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# Hormonal treatment increases the response of the reward system at the menopause transition: A counterbalanced randomized placebo-controlled fMRI study

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## KEYWORDS

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**Summary** Preclinical research using rodent models demonstrated that estrogens play neuroprotective effects if they are administered during a critical period near the time of cessation of ovarian function. In women, a number of controversial epidemiological studies reported that a neuroprotective effect of estradiol may be obtained on cognition and mood-related disorders if hormone therapy (HT) begins early at the beginning of menopause. Yet, little is known about the modulatory effects of early HT administration on brain activation near menopause. Here, we investigated whether HT, initiated early during the menopause transition, increases the response of the reward system, a key brain circuit involved in motivation and hedonic behavior. We used fMRI and a counterbalanced, double-blind, randomized and crossover placebo-controlled design to investigate whether sequential 17 $\beta$ -estradiol plus oral progesterone modulate reward-related brain activity. Each woman was scanned twice while presented with images of slot machines, once after receiving HT and once under placebo. The fMRI results demonstrate that HT, relative to placebo, increased the response of the striatum and ventromedial prefrontal cortex, two areas that have been shown to be respectively involved

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during reward anticipation and at the time of reward delivery. Our neuroimaging results bridge the gap between animal studies and human epidemiological studies of HT on cognition. These findings establish a neurobiological foundation for understanding the neurofunctional impact of early HT initiation on reward processing at the menopause transition.  
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## 1. Introduction

Successful healthy brain aging has become one of the most crucial public health challenges of our time. Understanding how efficacious preventive pharmacological treatments modulate specific brain circuits, such as the reward system, is an integral part of rising to this challenge to improve our health and quality of life at old age. In women, menopause is accompanied by a drop of estradiol level, which may, in part, be responsible for cognitive impairments (Steiner et al., 2003). Although controversial studies reported that hormone therapy (HT) may prevent the deleterious effects of aging on cognition, and reduces the risks of dementia, including Alzheimer's disease, mild cognitive impairment and mood-related disorders (Henderson et al., 2005; Whitmer et al., 2011), others have found that initiation of HT more than a few years after menopause is associated with an unchanged or increased risk of dementia and age-associated cognitive decline (Resnick et al., 2006; Shumaker et al., 2003). The timing of initiation of HT relative to the onset of menopause has been proposed to be one important factor explaining part of the discrepant observations regarding a neuroprotective effect of HT (Sherwin, 2005, 2012; Daniel, 2013). According to the 'critical time window period' hypothesis, HT effectively decreases cognitive decline in aging women when it is initiated around the time of menopause, but this beneficial effect is not observed when HT is administered decades later. In humans, it is difficult to test this critical time window hypothesis and to study how sex steroid hormones affect the aging reward system because endocrine and neural senescence overlap in time and are mechanistically intertwined in complex feedback loops. Recent findings in younger early menopausal women reported that HT brought no significant benefit or harm to cognition 7 years later (Espeland et al., 2013). In contrast, animal models provide strong evidence for the critical window hypothesis, since 17 $\beta$ -estradiol (the major estrogen in most mammals, referred to as estradiol) replacement enhances cognitive functions when initiated immediately after ovariectomy, but not after a long period of ovarian hormone deprivation (Rocca et al., 2011; Daniel, 2013). Moreover, non-human primate studies have revealed that sequential HT reverses age-related prefrontal cortex (PFC) cognitive impairment in ovariectomized rhesus monkeys and that PFC synaptic attributes altered with aging are rescued by sequential HT (Hao et al., 2006).

