., 2001). In this study, only women demonstrated reduced activity in mPFC and hypothalamic-hippocampal connectivity dependent on cortisol. The relationships between higher cortisol and lower brain activity and connectivity were not significantly related to variation in their estradiol levels. We and others have previously shown low estradiol levels associated with inability to regulate arousal in women with depression (e.g. Jacobs et al., 2014) and psychoses (Goldstein et al., 2015), and thus the substantial variability in estradiol levels in this sample is, in part, due to the inclusion of healthy and ill populations.

Among the men across groups, HPA axis responses were significantly associated with HPG responses, specifically testosterone in relation with cortisol and mPFC response to negative affect. Only men with low testosterone demonstrated a positive relationship between cortisol and mPFC, suggesting that the lack of anti-glucocorticoid action by androgens was associated with greater need for the inhibitory role of mPFC to regulate a stress response. This is consistent with earlier work demonstrating a relationship between low levels of free androgens and hyperactivity in mPFC in men with psychoses (Goldstein et al., 2015).

Dysphoric mood also modulated the neural-hormonal coupling. Among individuals with more severe dysphoric mood symptomatology, increased cortisol response was associated with lower BOLD in the hypothalamus and left hippocampus. In women with worse dysphoric mood, increased cortisol response was related to decreased hypothalamic-hippocampal connectivity. In contrast, among individuals with lower levels of dysphoric mood, increased cortisol response was associated with decreased BOLD in L mPFC. Future research is needed to replicate these findings with larger sample size that could determine the presence of a three-way interaction, including variability in HPG-HPA interactions.

There are a few potential limitations of this study. As reported in the Supplementary table 1, the cortisol responses (as calculated here) were low, potentially reflecting the anticipatory anxiety prior to the task or the decline in diurnal cortisol secretion during morning hours when the participants were scanned. We attempted to eliminate the potential effect of anticipatory anxiety by correcting for cortisol response at time 0 (blood draws 5 minutes after the onset of scanning session) in the cortisol response calculation. Further, while the initial analysis (Mareckova et al, 2016) demonstrated significant (FWE-corrected) activity in the hypothesized negative affective circuitry regions and the current study used only the robust and independently-derived regions of interest and all test, including the hypothesized hypothalamus seed for connectivity analyses, were hypothesis-driven based on previous work, we did not have the power to further correct for the multiple comparisons related to the number of ROIs and connectivity targets.

Overall, our findings provide insights into the importance of a sex-dependent lens in understanding neural – hormonal relationships in the face of even mild stress and show that the pathophysiology is shared across populations. The notion of shared traits across disorders has been underscored by the recent focus of NIMH on the Research Domain Criteria (RDoC) initiative (Insel et al, 2010). Our study contributes to understanding the regulation of arousal and response to negative affective stimuli across disorders and the importance of a sex-dependent lens therein. Individuals with increased cortisol responses were less able to recruit cortical regulatory regions and had decreased connectivity between hypothalamus and limbic systems regulating arousal. Sex differences in HPA and HPG dysregulation in response to negative affective stimuli in depression and psychoses, independently, have been previously reported. We extended these previous results here by demonstrating shared sex-dependent deficits in neural-hormonal responses to stress across healthy and clinical populations. These neural-hormone findings may contribute to the development of hormonal adjunctive therapeutics that are sex-dependent and shared across diagnoses, underscoring the importance of one's sex to precision medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Neural-hormonal responses to negative affect vary with sex and dysphoric mood.
- Elevated cortisol associated with lower OFC BOLD and HYPO-AMYG connectivity.
- Elevated cortisol associated with lower HYPO and HIPPO BOLD, dependent on dysphoric mood.
- In women, elevated cortisol associated with lower mPFC BOLD and HYPO-HIPPO connectivity.
- In women with dysphoric mood, elevated cortisol associated with lower HYPO-HIPPO connectivity.

Figure 1. Cortisol response to negative affective stimuli was larger in men than in women ($t_{(90)}=1.97$, $p=0.049$).

Figure 2.

Higher cortisol response was associated with lower BOLD in L OFC in both men and women. The exact location of the cortisol-related BOLD response is shown in **2A**. The negative relationship between cortisol response and BOLD response in L OFC is shown in **2B** ($F_{(1,87)}=7.13$, $p=0.009$, Adj $R^2=0.06$).

