

# FDG-PET analysis of amygdalar-cortical network covariance during pre- versus post-menopausal estrogen levels: Potential relevance to resting state networks, mood, and cognition

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## Abstract

**OBJECTIVES:** 1.) Expand the scope of neuroendocrine applications of functional neuroimaging techniques. 2.) Compare the covariance of amygdalar activity with that of the rest of the brain during pre- and post-menopausal levels of estrogen (E2). Based on the distribution of cortical E2 receptors and the neocortical regions where E2 has been shown to preferentially accumulate, we predict that E2 infusion will increase covariance of amygdalar activity with that of the temporal and frontal cortices. **DESIGN:** This basic physiology study employed a within-subject design. All participants were post-menopausal women (n =7). Analysis of covariance between whole brain and amygdalar regional cerebral glucose consumption (CMRglc) was conducted in a voxel-wise manner by means of the basic regression option in SPM2 and was applied to FDG-PET scans acquired at baseline and after a 24 hour graded E2 infusion. **SETTING:** an academic medical center; Massachusetts General Hospital, Boston, Massachusetts. **RESULTS:** E2 levels (mean ± sem) were significantly greater at 24 hours (257.9 pg/mL ± 29.7) than at 0 hours (28.1 pg/mL ± 3.4). Right amygdalar CMRglc showed a significant covariance with activity of three different regions of the temporal cortex during E2 infusion, but none at baseline. In addition, right amygdalar CMRglc covaried with that of the right medial and superior frontal gyri only during E2 infusion. **CONCLUSIONS:** In addition to suggesting changes in amygdalar-cortical network connectivity as a result of short-term E2 exposure, these analyses provide evidence that basic neuroendocrine research may benefit from further use of FDG-PET and other functional neuroimaging modalities for network level analyses.

## Abbreviations & Units

CMRglc	– regional cerebral metabolic glucose consumption
EEG	– electroencephalography
E2	– estradiol
FDG-PET	– fluoro-deoxy-glucose positron emission tomography
fMRI	– functional magnetic resonance imaging
FWHM	– full width half maximum
msec	– milliseconds
PMW	– postmenopausal women
pg/mL	– picograms per milliliter
ROIs	– regions of interest
sem	– standard error of the mean
SPM	– statistical parametric mapping
ug/kg/hr	– microgram/kilogram/hour
VLPFC	– ventrolateral prefrontal cortex

## INTRODUCTION

As a complement to research questions at the molecular and cellular level, basic neuroendocrine research is progressively addressing more questions at the systems or network level (Kawakami *et al.* 1979; DeVoogd, 1991; Ball *et al.* 2004; Campbell, 2007; Romano-Torres *et al.* 2007). While conventional neuroendocrine techniques for network level analyses offer certain methodological advantages, these techniques also frequently involve sacrificing the animal or lesioning a part of the brain. EEG circumvents these methodological issues, but does not provide the spatial resolution achieved by functional neuroimaging modalities such as PET and fMRI. Thus, PET and fMRI techniques have received increased application in the context of animal studies evaluating basic neuroendocrine physiology paradigms and probably even greater application in the context of behavioral psychoneuroendocrine paradigms in humans (Dreher *et al.* 2007; Goldstein *et al.* 2005; Ottowitz *et al.* 2004a; Ganguli *et al.* 2002; Shaywitz *et al.* 1999). Despite the increased purview of these applications, there has been very little use of functional neuroimaging techniques to evaluate basic neuroendocrine physiological paradigms in humans (Reiman *et al.* 1996; Ottowitz *et al.* 2004b).

In this regard, different lines of evidence point to the amygdala as a key component of cortical-subcortical circuits whose function may be substantially influenced by estrogen (E2). From an anatomical perspective, both the amygdala and cortex contain a significant density of E2 receptors (Osterlund *et al.* 2000; Shugrue *et al.* 1997; Taylor and Al-Azzawi, 2000) and extensive projections exist between the amygdala and cortex (Amaral and Price, 1984; Swanson and Petrovich, 1998). From a functional or physiological perspective, animal studies have shown that infusion of E2 into the amygdala affects activity of amygdalar projection sites, and conversely, that application of E2 to structures receiving amygdalar projections affects amygdalar activity (Lehmann and Erskine, 2005; Li *et al.* 1992; Dudley *et al.* 1990; Wong and Moss, 1992). Neuroimaging studies in humans have shown that activations of the amygdala and cortex change with E2 levels across the menstrual

cycle (Dreher *et al.* 2007; Goldstein *et al.* 2005), providing evidence that E2 may also coordinate activity between the amygdala and cortex in humans.

