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Evaluation of prefrontal—hippocampal effective connectivity following 24 hours of estrogen infusion: An FDG-PET study

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Summary

Although several functional neuroimaging studies have addressed the relevance of hormones to cerebral function, none have evaluated the effects of hormones on network effective connectivity. Since estrogen enhances synaptic connectivity and has been shown to drive activity across neural systems, and because the hippocampus and prefrontal cortex (PFC) are putative targets for the effects of estrogen, we hypothesized that effective connectivity between these regions would be enhanced by an estrogen challenge. In order to test this hypothesis, FDG-PET scans were collected in eleven postmenopausal women at baseline and 24 h after a graded estrogen infusion. Subtraction analysis (SA) was conducted to identify sites of increased cerebral glucose uptake (CMR_{glc}) during estrogen infusion. The lateral PFC and hippocampus were *a priori* sites for activation; SA identified the right superior frontal gyrus (RSFG; MNI coordinates 18, 60, 28) (SPM2, Wellcome Dept. of Cognitive Neurology, London, UK) as a site of increased CMR_{glc} during estrogen infusion relative to baseline. Omnibus covariate analysis conducted relative to the RSFG identified the right hippocampus (MNI coordinates: 32, -32, -6) and right middle frontal gyrus (RMFG; MNI coordinates: 40, 22, 52) as sites of covariance. Path analysis (Amos 5.0 software) revealed that the path coefficient for the RSFG to RHIP path differed from zero only during E2 infusion ($p < 0.05$); moreover, the magnitude of the path coefficient for the RHIP to RMFG path showed a significant further increase during the estrogen infusion condition relative to baseline [$\Delta\chi^2 = 4.05$, $\Delta d.f. = 1$, $p = 0.044$]. These findings are consistent with E2 imparting a stimulatory effect on effective connectivity within prefrontal—hippocampal circuitry. This holds mechanistic significance for resting state network interactions and may hold implications for mood and cognition.

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Conflict of interest

No author involved in this study has any financial involvements relevant to their efforts on this study.

Keywords

Effective connectivity; Estradiol; Neural networks; Depression; Verbal memory; Postmenopausal women

1. Introduction

While early conceptions of brain function based on neuro-behavioral investigations of patients with solitary brain lesions (e.g. case studies of cerebral infarction) might have encouraged a regional approach to the conceptualization of neurobehavioral processes, current theories of brain function are oriented toward network level analyses. Indeed, effective connectivity analyses are governed by the concept that brain processes are mediated by coordinated activations and deactivations driven between remote, yet interacting brain regions (Friston, 1994; McIntosh and Gonzalez-Lima, 1994).

As basic as cognition and emotion are to cerebral function, hormones also hold primary relevance for cerebral processes. The classic neuroendocrine model introduced by Phoenix et al. (1959) divided the neuronal effects of hormones into organizational and activating effects. Since the introduction of this classic conception, animal neuroendocrine research has further delineated our understanding of the effects of hormones at the cellular, intercellular, and network level. At the cellular level, studies in rats and non-human primates have shown that estrogen (E2) affects neuronal structure through induction of dendritic spines (Gould et al., 1990) and affects function by altering neuronal excitability (Akaishi and Sakuma, 1985; Kow et al., 2005). At the intercellular level, application of E2 to synapses in the hypothalamic arcuate nucleus has been shown to increase the number of excitatory dendritic spines encroaching upon arcuate neurons, decrease the number of inhibitory synapses, and increase the firing rate of arcuate neurons (Parducz et al., 2002). At the network level, it has been shown that infusion of E2 into the amygdala of female mice increases the number of tuberoinfundibular neurons showing an excitatory response to transmissions received from the accessory olfactory body (Li et al., 1992).