Many studies have focused on effects of HT on disease outcomes, such as increase risk of heart attack, breast cancer or coronary artery disease, but little is known about the effects of HT on the human brain. Indeed, there are limited studies of sequential HT intervention at the functional brain level around menopause and it is unknown how the reward system, involved in motivation and hedonic

behavior, is modulated by HT at perimenopause. Achieving an understanding of how the reward circuit changes with HT administration is of public health importance given that HT continues to be widely prescribed for managing menopausal symptoms. This brain system is known to show reduced BOLD response and lower number of dopaminergic receptors with aging (Dreher et al., 2008; Wong et al., 1988) and there are strong links between decline in striatal dopamine activity and cognitive dysfunctions as age increase (Volkow et al., 1998). Moreover, interventions that enhance dopamine activity enhance working memory capacity in aged monkeys (Castner and Goldman-Rakic, 2004), suggesting possible beneficial effects on performance and quality of life in healthy aging. A better understanding of these hormonal influences also has crucial implications for understanding sex-related differences on prevalence, course and treatment response characteristics of neurological and neuropsychiatric disorders in which dopaminergic abnormalities play a prominent role.

From a neuroanatomic point of view, there are reasons to believe that HT may modulate the reward circuitry near menopause. Indeed, ovarian steroids have widespread neurophysiological effects, including on the dopaminergic system, and estrogen and progesterone receptors are densely present along rodents' midbrain dopaminergic neurons and other components of the reward system, such as the striatum (McEwen, 2002; Brailou et al., 2007; Laflamme et al., 1998). A number of preclinical data attest to a neuroregulatory role of estradiol on the reward system. For example, estrogen facilitates the effect of amphetamine or apomorphine on dopamine release and locomotor activity in rats unilaterally lesioned by 6-hydroxydopamine (Becker and Cha, 1989), and this activity is responsive to natural fluctuations in estradiol and is increased during late proestrus and early estrus (Becker et al., 1982). Although recent neuroimaging data demonstrate neuroregulatory effects of sex steroid hormones in young women on the reward system and on brain regions engaged in processing emotions, the evidence of these influences were indirect because estrogen and progesterone are simultaneously present in women of reproductive age (Dreher et al., 2007; Goldstein et al., 2005; Andreano and Cahill, 2010; Ossewaarde et al., 2011; Alonso-Alonso et al., 2011). In contrast, menopause provides a unique model to study how naturally low endogenous baseline estradiol levels influence reward-related functions and how HT may restore these functions.

Here, we investigated how HT, a sequential administration of 17 $\beta$ -estradiol followed by progesterone, influenced activity of the reward system in a carefully selected group of women at the end of the menopause transition, using a counterbalanced, randomized, crossover, double-blind and placebo-controlled fMRI design study (Fig. 1). We chose to initiate HT early in this group of women (the time between

the last menses and HT initiation was less than a year) to maximally take advantage of a possible beneficial effect of HT on the reward system. Importantly, none of the women had ever taken HT before inclusion in the study. Each woman with a loss of ovarian hormones function was scanned once after a placebo period and once after HT. The order of the scans was counterbalanced across women. In each treatment condition, women performed an event-related fMRI reward task consisting in viewing four types of slot machines varying monetary reward probability of being rewarded 20€ or 0€. They simply had to press one of four specific response buttons corresponding to each slot machine at the time of their presentation and at the time of outcome delivery.

Because previous fMRI studies in young participants have documented that distinct reward anticipation- and outcome-processing phases are associated with differential patterns of ventral striatal and ventromedial prefrontal cortex activity (Dreher et al., 2006), we hypothesized that HT could restore or increase activity of these reward-related brain areas.