In this study, we investigate the basic physiological capacity for an exogenous E2 challenge to alter network level interactions in humans by evaluating covariance of resting state activity (CMRglc: regional cerebral glucose consumption) between the amygdala and cortex during baseline post-menopausal E2 levels vs. levels typical of the follicular phase in pre-menopausal women.

Bixo *et al.* (1995) have shown the prefrontal cortex to be the neocortical region showing the greatest tendency to concentrate E2, whereas Osterlund and co-workers (2000) have shown that the most distinct collection of neocortical E2 receptors lie in the temporal lobe. Thus, we predicted that, relative to postmenopausal E2 levels, covariance of activity between the amygdala and frontal and temporal cortices would increase during E2 levels characteristic of the pre-menopausal follicular phase.

## MATERIAL AND METHODS

### Overview

This study was one component of a three-part basic physiology paradigm evaluating the effects of E2 on 1.) hypothalamic-pituitary regulatory processes 2.) prefrontal-hippocampal effective connectivity, and 3.) amygdalar-cortical network covariance (the current study). No component of our study involved evaluation of cognition, emotion, nor treatment effects of E2.

In order to control for subject effects and the effects of endogenous E2, the same postmenopausal women were evaluated at E2 levels characteristic of the menopause vs. levels characteristic of the pre-menopausal follicular phase, i.e. as achieved by means of E2 infusion. E2 is known to enhance synaptic connectivity within 12–24 hours of administration (Gazzaley *et al.* 2002) and formation of dendritic spines is known to recruit basic metabolic processes (D'Ambrosi *et al.* 2001; Skladchikova *et al.* 1999). Accordingly, FDG-PET scans were acquired at the 0 hour baseline and after 24 hours of a continuous E2 infusion.

To identify neuroanatomical regions whose activity co-varied with that of the amygdala, whole brain covariate analyses were conducted by means of regressing amygdalar ROI values against the CMRglc of each voxel of the brain for all subjects. This was done separately for both the 0 and 24 hour scans. To enhance anatomical localization of boundaries for the amygdalar regions of interest (ROIs), structural MRI scans were also collected for each subject.

As a point of clarification, because the point of the study was to evaluate the effects of E2 on amygdalar-cortical network interactions, there is no dedicated analysis of the effects of E2 on regional brain activity. In other words, this study evaluated how different parts of the brain “talk to each other” under the influence of

different E2 levels; this study did not evaluate the global effects of E2 on brain activity.

### Subjects

Participants were recruited through the General Clinical Research Center of Massachusetts General Hospital (MGH). Only postmenopausal women were studied. The study was approved by the Federal Drug Administration and Partners Institutional Review Board and written, informed consent was provided prior to any study procedures. Admission to the General Clinical Research Center was preceded by general history and physical exam, and a laboratory evaluation of basic chemistry and complete blood count.

### Hormone Preparation, Infusion, Collection, and Statistical Analysis

The E2 infusion was prepared as previously described (Taylor *et al.* 1995). Infusion rates were 0.1 ug/kg/hr for the initial 12 hours and 0.135 ug/kg/hr for the following 12 hours. Infusions were administered via a closed system, non-PVC fluid-path IV tubing (Accuset, IMED Corp., San Diego, CA). A Micro 965 Volumetric Infusion Pump was used to control the infusion rate (IMED Corp.).

Blood samples were collected for E2 at baseline and 24 hrs. E2 was measured using a two-site monoclonal non-isotopic system according to the manufacturer's instructions (AxSYM, Abbott Laboratories, Abbott Park, IL, USA). The inter-assay coefficients of variation for the E2 assay were 9.2, 5.4 and 9.6% at E2 concentrations of 85, 300, and 700 pg/mL (312.0, 1101, and 2570 pmol/L), respectively.

Matlab 7 was used to conduct paired t-tests evaluating for differences in E2 levels across the two scanning sessions.