In addition to the effects of E2 on the hypothalamus and amygdala, E2 has been shown to increase synaptic connectivity and to have activating effects in both the prefrontal cortex (PFC) and hippocampus (Reiman et al., 1996; Maki and Resnick, 2000; Gould et al., 1990; Wallace et al., 2006). Studies evaluating the effects of E2 on the hippocampus and PFC, however, have not involved network analyses. That is, as far as we know, there are no studies that have evaluated the effects of E2 on prefrontal—hippocampal functional and effective connectivity. Moreover, there are no models that identify which regions of each structure may be most responsive to the effects of E2, or whether E2 has the same degree of effect on each circuit component of a given prefrontal—hippocampal network.

In the current study, we evaluate the effects of a 24-h graded E2 infusion on network effective connectivity between the hippocampus and PFC in postmenopausal women (PMW) in the resting state, i.e. in the absence of active cognitive or emotional challenge. Because the hippocampus and prefrontal cortex (especially the dorsolateral and ventrolateral aspects of the PFC: Berman et al., 1997; Shaywitz et al., 1999; Stevens et al., 2005; Eberling et al., 2000; Joffe et al., 2006) have been shown to be activated by E2 and to show an increase in synaptic connectivity within 12–24 h of E2 exposure (Gazzaley et al., 2002), we predicted that E2 would enhance effective connectivity between the lateral PFC and the hippocampus. This hypothesis will be conducted in the context of developing an initial network model for the effects of E2 on prefrontal—hippocampal circuitry.

2. Methods

2.1. Subjects

As part of a larger paradigm, eleven healthy subjects were recruited from the community. In order to minimize the relevance of endogenous estrogen, only postmenopausal women were studied. The study was approved by the FDA and Partners IRB and written, informed consent was provided prior to any study procedures. Admission to the General Clinical Research Center of Massachusetts General Hospital was preceded by general history and physical exam, and a laboratory evaluation of basic chemistry and complete blood count.

2.2. Hormone preparation, infusion, collection, and assessment

The estradiol (E2) infusion was prepared as previously described (Taylor et al., 1995). Based on the results of prior studies, in the first few subjects E2 was infused at a rate of $0.15 \mu\text{g}/(\text{kg h}) \times 12 \text{ h}$, and then $0.203 \mu\text{g}/(\text{kg h}) \times 12 \text{ h}$. This resulted in E2 levels above the target range and subsequent infusion rates were reduced to $0.1 \mu\text{g}/(\text{kg h})$, and then $0.135 \mu\text{g}/(\text{kg h})$, respectively, in the remaining eight subjects. 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 (IMED Corp.) was used to control the infusion rate.

Blood samples were collected for E2 at baseline and 24 h. 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 interassay CVs 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. The lower range of the sensitivity for the E2 assay was 20 pg/mL. Paired *t*-tests were conducted to evaluate for differences in E2 levels across the scanning sessions.

2.3. PET scanning and image reconstruction

[18F] fluorodeoxyglucose-PET scans were obtained on all subjects at baseline and after 24 h of estrogen infusion. PET images were acquired using a Siemens HR+, 32-ring, 63-slice body tomography. In both transverse and axial directions, the intrinsic spatial resolution of the scanner is 4.5 mm full width half maximum. 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 h. After fasting for approximately 6 h, subjects were injected with 5–6 mCi of 18F-FDG, while seated in a dimly lighted room with eyes closed; subjects were instructed simply to rest. Forty-five minutes after radiopharmaceutical administration, subjects were positioned supine in the PET scanner, with head position stabilized, and a single 20 min emission measurement was acquired.

2.4. Subtraction and covariate analyses

For FDG-PET paradigms, subtraction analyses are the standard for identifying sites of activation relevant to the experimental intervention, in our case, the E2 infusion. Several studies have already shown the lateral PFC (and hippocampus) to be activated by E2 (Berman et al., 1997; Shaywitz et al., 1999; Joffe et al., 2006; Stevens et al., 2005; Eberling et al., 2000), and we thus conducted our own SA to further isolate the site of greatest activation in the lateral PFC (and/or hippocampus) specific to our cohort.