## 2. Methods

### 2.1. Subjects

Fifteen healthy, right-handed non-smoking perimenopausal caucasian women were recruited through advertisement in local newspapers. Two of them were excluded from the analyses because of problems encountered during scanning. The mean age of the thirteen remaining women was  $52.3 \pm 2.2$  years old (range 48–55 y.o.). Women were all at the end of their menopause transition ( $8.7 \pm 1.3$  months after the last menstrual period) at the time of the first scan. The menopause transition, also called perimenopause, is the period lasting up until menopause, the point when the ovaries stop releasing eggs. By definition, perimenopause ends when a woman has gone 12 months without having her period. The perimenopausal status was confirmed on the inclusion day by low plasma estradiol levels ( $28 \pm 22.3$  pmol/L) and by increased follicular stimulating hormone (FSH) level ( $>30$  IU/L) for each woman. One important inclusion criteria was that the time between the last menses and HT initiation was less than a year (Fig. 1). Importantly, none of the women had ever taken HT before inclusion in the study. In addition, all the women included did not experience menstrual cycle recovery after completion of the study. Women were initially screened by phone interview. This initial interview was followed by a full clinical interview and a physical exam (MP). At this time, all women exhibited menopause-associated symptoms, such as vasomotor symptoms. Exclusion criteria included any current use of estrogen or progestin, any use of contraceptives since less than a year, current or previous mental disorders, depressive tendency assessed by the Beck Depression Inventory (BDI), pathological gambling assessed by the South Oaks Gambling Screen (SOGS), hypertension, diabetes, elevated cholesterol, breast cancer and MRI contraindications. All women were French native speakers and had at least a high school level of education. After complete description of the study to the subjects, written informed consent

was obtained. The study was approved by the Lyon ethics committee (CPPPRB no. 06/038).

### 2.2. Hormonal and placebo treatment

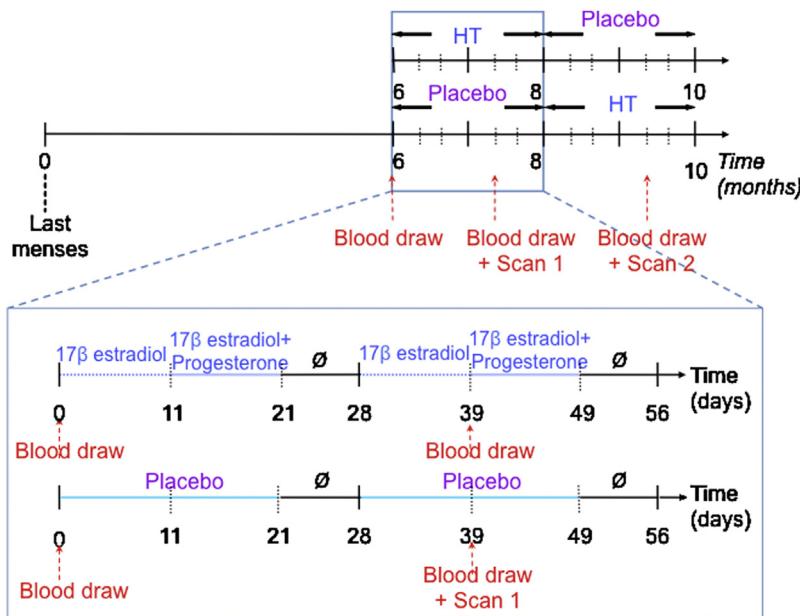
Our choice of the hormone therapy (sequential estradiol-plus-progestin) aimed to reproduce physiological sex-steroid hormone environment (Anderson et al., 2004; Rossouw et al., 2002) and was also based on the fact that unopposed estradiol (the supplementation of endogenous estradiol without a progestagen) could result in endometrial hyperplasia, a precursor to endometrial cancer (Anderson et al., 2004). Each woman received micronized preparations of  $17\beta$ -estradiol subsequently combined with progesterone and a placebo in a random and counter-balanced order (6 women started the experiment with 2 months of HT while the other 7 women started it after taking a placebo for 2 months) (Fig. 1). Women received for 21 days a daily pill containing  $17\beta$ -estradiol (2 mg/day) that was associated to oral progesterone (100 mg/day) from day 12 to day 21. This was followed by a week washout period to reproduce the normal menstrual cycle (during periods both estradiol and progesterone levels are very low and close to the values observed during menopause). A similar hormonal substitution was repeated during the following next month. Women had to take their pills every day at the same time. On the scanning days, women took their pills 1 h before the session. HT and placebo pills were produced by our Pharmacy Division (Bron) and had the exact same visual aspect and doses.