### PET Scanning and Image Reconstruction

[18F]fluorodeoxyglucose-PET (FDG-PET) scans were obtained on all subjects at baseline and after 24 hours of estrogen infusion. PET images were acquired using a Siemens HR+, 32-ring, 63-slice body tomograph. In both the transverse and axial directions, the intrinsic spatial resolution of the scanner is 4.5 mm full width half maximum (FWHM). The slice geometry includes 63 slices with 2.25 mm separation and a total axial field of 16.5 mm.

Scan acquisition began on the day of admission, at 15:00 hours. After having fasted for approximately 6 hours, subjects were injected with 5–6 millicuries of [18]FDG, while seated in a dimly lighted room. Forty-five minutes after radiopharmaceutical administration, subjects were positioned supine on the PET scanner bed. A vacuum formable reusable polystyrene bead-filled pillow was used to minimize potential head movements and to align each subject's head so that slice acquisition occurred parallel to the canthomeatal line. After correct head positioning was achieved, a single 20

min emission measurement was acquired. Subsequently, after 24 hours of a graded E2 infusion conducted in accord with the aforementioned protocol, the second FDG-PET scan was collected.

Reconstruction of images was conducted by means of a conventional filtered back-projection algorithm to an in-plane resolution of 4.5 mm FWHM. Projection data were corrected for random coincidences and scattered radiation, non-uniformity of detector response, and dead time. Attenuation correction was conducted by means of transmission images acquired with a rotating pin source containing germanium 65.

### MRI Acquisition and Coregistration with PET Images

To enhance anatomical accuracy for identification of amygdalar regions of interest, high-resolution T1-weighted 3D MRI scans were acquired for each subject. MRI sagittal scout images and subsequent contiguous axial three-dimensional T1-weighted spoiled gradient echo pulse sequences were acquired using a General Electric 1.5 Tesla Signa scanner (Milwaukee, WI). Acquisition parameters included a repetition time of 30 msec, echo time=9 msec, flip angle = 25 degrees, band width 15.63, number of scan locs = 124, field of view = 22, matrix = 256 × 192, number of excitations = 1, frequency direction = A/P, phase direction = L/R, and phase FOV 0.75. Water was used as the auto center frequency.

Coregistration of each subject's PET and MRI scans was performed by means of the standard coregistration protocol included in SPM2 (Wellcome Department of Cognitive Neurology, London).

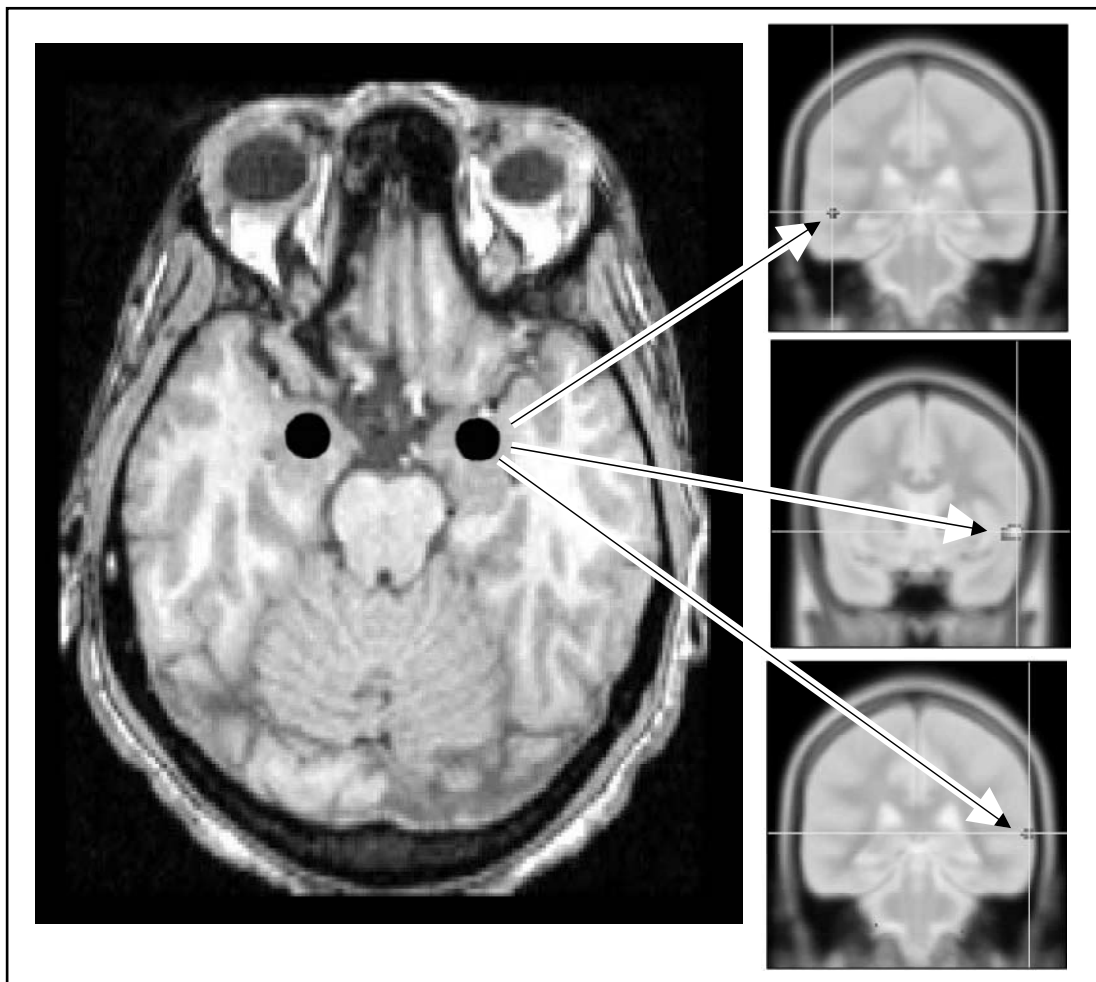
### Amygdalar Regions of Interest; Definition of, and Data Acquisition