Analysis of FDG-PET data was conducted following the theory of statistical parametric mapping (SPM) through use of the SPM2 software package (Wellcome Department of Cognitive Neurology, London UK). Subtraction analysis (SA) entailed a whole brain voxel-wise paired *t*-test conducted across the no-dose baseline and E2 infusion scans (i.e. at 0 vs. 24 h). This was done to identify sites of increased (and/or decreased) resting state metabolism

(CMRglc: regional cerebral glucose consumption) during E2 infusion relative to baseline. From the subtraction analysis, the prefrontal or hippocampal site showing the greatest increase (or decrease) in resting state activity (i.e. greatest z-score) was used as the site to generate an 'activation cluster' region of interest (i.e. Activation ROI) by means of the MarsBaR toolbox in the SPM2 software package. Because our E2 infusion scan did not have a time and placebo controlled scan as the subtraction comparison, we further evaluated a plot of the Activation ROI activity as a function of E2 levels during the no-dose baseline and E2 infusion scans to check whether activity of the Activation ROI correlated with E2 values during E2 infusion but not during the no-dose baseline; the strong possibility of a non-linear association between E2 values and activity of the Activation ROI precluded merely including E2 values as a covariate in a regression analysis (Bonsall et al., 1978; Yen and Lein, 1976; Fillenbaum et al., 2001; Brinton et al., 1997).

The Activation ROI was used as the seed region for a standard functional connectivity (seed region) analysis to identify prefrontal and/or hippocampal regions whose activity covaried with the Activation ROI; this covariate analysis was conducted merely to identify candidate sites for subsequent effective connectivity analysis. This procedure was approached by means of the simple regression option of SPM2 and entails a voxel by voxel searching of the entire brain volume for voxels that register a significant covariance with the specified ROI, in our case the Activation ROI (Cordes et al., 2000; Dougherty et al., 2004).

Significance thresholds were set at a p value of 0.001 and a minimum of 5 contiguous voxels. Because E2 has already repeatedly been shown to have activating effects on the lateral PFC and hippocampus, and these were clear sites of *a priori* interest, this thresholding was employed as a rudimentary control for multiple comparisons (Wager et al., 2007).

2.5. Effective connectivity analysis

After confirming the underlying circuit connections for the regions corresponding to the Activation ROI and its sites of covariance (Schmahmann and Pandya, 2006), the MarsBaR toolbox of SPM2 was used to collect ROI values from these sites for the effective connectivity analysis (Brett et al., 2002). Effective connectivity analysis, approached within the theoretical framework of structural equation modeling (McIntosh and Gonzalez-Lima, 1994), was conducted by means of the Amos 5.0 software package. Of the several possible permutations for path diagrams between the candidate regions, we limited our analysis to paths originating in the Activation ROI. This allowed for identification of connectivity changes in a circuit driven (i.e. activated) by E2. Goodness of fit statistics were applied to baseline data to identify which of the candidate models fit the data best (Hu and Bentler, 1999). Model selection initially involved likelihood ratio testing of the two 3-path "full models" to the corresponding nested 2-path models (Fig. 1). This was done to allow for selection of the most parsimonious model.

To determine whether there were significant differences in the path coefficients of the best fitting model across the two conditions, a stacked model comparison of constrained and unconstrained models was conducted. The "unconstrained" analysis refers to the model in which there are no constraints across the path coefficients, i.e. both of the coefficients are free to vary across the timepoints. In the "constrained" model, each path coefficient is separately constrained to be the same across both timepoints. For example, in the "b1 constrained" model (Table 1), only the b1 path is constrained to be the same across the two conditions and in the "b2 only" model, only the b2 path is constrained to be the same across the conditions. As labeled in Fig. 1, 'b1' is the path between the RSFG and RHIP, and 'b2' is the path between the RHIP and RMFG. If any constrained model fits significantly worse than the baseline unconstrained model (as determined by $\Delta\chi^2/\Delta d.f.$), then it is argued that the path coefficients are of a different magnitude across the specified conditions.