### 2.3. Quality of life assessment

On the first day of the visit and at the end of each week of our 16 weeks study, women filled the Women Health Questionnaire (WHQ). The WHQ is a 36-item questionnaire assessing nine domains of physical and emotional health experienced by mid-aged women. More specifically, the following domains are covered by the questionnaire: depressive mood, somatic symptoms, memory/concentration, vasomotor symptoms, anxiety/fear, sexual behavior, sleep problems, menstrual symptoms and attractiveness. The WHQ measures a range of domains of symptom experience relevant to the menopause, such as vasomotor symptoms and other symptoms, which are associated with psychosocial factors, general health and/or aging, such as sleep, sexual problems and cognitive difficulties. The scores obtained for each domain were averaged for each subject and for each stage of the experiment, i.e. during HT and during placebo administration. The higher the score was, the lower the subjects' well-being.

### 2.4. Reward task

Each woman was scanned twice: once under HT and once under placebo (on day 11 of the second and of the fourth month). Women performed an event-related monetary reward task consisting in viewing four types of slot machines varying monetary reward probability ( $P=0$ ,  $P=0.25$ ,  $P=0.5$  and  $P=0.75$ ) of being rewarded 20€ or 0€ (Fig. 2) (Dreher et al., 2006). They had to press one of four specific response



**Figure 1** Study schematic and timing of the fMRI pharmacological procedure. Women were enrolled in a double blind, randomized, placebo-controlled crossover study. One important inclusion criteria was that the time between the last menstrual cycle and the HT initiation was less than a year (the figure shows an example when HT starts 6 months after the last menses). Each woman was scanned twice: once under sequential HT and once under placebo (on day 11 of the second and on day 11 of the fourth month). Women took a daily pill of either HT (or a placebo) for two cycles of 28 days each, followed by two consecutive months of placebo (respectively HT). For the first 11 days of a 'restored' menstrual cycle (under HT), pills contained 17 $\beta$  estradiol (2 mg/day). From the 12th to the 21th day, the pills contained progesterone (100 mg/day), in addition to 17 $\beta$  estradiol. This was followed by a week washout period. This cycle was repeated for a second month (see blue rectangle). After these two months, a new cycle started: women receiving HT first were administrated with a placebo containing 2 mg/day of inactive substance for the first 11 days and 102 mg/day for the following 10 days. This cycle was repeated during a last cycle of 28 days. The order of receiving HT and placebo was randomly assigned and counter-balanced. Blood samples were collected at the beginning of the study and on each day of scanning (once under HT and once under placebo). Hormonal dosage indicates time of blood draw followed by hormonal assay. For interpretation of the references to color in this figure, the reader is referred to the web version of the article. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

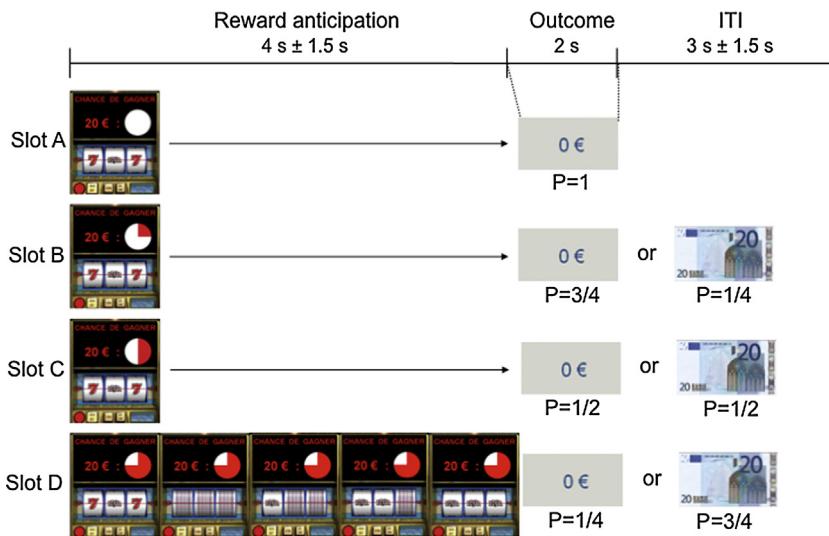
buttons corresponding to each slot machine at the time of their presentation and at the time of outcome delivery. These motor responses enabled us to ensure that women were paying attention to the different types of slot machines and to their outcome. The visuo-motor association between each slot machine and the corresponding response button was learned outside of the scanner during a training session. Each trial consisted in an anticipation phase (delay with three spinners rolling around: 4 s  $\pm$  1.5 s) and an outcome phase (20€/0€) followed by an Inter-Trial Interval (3 s  $\pm$  1.5 s). There were 5 runs, each composed of 48 trials (12 trials for each type of slot machine). Each of the 4 possible slot machines appeared pseudo-randomly during each run. The exact probability of each potential outcome was reached at the end of each run for each slot machine. The order of the runs was randomized between subjects. Subjects were informed that they would earn extra-money for playing with the slot machines, in addition to the money earned for participating in the HT study. Subjects were told that the money effectively earned for playing the slot machines would be proportional to the total amount of money experienced during the experiment, but this coefficient of proportionality was kept unknown to the subjects.