The MarsBaR toolbox (Brett *et al.* 2002) of the SPM2 software package (Wellcome Department of Cognitive Neurology, London) was used to define bilateral amygdalar regions of interest (ROIs) on each subject's MRI scan. A central, spherical portion of the amygdala was sampled in accord with anatomical guidelines of Bronen and Cheung (1991). ROI dimensions were standardized at 6 mm diameter across all subjects in order to eliminate any variability in ROI counts that might be caused by variations in ROI volume (Figures 1 and 2, left sided panels).

After definition of amygdalar ROIs, and co-registration of corresponding PET and MRI scans, the MarsBaR toolbox of SPM2 was used to extract amygdalar ROI values from the PET images (for each subject, for both imaging sessions).

### Whole Brain-Amygdalar ROI Covariate Analyses

Amygdalar ROI values were used in a standard seed analysis (Cordes, *et al.* 2000; Della-Maggiore, *et al.*, 2000; Dougherty *et al.* 2004), where the covariance between the amygdalar CMRglc and all other regions of the brain is calculated voxel by voxel. This allowed us to



**Figure 1. Sites of covariance of right amygdalar and temporal lobe CMRglc during estrogen infusion.** Left Panel: Transverse structural MRI section of the brain, imaged at the level of the midbrain and temporal lobe showing spherical amygdalar regions of interest (ROIs). To facilitate anatomical placement, ROIs were drawn on each subject's high resolution MRI scan, which was co-registered to their PET scans; the right sided panels include examples of PET images. Panels on Right: Regions in the temporal lobe showing covariance with the right amygdala included the left middle temporal gyrus at MNI coordinates -52, -32, -2 (top right), and the anterior and posterior aspects of the right superior temporal gyrus at MNI coordinates 58, 8, -4, (middle right), and 68, -34, 8, (bottom right), respectively. These covariances were significant only during estrogen infusion. Both the MRI and PET images are positioned in neurological convention, i.e. the right hemisphere is located on the right side of the image. (CMRglc = regional cerebral glucose consumption).

search the entire brain for regions that were positively or negatively correlated with activity of the amygdala. The data across the whole brain analysis was thresholded at  $p=0.001$  (uncorrected), and an extent threshold of 5 contiguous voxels was applied to provide rudimentary control for multiple comparisons (Wager *et al.* 2007; Friston *et al.* 1994).