Figure 3.

Higher cortisol response was associated with lower BOLD in L mPFC (see **3A** for the exact location) in women ($t_{(42)}=-2.08$, $p=0.04$, $R^2=0.10$) but not men ($t_{(48)}=1.50$, $p=0.14$; **3B**). Men with low ($t_{(24)}=2.29$, $p=0.03$, $R^2=0.19$) but not high ($t_{(23)}=-0.48$, $p=0.64$) levels of free androgens showed a positive relationship between cortisol response and BOLD in L mPFC (**3C**).

Figure 4.

In individuals with higher levels of dysphoric mood, increased cortisol response was associated with lower BOLD in HYPO ($t_{(34)}=-2.61$, $p=0.01$, $R^2=0.17$, **Figure 4A**) and L HIPP ($t_{(34)}=-3.31$, $p=0.002$, $R^2=0.25$, **Figure 4B**) and no changes in L mPFC ($t_{(34)}=1.77$, $p=0.09$). In contrast, in individuals with lower levels of dysphoric mood, increased cortisol response was associated with no changes in BOLD in HYPO ($t_{(32)}=-0.05$, $p=0.96$) and L HIPP ($t_{(31)}=0.45$, $p=0.65$) but with decreased BOLD in L mPFC ($t_{(32)}=-2.73$, $p=0.01$, $R^2=0.19$, **Figure 4C**).

Figure 5. Increased cortisol response was associated with lower HYPO-L AMYG connectivity in all subjects (**5A, 5B**; $z=3.36$, $FWE p=0.01$) and lower HYPO-L HIPPO connectivity in women but not men (**5C, 5D**; $z=4.39$, $FWE p=0.001$).

Table 1

Demographics and clinical characteristics of the sample

	Males (n=51)	Females (n=48)	Between Group Comparison
Ethnicity			
% Caucasian (n)	68.63% (35/51)	52.08% (25/48)	No difference
% Other (n)	3.92% (2/51)	14.58% (7/48)	
% Missing (n)	27.45% (14/51)	33.33% (16/48)	
Handedness			
% Right-handed (n)	90.20% (46/51)	93.75% (45/48)	No difference
Age			
in years; M (SD)	45.02 (3.88)	44.33 (4.77)	No difference
BMI			
M (SD)	29.17 (5.15)	29.86 (6.89)	No difference
Parental SES^a			
% Lowest SES quartile (n)	23.53% (12/51)	20.83% (10/48)	No difference
% Lower middle SES quartile (n)	23.53% (12/51)	18.75% (9/48)	
% Higher middle SES quartile (n)	21.57% (11/51)	25.00% (12/48)	
% Highest SES quartile (n)	19.61% (10/51)	20.83% (10/48)	
% Missing (n)	11.76% (6/51)	12.50% (6/48)	
Education level			
% Without completed high school (n)	9.8% (5/51)	4.17% (2/48)	No difference
% Completed high school (n)	21.57% (11/51)	14.58% (7/48)	
% More than high school (n)	66.67% (34/51)	77.08% (37/48)	
% Missing (n)	1.96% (1/51)	4.17% (2/48)	
WAIS vocabulary, age-scaled			
M (SD)	11.18 (3.35)	10.09 (3.08)	No difference
WAIS block design, age-scaled			
M (SD)	11.67 (3.35)	9.74 (2.98)	Males > Females
DSM-based diagnosis			
% MDD in remission (n)	25.49% (13/51)	29.17% (14/48)	No difference
% Psychosis (n)	31.37% (16/51)	31.25% (15/48)	
% Healthy controls (n)	43.14% (22/51)	39.58% (19/48)	
Psychotropic medication			
% on psychotropic medication (n)	35.29% (18/51)	31.25% (15/48)	No difference
Substance use disorder			
% with any substance use disorder (n)	64.72% (33/51)	16.67% (8/48)	Males > Females

^aParental socioeconomic status (SES) was assigned a single, continuous score for education, occupation, and family income according to the system used for the United States Bureau of the Census (Myrianthopoulos & French, 1968). This composite index ranged from 0.0 (low) to 9.5 (high).

Myrianthopoulos, N.C., & French, K.S. (1968). An application of the US Bureau of the Census socioeconomic index to a large, diversified patient population, *Soc Sci Med*, 2, 283–299.

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