After completing the two fMRI scans, all women received the same amount of money for participating (300€).

## 2.5. fMRI data acquisition

Scanning was performed on a 1.5 Tesla Siemens Sonata Maestro. During the reward task, for each participant, and on each scanning day, 5 time series of 187 whole-brain functional scans were obtained with a gradient-echo, T2\*-weighted, echo-planar scanning sequence (EPI T2\*-weighted) to measure the blood oxygenation level-dependent (BOLD) change, with the following parameters: repetition time = 2.5 s, echo time = 60 ms, flip angle = 90°; field of view = 22 cm, acquisition matrix = 64  $\times$  64, 26 axial slices, thickness 4 mm. Head movement during scanning was minimized with a pillow and additional padding.

A T1-weighted structural image was also acquired for each subject at the end of the experiment with a magnetization-prepared rapid-acquisition gradient echo (MPRAGE) gradient echo sequence (repetition time TR = 1970 ms, echo time TE = 3.93 ms, inversion time TI = 1100 ms, FOV = 256 mm, acquisition matrix = 256  $\times$  256,



**Figure 2** Reward fMRI task. Four types of slot machines ((A)–(D)) were presented pseudo-randomly. The pie charts of each slot machine indicated the probabilities of winning 20€ (red part) or nothing (white part). Each trial consisted of a delay with slot machines' spinners rolling around (anticipation phase: 4 s ± 1.5 s) followed by an outcome phase (reward: 20€ or no reward delivery: 0€, 2 s) and an inter-trial interval (ITI) of 3 s ± 1.5 s. Subjects indicated which slot machine was presented by pressing one of 4 specific buttons both at the cue and at the outcome, regardless of winning or not. For interpretation of the references to color in this figure, the reader is referred to the web version of the article. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

slice thickness = 1 mm, number of slices = 26, in-plane resolution = 1 × 1 × 1 mm<sup>3</sup>, bandwidth = 130 Hz/pixel) along the anterior/posterior commissure (AC/PC) line and covering the whole brain.

## 2.6. Hormone levels

Four venous blood samples were drawn to measure estradiol plasma level: one before the study to check the perimenopausal status, one on the first day of inclusion in the study and the last two before each fMRI session. The samples were centrifuged, aliquoted, and stored at –70°C until time of assay. Hormone measurements were performed on-site using in-house validated assays (Laboratoire de Radioanalyse, Centre de Biologie Est, Groupe Hospitalier Est, Hôpitaux Civils de Lyon) (Rinaldi et al., 2002a,b). The samples were frozen and assays were performed on plasma thawed for a maximum period of 15 days. Steroids are “relatively strong” molecules that are resistant to long-term storage at –70°C. Serum 17β-estradiol was measured by tritiated radioimmunoassay after diethylether extraction. The anti-estradiol antibody was raised by immunization of rabbit with estradiol-6-CMO-BSA. The antibody cross-reacted by 8.8% with 6-OH-estradiol, 3.0% with 16-ceto-17β-estradiol, 1% with estrone, 0.15% with estriol, 0.4% with 17α-estradiol, 0.1% with ethynodiol-estradiol and <0.001% with testosterone or 17α-OH-progesterone. Assays of steroids are competitive assays. In the same run, the coefficients of variation (between 0.8 and 5.0%) are not identical for different concentration levels (slope of standard curve inconstant). In inter-assay, we observe the same phenomenon with higher values of CV. So, the inter-assay coefficient of variation is different when measuring 21 pmol/L (usually high for low concentration) with an