## RESULTS

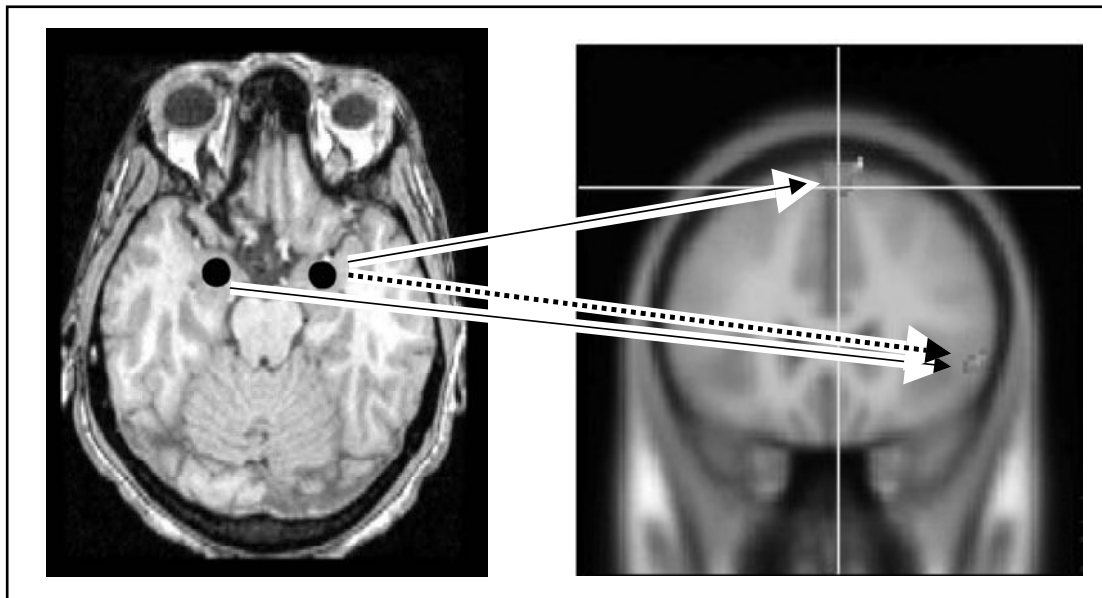
**Subjects.** Participants ranged in age from 48–76 years and had a mean age of 64.2 years. All subjects were at least one year from menopause. All subjects lived independently and were free of active medical and psychiatric disorders. No subjects were receiving E2 at the time of the study, nor within at least the year prior to the study.

### Steroid Hormone Levels

E2 levels [mean (sem)] increased from 28.1(3.4) pg/mL at baseline, to 257.9(29.7) pg/mL at 24 hours. E2 levels at 24 hours differed from baseline ( $p<0.05$ ).

### Sites of Amygdalar-Cortical Covariance

All brain regions whose CMRglc showed significant covariance with amygdalar CMRglc at time 0, and/or at time 24 hours, are listed in Table 1. In brief summary of the findings of relevance to our *a priori* hypothesis, it is notable that there were no sites of significant covariance between amygdalar CMRglc and CMRglc of the temporal cortex at baseline E2 levels, whereas three sites of significant covariance between the amygdala and temporal cortex were apparent during E2 infusion (Figure 1). Figure 2 highlights amygdalar sites of covariance with the prefrontal cortex that were present at 0 and 24



**Figure 2.** Sites of covariance of CMRglc between each amygdala and the prefrontal cortex during estrogen infusion.

Left Panel: Transverse structural MRI section of the brain, imaged at the level of the midbrain and temporal lobe showing spherical amygdalar regions of interest (ROIs). Right Panel: Prefrontal sites of covariance with the right amygdala included the right superior frontal gyrus (MNI coordinates 8, 48, 48; identified by the crosshairs) and the right ventrolateral prefrontal cortex (VLPFC), (MNI coordinates 54, 28, 0 at 0 hours, and 54, 22, 0 at 24 hours). The dashed arrow indicates a significant covariance that was present during both baseline scanning and scanning after 24 hours of estrogen infusion, whereas the solid arrows indicate a significant covariance that was present only after 24 hours of estrogen infusion. The top arrow indicates a significant covariance present between the right amygdala and right medial frontal gyrus/superior frontal gyrus (MNI coordinates 8, 48, 48), whereas the bottom arrow indicates a significant covariance between the left amygdala and right VLPFC (MNI coordinates 54, 22, 0). Both the MRI and PET images are positioned in neurological convention, i.e. the right hemisphere is located on the right side of the image. (CMRglc = regional cerebral glucose consumption).