3. Results

3.1. Subjects

Subjects ranged in age from 48 to 76 years and had a mean age of 64.2 years. All subjects were at least 1 year from menopause. No subjects had a prior history of E2 therapy. All subjects lived independently and were free of active medical and psychiatric disorders.

3.2. Steroid levels

E2 levels increased from 30.5 ± 3.7 pg/mL (mean $\pm$ S.E.M.) at baseline [range: 20–53 pg/mL (four of these values were ≤ 20 pg/mL)] to 347.1 ± 51.4 pg/mL (range: 156–627 pg/mL) at 24 h. E2 levels at 24 h differed from baseline ($p < 0.001$).

3.3. Subtraction and covariate analysis

In terms of *a priori* sites of increased CMRglc, the 24 h minus 0 h subtraction revealed sites of increased activity in the right superior frontal gyrus (RSFG) (MNI coordinates: 18, 60, 28) and the right middle frontal gyrus (RMFG) (MNI coordinates: 52, 18, 38) during E2 infusion relative to baseline. Conversely, there were no deactivations at 24 h. Of these sites, the *z*-score for the RSFG was 3.81 and the *z*-score for the RMFG was 3.42. There were no hippocampal activations or deactivations at 24 h, even with searching to a *p* value of 0.005.

Thus, the RSFG was the *a priori* site showing the greatest increase in CMRglc. This site has been shown by a different component of our lab to be activated by E2 [even though employing a different paradigm and a different cohort of postmenopausal women, Joffe et al. (2006) showed essentially identical RSFG coordinates (18, 58, 28) to be associated with activation by E2]. Further evidence that activation of the RSFG was due to the E2 infusion was provided by plots of RSFG activity as a function of E2 levels. These plots revealed an obvious “U” shaped relationship between RSFG activity and E2 values following the E2 infusion, but no sort of any relationship was apparent during the no-dose baseline (data not shown; these findings are consistent with other investigators who have shown a non-linear, i.e. an U-shaped, quadratic, or bimodal association of network level observations with E2 levels: Bonsall et al., 1978; Yen and Lein, 1976; Fillenbaum et al., 2001; Brinton et al., 1997).

This site (the RSFG) was used as our seed site for our functional connectivity covariate analyses. Relative to the RSFG Activation ROI (59 voxels), the right hippocampus (RHIP, 81 voxels) (posterior aspect, MNI coordinates: 32, -32, -6), and right middle frontal gyrus (62 voxels) (MNI coordinates: 40, 22, 52) were identified as sites of covariance; these regions did not show significant covariance during no-dose baseline.

3.4. Effective connectivity analysis

Likelihood ratio testing revealed no difference between model fit for the 3-path full models and corresponding 2-path nested models. Although our small sample size afforded limited power to identify the most definitive model, this is the first time this type of neuroendocrine study has been conducted and our goal was to simply identify the most parsimonious model appropriate for an analysis. Thus, the 2-path models were further evaluated for goodness of fit.

Of the three models characterized by 2 paths, Model C fit the data best (Comparative Fit Index = 1, RMSEA = 0). Subsequent evaluation of the path coefficients of Model C (i.e. b_1 and b_2 , see Fig. 1) revealed that b_1 and b_2 both differed from zero during E2 infusion, but not during no-dose baseline ($P < 0.05$). Thus, stacked model comparison was conducted to evaluate whether the 24 h path coefficient also differed from the corresponding path coefficient at 0 h (i.e., as opposed to merely differing from zero). When both path coefficients were evaluated

for the E2 condition relative to baseline, the b1 path did not show any difference between conditions, but the b2 path did show a significant difference between the E2 infusion condition and the no-infusion baseline condition (Fig. 1 and Table 1), ($\Delta\chi^2 = 4.05$, d.f. = 1, $p = 0.044$).

4. Discussion

Our study had two objectives. First, our investigation involved an initial evaluation of a three-step analytic process aiming at effective connectivity analyses of a resting state hormonal challenge paradigm. Specifically, we used FDG-PET to identify a prefrontal or hippocampal site of increased CMRglc during E2 infusion relative to baseline, subsequently identified sites that showed covariance with the region of increased CMRglc, and finally, applied a path analysis to the model that most parsimoniously represented the interactions between these regions. Secondly, our study evaluated the hypothesis that E2 would enhance effective connectivity between the hippocampus and PFC. Our findings identified the RSFG as the prefrontal site of greatest increase in CMRglc during E2 infusion, and our path analysis of this site (i.e. the RSFG) with its sites of covariance (i.e. RHIP and RMFG) showed a significant difference (from zero) for the “b1” path from the RSFG to the RHIP during E2 infusion, but not baseline, and stacked model comparison showed a significant increase in the magnitude of the path coefficient for the “b2” path from the RHIP to the RMFG during the E2 infusion condition relative to baseline. These findings are consistent with E2 imparting a stimulatory effect on effective connectivity within prefrontal—hippocampal circuitry.

The basic physiological capacity for hormones to influence and even drive activity across neural systems has been demonstrated in several different contexts (Phoenix et al., 1959; Ball et al., 2004; Li et al., 1992; Frohlich et al., 2002). In this regard, our findings may most directly address the effect that hormones have on the activity within resting state networks (Fox and Raichle, 2007; De Luca et al., 2006). For example, a parallel from a basic research study is provided by Yoshida and co-workers (1994) who evaluated the effects of E2 on circuit activity across limbic structures in rats in the anesthetized state. Their study showed that E2 can dampen activity of amygdalar projections originating from the hypothalamic medial preoptic area, but not those emerging from the lateral septum. Parallel to this observation, our finding that E2 had a greater effect on projections from the hippocampus to the RMFG (i.e. differences upon stacked model comparison), than on projections from the RSFG to the hippocampus (although this path coefficient was different from zero only during E2 infusion, stacked model comparison did not show a significant difference) suggests that E2 may drive or influence activity across neural systems in a highly specific manner, i.e. possibly having a greater impact on specific circuit subcomponents. This similarity in our findings with findings from the animal literature may inform the general relevance of our study to the phenomenon of resting state networks. For example, regular fluctuations in E2 (as associated with the menstrual cycle) may have very specific effects on how activity of different structures is related during the resting state.

Our findings also hold implications for cognitive and emotional processes mediated by the PFC and hippocampus. For example, depressive symptoms have been shown to be associated with reduced E2 levels (Young et al., 2000), whereas resolution of depressive symptoms have been shown to be associated with increased synaptic connections in prefrontal and hippocampal systems (Santarelli et al., 2003; Jay et al., 2004; Warner-Schmidt and Duman, 2006). Thus, our findings that administration of E2 enhances effective connectivity between the PFC and hippocampus may hold mechanistic relevance to the depressive mood associated with reduced E2 levels, i.e. reduced E2 levels may predispose for both reduced synaptic connectivity and propagation of activity between the PFC and hippocampus, which may then manifest as a depressed mood. Similarly, changes in E2 levels, as associated with the perimenopause, may also impact mood by inducing fluctuations in prefrontal—hippocampal connectivity.

In terms of cognition, verbal memory is the neuropsychological function most repeatedly shown to be enhanced by estrogen replacement therapy in postmenopausal women (Maki, 2005; Zec and Trivedi, 2002; Ottowitz and Halbreich, 1995). Although verbal memory is more dependent on interactions between the left hippocampus and PFC (Eichenbaum, 2000), verbal memory performance may be enhanced by bilateral activation of the hippocampus and PFC (Brassen et al., 2006). Our findings that E2 enhances connectivity between the right hippocampus and right PFC suggest that E2 may enhance verbal memory performance by means of recruiting a bilateral cooperation between prefrontal and hippocampal systems during verbal memory tasks.