**Table 1.** Voxel-wise Covariance of Whole Brain vs. Amygdalar CMRglc at Baseline and During Estrogen Infusion

ESTROGEN DOSING and Amygdalar Lateralization	Neuroanatomical Region	MNI Coordinates	Z score
BASELINE, POSTMENOPAUSAL ESTROGEN LEVELS			
<b>Left Amygdala:</b>	L precuneus	-20, -62, 44	(+) 4.36
	R cuneus	4, -62, 4	(-) 3.44
	L pulvinar	-20, -26, -2	(+) 3.37
<b>Right Amygdala:</b>	R precentral gyrus	58, -4, 32	(-) 3.11
	R VLPFC	54, 28, 0	(-) 2.93
24 HOURS of ESTROGEN INFUSION			
<b>Left Amygdala:</b>	R VLPFC	58, 28, -2	(-) 4.44
	R lingual gyrus	12, -86, -2	(-) 4.15
	R cuneus	2, -68, 12	(-) 3.80
<b>Right Amygdala:</b>	R inferior occipital gyrus	38, -82, -10	(+) 4.79
	R superior temporal gyrus	58, 8, -4	(-) 4.11
	R VLPFC	54, 22, 0	(-) 3.25
	L middle temporal gyrus	-52, -32, -2	(+) 3.24
	L precentral gyrus	-28, -6, 72	(-) 3.01
	R MedFG/SFG	8, 48, 48	(-) 2.97
	R superior temporal gyrus	68, -34, 8	(-) 2.97

CMRglc: regional cerebral glucose consumption. MNI: Montreal Neurological Institute. R: right. L: left. VLPFC: ventrolateral prefrontal cortex. MedFG/SFG: interface of the medial frontal gyrus and superior frontal gyrus. Note that a (-) z-score corresponds to negative covariance between CMRglc of the identified anatomical site and the amygdala; a (+) z-score corresponds to positive covariance between CMRglc of the identified anatomical site and the amygdala.

hours (the right amygdala and VLPFC) versus sites of covariance that were present only at 24 hours (the right amygdala and medial/ superior frontal gyri, and the left amygdala and right VLPFC).

## DISCUSSION

To the best of our knowledge, there have been no previous neuroimaging studies evaluating the effects of a hormonal challenge on amygdalar-cortical network interactions. In this regard, our study was primarily oriented toward highlighting an integration of methodologies that may hold general significance for a range of basic neuroendocrine research questions. For example, the amygdala is a putative site of neuroendocrine significance, and may serve as a neuroendocrine effector site for many parts of the brain. Thus, in terms of potential application to animal studies, *in vivo* methodologies that enable investigation of the effects of hormones on the amygdala in the context of the entire (intact) brain may provide important advantages in comparison to techniques that isolate specific brain regions and lose the capacity to evaluate the comprehensive interconnectedness of the brain in its native state.

In terms of increased application in the setting of human research, our study has highlighted the potential for functional neuroimaging techniques to evaluate not just behavioral psychoneuroendocrine paradigms (e.g. the effects of hormones on cognition and emotion), but also basic neuroendocrine paradigms evaluating the effects of hormones on physiological parameters such as network connectivity. Our study prioritized this agenda by evaluating subjects in the absence of active cognitive or emotional challenge, i.e. subjects were evaluated in the resting state. Even though theories involving the brain's so-called "resting state" are still evolving, a reasonable consensus is emerging that networks of coordinated activations and deactivations exist across specific brain regions at rest, (i.e. networks that reliably characterize the resting state in the absence of active cognitive or emotional challenge) (Raichle *et al.* 2001; Damoiseaux *et al.* 2006). While FDG-PET had classically been the primary neuroimaging modality for evaluation of the resting state (Mazziotta *et al.* 1981; Mazoyer *et al.* 2001), fMRI has clearly become the modality of choice for evaluation of resting state networks (Fox and Raichle 2007). In this regard, Salvador *et al.* (2008) have used fMRI to show that the amygdala may be a core component of a limbic resting state network. Parallel to this, our study has shown that E2 may affect the degree to which the amygdala coordinates its activity with certain structures during the resting state. For example, our findings show that at postmenopausal E2 levels (baseline), there were five brain regions showing significant covariance of resting state CMRglc with amygdalar CMRglc, whereas during E2 infusion, there were ten cortical sites showing significant covariance with the amygdala. Consistent with our *a priori* hypoth-

esis, the frontal and temporal cortical regions showed a substantial increase in covariance with amygdalar activity. For example, at baseline E2 levels, there were no temporal regions showing significant covariance with the amygdala, whereas during E2 infusion, there were three sites. Similarly, there was only one prefrontal site of covariance with the amygdalae at 0 hours, but three at 24 hours.