In closing, while these findings provide preliminary evidence that a simple hormonal challenge can alter prefrontal—hippocampal network effective connectivity, different aspects of our study deserve further attention in future paradigms. First, although our findings were all specific to the E2 infusion scan relative to a no-dose baseline scan, further investigation of these initial findings warrants comparison to a placebo controlled scanning session. For example, familiarity with the scanner or hospital setting may have contributed to enhancing connectivity between the hippocampus and PFC. Secondly, although our age bracket was limited to postmenopausal women, further investigation of the effects of age on hormone associated network interactions may reveal that these effects are more prominent in the early menopause. Finally, the three-part analytic strategy employed by our study was successful in identifying candidate regions for our path analysis, but alternate approaches may also be appropriate for this inquiry. Moreover, structural equation modeling is merely one means of evaluating effective connectivity. The effects of hormones on effective connectivity also deserve investigation from other techniques evaluating effective connectivity, e.g. dynamic causal modeling (Friston et al., 2003).

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References

- Akaishi T, Sakuma Y. Estrogen excites oxytocinergic, but not vasopressinergic cells in the paraventricular nucleus of female rat hypothalamus. *Brain Res* 1985;335:302–305. [PubMed: 4005557]
- Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Ritters LV, Balthazart J. Seasonal plasticity in the song control system: multiple brain sites of steroid hormone action and the importance of variation in song behavior. *Ann. N. Y. Acad. Sci* 2004;1016:586–610. [PubMed: 15313796]
- Berman KF, Schmidt PJ, Rubinow DR, Danaceau MA, Van Horn JD, Esposito G, Ostrem JL, Weinberger DR. Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. *Proc. Natl. Acad. Sci. U.S.A* 1997;94:8836–8841. [PubMed: 9238064]
- Bonsall RW, Zump D, Michael RP. Menstrual cycle influences on operant behavior of female rhesus monkeys. *J. Comp. Physiol. Psychol* 1978;92:846–855. [PubMed: 103942]
- Brassen S, Weber-Fahr W, Sommer T, Lehmbeck JT, Braus DF. Hippocampal—prefrontal encoding activation predicts whether words can be successfully recalled or only recognized. *Behav. Brain Res* 2006;171:271–278. [PubMed: 16725214]
- Brett, M.; Anton, J.L.; Valabregue, R.; Poline, B.P. Region of interest analysis using an SPM toolbox. Presented at the 8th International Conference on Functional Mapping of the Human Brain; June 2–6; Japan. Sendai; 2002.

- Brinton RD, Tran J, Proffitt P, Montoya M. 17-Beta-estradiol enhances the outgrowth and survival of subcortical neurons in culture. *Neurochem. Res* 1997;22:1339–1351. [PubMed: 9355106]
- Cordes D, Haughton VM, Arfanakis K, Wendt GJ, Turski PA, Moritz CH, Quigley MA, Meyerand ME. Mapping functionally related regions of brain with functional connectivity MR imaging. *Am. J. Neuroradiol* 2000;21:1636–1644. [PubMed: 11039342]
- De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM. fMRI resting state networks define distinct modes of long distance interactions in the human brain. *NeuroImage* 2006;29:1359–1367. [PubMed: 16260155]
- Dougherty DD, Rauch SL, Deckersbach T, Marci C, Loh R, Shin LM, Alpert NM, Fischman AJ, Fava M. Ventromedial prefrontal cortex and amygdala dysfunction during an anger induction positron emission tomography study in patients with major depressive disorder with anger attacks. *Arch. Gen. Psychiatry* 2004;61:795–804. [PubMed: 15289278]
- Eberling JL, Reed BR, Coleman JE, Jagust WJ. Effect of estrogen on cerebral glucose metabolism in postmenopausal women. *Neurology* 2000;55:875–877. [PubMed: 10994014]
- Eichenbaum H. A cortical—hippocampal system for declarative memory. *Nat. Rev. Neurosci* 2000;1:41–50. [PubMed: 11252767]
- Fillenbaum GG, Hanlon JT, Landerman LR, Schmader KE. Impact of estrogen use in decline on cognitive function in a representative sample of older community-resident women. *Am. J. Epidemiol* 2001;153:137–144. [PubMed: 11159158]
- Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat. Rev. Neurosci* 2007;8:700–711. [PubMed: 17704812]
- Friston KJ. Functional and effective connectivity in imaging: a synthesis. *Hum. Brain Mapp* 1994;2:56–78.
- Friston KJ, Harrison L, Penny W. Dynamic causal modeling. *NeuroImage* 2003;19:1273–1302. [PubMed: 12948688]
- Frohlich J, Morgan M, Ogawa S, Burton L, Pfaff D. Statistical analysis of hormonal influences on arousal measures in ovariectomized female mice. *Horm. Behav* 2002;42:414–423. [PubMed: 12488108]
- Gazzaley A, Kay S, Benson DL. Dendritic spine plasticity in hippocampus. *Neuroscience* 2002;111:853–862. [PubMed: 12031408]
- Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci* 1990;10:1286–1291. [PubMed: 2329377]
- Hu L-T, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct. Equation Model.: Multidisciplinary J* 1999;6:1–55.
- Jay TM, Rocher C, Hotte M, Naudon L, Gurden H, Spedding M. Plasticity at hippocampal to prefrontal cortex synapses is impaired by loss of dopamine and stress: importance for psychiatric diseases. *Neurotoxicol. Res* 2004;6:233–244.
- Joffe H, Hall JE, Gruber Staci, Sarmiento Ingrid A, Cohen LS, Yurgelun-Todd D, Martin KA. Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause* 2006;13:411–422. [PubMed: 16735938]
- Kow LM, Easton A, Pfaff DW. Acute estrogen potentiates excitatory responses of neurons in rat hypothalamic ventromedial nucleus. *Brain Res* 2005;1043:124–131. [PubMed: 15862525]
- Li CS, Kaba H, Saito H, Seto K. Oestrogen infusions into the amygdala potentiate excitatory transmission from the accessory olfactory bulb to tuberoinfundibular arcuate neurones in the mouse. *Neurosci. Lett* 1992;143:48–50. [PubMed: 1436681]
- Maki PM. Estrogen effects on the hippocampus and frontal lobes. *Int. J. Fertil. Womens Med* 2005;50:67–71. [PubMed: 16334413]
- Maki PM, Resnick SM. Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition. *Neurobiol. Aging* 2000;21:373–383. [PubMed: 10867223]
- McIntosh AR, Gonzalez-Lima F. Structural equation modeling and its application to network analysis in functional brain imaging. *Hum. Brain Mapp* 1994;2:2–22.
- Ottowitz WE, Halbreich U. Mood and cognitive changes following estrogen replacement therapy in postmenopausal women. *CNS Drugs* 1995;4:161–167.