In complement to the relevance of these findings to resting state networks, the induction of covariance of activity within amygdalar-temporal circuits holds relevance to behavioral states that are necessarily present even when the brain is at rest. For example, the amygdala and temporal pole are both putative sites of relevance to mood (Beauregard *et al.* 2006) and in contrast to the relatively fleeting nature of an individual's affect or transient emotional state, mood is an emotional state persisting across time (Moran, 2003). Thus, mood must (at least in part) rely on the resting state activity of structures such as the amygdala. Indeed, FDG-PET has been used to show that resting state metabolism of the amygdala is elevated in abnormal mood states such as depression (Drevets *et al.* 2002). Of further relevance to our paradigm, it is especially notable that a depressed mood in women has been shown to be associated with reduced E2 levels (Young *et al.* 2000), and that the transition to menopause (with its associated fluctuations in E2 levels), is accompanied by a notable increase in depressive symptoms (Hay *et al.* 1994). Because our findings have shown that E2 levels typical of the pre-menopause (the levels attained during 24 hour scanning) induce a covariance between activity of the amygdala and temporal cortex that is not present during levels typical of the menopause (our baseline scanning E2 levels), it can be speculated that significant reductions in E2 levels may contribute to depressive disorders by means of predisposing for dampened or aberrant amygdalar-cortical network interactions within the temporal lobe.

In addition to the enhanced covariance of activity between the amygdala and temporal cortex during E2 infusion, it is notable that our E2 infusion was associated with an increased covariance between the amygdala and prefrontal cortex. Specifically, at baseline postmenopausal E2 levels, although covariance of CMRglc was noted between the right amygdala and right ventrolateral PFC (VLPFC), only during E2 infusion was significant covariance noted between both the left and right amygdala and right VLPFC. In addition, only during E2 infusion did activity of the right amygdala covary with that of the right medial and superior frontal gyri (Rmed/SFG) (Table 1 and Figure 2). The right superior frontal gyrus and right VLPFC have both been shown to mediate working memory (Petrides, 2005; Shaywitz *et al.* 1999), a neuropsychological domain repeatedly shown to be enhanced by E2 (Maki, 2005; Lacreuse *et al.* 2004). Several studies have shown that the amygdala may contribute to working memory (Pegna *et al.* 2002; Barros *et al.* 2002); thus, our findings suggest that the

capacity for the amygdala to do so may be enhanced by E2.

Different limitations of our study deserve mention. For example, our definition of the amygdalar ROIs was conducted in a rather straightforward manner, e.g. simple placement of a sphere in the approximate center of the amygdala. Because different regions of the amygdala have different projection sites, studies employing more detailed ROI placement criteria may identify different sites of covariance. Our paradigm, did however, use the high resolution MRI co-registration techniques available at the time our study was conducted. As high-resolution MRI techniques are developed further, future protocols will presumably allow for greater anatomical precision. In addition to issues of anatomical resolution, the generalizability of our findings are limited by our small sample size; thus, our preliminary findings certainly require replication.

Nonetheless, despite the aforementioned limitations, our study describes one potential manner in which functional neuroimaging techniques can serve as an important adjunct to evaluate the basic physiological effects of hormones on neural networks. Furthermore, our study provides preliminary evidence that E2 may have a significant impact on amygdalar-cortical network interactions, especially those characterizing the resting state. Modifications and extensions of our paradigm, including use of fMRI technology, may benefit a myriad of basic neuroendocrine and neurophysiological research enquiries such as the investigation of hypothalamic-pituitary-ovarian and adrenal (i.e. HPO and HPA) regulatory processes, the effects of hormones on functional and effective connectivity, and the relevance of hormones to resting state networks.

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