- Parducz A, Hoyk Z, Kis Z, Garcia-Segura LM. Hormonal enhancement of neuronal firing is linked to structural remodelling of excitatory and inhibitory synapses. *Eur. J. Neurosci* 2002;16:665–670. [PubMed: 12270042]
- Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* 1959;65:369–382. [PubMed: 14432658]
- Reiman EM, Armstrong SM, Matt KS, Mattox JH. The application of positron emission tomography to the study of the normal menstrual cycle. *Hum. Reprod* 1996;11:2799–2805. [PubMed: 9021395]
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman RS, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 2003;301:805–809. [PubMed: 12907793]
- Schmahmann, JD.; Pandya, DN. White Matter Fiber Bundles by Cortical Region of Origin. In: Schmahmann, JD.; Pandya, DN., editors. *Fiber Pathways of the Brain*. New York: Oxford University Press; 2006. p. 378–380.
- Shaywitz SE, Shaywitz BA, Pugh KR, Fulbright RK, Skudlarski P, Mencl WE, Constable RT, Naftolin F, Palter SF, Marchione KE, Katz L, Shankweiler DP, Fletcher JM, Lacadie C, Keltz M, Gore JC. Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *JAMA* 1999;281:1197–1202. [PubMed: 10199429]
- Stevens MC, Clark VP, Prestwood KM. Low-dose estradiol alters brain activity. *Psychiatry Res* 2005;139:199–217. [PubMed: 16084069]
- Taylor AE, Whitney H, Hall JE, Martin K, Crowley WF. Midcycle levels of sex steroids are sufficient to recreate the follicle stimulating hormone but not the leutenizing hormone mid-cycle surge: evidence for the contribution of other ovarian factors to the surge in normal women. *J. Clin. Endocrinol. Metab* 1995;80:1541–1547. [PubMed: 7744998]
- Wallace M, Luine V, Arellanos A, Frankfurt M. Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. *Brain Res* 2006;1126:176–182. [PubMed: 16934233]
- Wager, TD.; Hernandez, L.; Jonides, J.; Lindquist, M. Elements of functional neuroimaging. In: Cacioppo, JT.; Tassinary, LG.; Berntson, GG., editors. *Handbook of Psychophysiology*. Cambridge: Cambridge University Press; 2007. p. 19–55.
- Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus* 2006;16:239–249. [PubMed: 16425236]
- Yen SS, Lein A. The apparent paradox of the negative and positive feedback control system on gonadotropin secretion. *Am. J. Obstet. Gynecol* 1976;126:942–954. [PubMed: 793393]
- Yoshida M, Suga S, Sakuma Y. Estrogen reduces the excitability of the female rat medial amygdala afferents from the medial preoptic area but not those from the lateral septum. *Exp. Brain Res* 1994;101:1–7. [PubMed: 7843289]
- Young EA, Midgley AR, Carlson NE, Brown MB. Alteration in the hypothalamic—pituitary—ovarian axis in depressed women. *Arch. Gen. Psychiatry* 2000;57:1157–1162. [PubMed: 11115329]
- Zec RF, Trivedi MA. The effects of estrogen replacement therapy on neuropsychological functioning in postmenopausal women with and without dementia: a critical and theoretical review. *Neuropsychol. Rev* 2002;12:65–109. [PubMed: 12371603]

Fig. 1.

Model selection. After likelihood ratio testing revealed no difference in fit between the 'full models' (3-path) and corresponding 2-path nested models, the parsimonious 2-path diagrams were evaluated by goodness of fit statistics. Model C was the best fitting model (CFI = 1, RMSEA = 0). Consequently, this model was also evaluated by means of constrained and unconstrained stacked model comparison. [HIP = right hippocampus, MNI coordinates (32, -32, -6), 81 voxels; SFG = right superior frontal gyrus, MNI coordinates (18, 60, 28), 59 voxels; MFG = right middle frontal gyrus, MNI coordinates (40, 22, 52), 62 voxels; path b1 = projection from the SFG to the HIP; path b2 = projection from HIP to the MFG]. For purposes of simplifying the figure, error terms are not included in the path diagrams.

Table 1

Stacked model comparison of paths b1 and b2 of Model C from Fig. 1.

Path of interest (0 h vs. 24 h)	$\Delta\chi^2$	$\Delta d.f.$	<i>p</i> value
b1 Constrained	0.34	1	ns
b2 Constrained	4.05	1	0.044

After confirming that each path coefficient differed from zero during estrogen infusion but not during baseline, each path was also subjected to stacked model comparison to further determine whether the path coefficients corresponding to the estrogen infusion and baseline scans also differed from each other (i.e. as opposed to merely differing from zero).

$\Delta\chi^2$ Change in chi-square, $\Delta d.f.$: change in degrees of freedom and ns: not significant.