optimal inter-assay coefficient of variation for measuring an estradiol concentration of 100 pmol/L. These in-house steroid immunoassay were research assays previously developed in the laboratory (Rinaldi et al., 2002a,b). The detection limit was 11 pmol/L and the inter-assay coefficient of variation was 12.1% for 21 pmol/L, 6.0% for 100 pmol/L and 9.4% for 169 pmol/L. For concentrations between 11 and 1000 pmol/L, the intra-assay coefficients of variation were less than 5%. An ANOVA was performed with HT/placebo as independent factor to test the difference between estradiol levels on the two scanning days.

## 2.7. fMRI methods

### 2.7.1. Images analysis

The fMRI data from both scanning sessions (HT and placebo) were preprocessed and analyzed using the SPM5 software (Wellcome Department of Cognitive Neurology, London, UK, <http://www.fil.ion.ucl.ac.uk/spm/>). For each subject, the first four functional volumes of each run were removed, and the remaining images were corrected for slice-timing artifacts and spatially realigned. The functional images were then normalized to the Montreal Neurological institute (MNI) stereotaxic space using the EPI template of SPM5 and spatially smoothed with a 8 mm full-width at half-maximum isotropic Gaussian kernel.

After preprocessing, a random-effects, epoch-related statistical analysis was performed in a two-level procedure. At the first level, a separate general linear model was specified for each participant for each of the sessions. A set of boxcar functions, modeling the duration of each event separately, that is the presentation of the slot machine (during the anticipation phase: 4 s ± 1.5 s) and at the outcome (2 s), was convolved with a canonical hemodynamic response

**Table 1** BOLD response during reward anticipation and at the time of rewarded outcome during HT and placebo alone. Abbreviations: L: left; R: right.

Anatomical structure	Reward anticipation (HT alone)				Rewarded outcome (HT alone)			
	MNI coordinates			Z value	MNI coordinates			Z-value
	x	y	z		x	y	z	
<b>Frontal</b>								
Ventromedial PFC					-6	45	9	4.06
Posterior cingulate cortex					-3	-42	18	3.86
Pre-SMA	9	-3	48	3.31				
Superior frontal gyrus								
Left ventrolateral PFC								
<b>Striatum</b>								
R putamen	27	21	9	3.84				
<b>Visual cortex</b>								
R m. occipital gyrus					18	-102	6	5.33
L m. occipital gyrus					-15	-87	-18	5.41
Midbrain	6	-27	-18	4.03				
Anatomical structure	Reward anticipation (placebo)				Rewarded outcome (placebo)			
	MNI coordinates			Z-value	MNI coordinates			Z-value
	x	y	z		x	y	z	
<b>Frontal</b>								
Ventromedial PFC								
Posterior cingulate cortex								
Pre-SMA								
Superior frontal gyrus					-15	57	30	4.51
Left ventrolateral PFC					-39	24	-15	4.7
<b>Striatum</b>								
R putamen								
<b>Visual cortex</b>								
R m. occipital gyrus	51	-57	-12	3.86	18	-102	6	4.94
L m. occipital gyrus					-27	-99	12	4.88
Midbrain								

function. Within-subject time series modeling accounted for the following 11 regressors: four during the delay period and seven regressors at the outcome (3 rewarded and 3 non-rewarded outcomes of potentially rewarded slot machine, plus the non-rewarded outcome of the never rewarded slot machine). In addition, the 6 ongoing motion parameters estimated during realignment were included as regressors of no interest. The data were high-pass filtered (128 s cutoff) to remove low-frequency drifts. In the current analyses, we assessed both the anticipatory (delay) and outcome phases. Contrast images were calculated for each participant in both sessions. The individual contrast images were then entered into a second-level random-effects analysis to assess the group effect of HT on brain activity related to the effects of reward anticipation and of rewarded outcome, as assessed by the two comparisons:

- (1) Anticipation of potentially rewarded slot machines:  $\text{delay}_{(\text{slots: } P=0.25+P=0.5+P=0.75)} > \text{delay}_{(\text{slot: } P=0)}$ .
- (2) Response at the time of rewarded outcome relative to no reward delivery of the potentially rewarded

slot machines:  $20\text{€}_{-\text{slot}, P=25} + 20\text{€}_{-\text{slot}, P=50} + 20\text{€}_{-\text{slot}, P=75} > 0\text{€}_{-\text{slot}, P=25} + 0\text{€}_{-\text{slot}, P=50} + 0\text{€}_{-\text{slot}, P=75}$ .

First, a one-sample *t*-test was used to investigate the statistical maps for the anticipation and outcome contrasts in the HT and placebo conditions separately (Table 1). Then, a paired *t*-test was used to statistically assess drug effects (HT versus placebo) on resulting neural activity (Table 2).

Because we were testing specific *a priori* hypotheses about the pattern of activation of specific components of the reward system, including the ventral striatum and ventromedial prefrontal cortex, and because gonadal sex hormone effects of HT administration were expected to be small at the brain system-level, the statistical maps were thresholded at  $P < 0.001$ , uncorrected, with a minimum cluster size of 8 voxels. Moreover, note that the activity of the brain reward regions observed in the comparison HT > placebo remained statistically significant after correction for multiple comparisons based on the false discovery rate (FDR) with a threshold of  $P < 0.05$ , using reduced search volumes. This small volume correction for multiple comparisons was

**Table 2** Between treatments (HT versus placebo) comparison during reward anticipation and at the time of rewarded outcome.

Anatomical structure	Reward anticipation (HT > placebo)						Rewarded outcome (HT > placebo)					
	MNI coordinates			Z-values			MNI coordinates			Z-values		
	x	y	z				x	y	z			
Anterior medial PFC												
Right caudate nucleus	24	18	15	3.80			0	45	6	3.35		
Right putamen	30	18	-3	3.69								

performed using spherical search volumes centered at peak coordinates from a meta-analysis (i.e. independent data) with 10-mm spheres for the caudate nucleus ( $xyz=20, 8, 10$ ) and vmPFC ( $xyz=0, 38, 8$ ) (Diekhof et al., 2012).

Finally, we also performed a correlational analysis between gonadal steroids and brain regions activated during reward anticipation and at the time of reward delivery. To do so, we entered the Log estradiol levels of each woman under HT as a covariate in regression analyses. Only estradiol levels under HT were considered because estradiol levels were low and showed small between-subjects variations in the placebo condition.

Parametric fMRI analyses with reward probability as regressor were also conducted but the results not reported.

### 2.7.2. Activations localization and region of interest analyses

Anatomic labeling of activated regions was performed using the SPM Anatomy toolbox ([http://www2.fz-juelich.de/inm/inm-3/spm\\_anatomy\\_toolbox](http://www2.fz-juelich.de/inm/inm-3/spm_anatomy_toolbox)) and the probabilistic atlas of Hammers. Reported coordinates conform to the Montreal Neurological Institute (MNI) space. For illustrative purposes only, we extracted and plotted the activity corresponding to the reward anticipation and rewarded outcome phases for the HT and placebo conditions in 10 mm-radius spheres centered on the highest peak of putamen activity during reward anticipation and of vmPFC activity at the time of rewarded outcome. These regions of interest, conducted with the extension of SPM MarsBaR (<http://marsbar.sourceforge.net/>), were defined functionally from the whole-brain analysis based on a super group of mean activity across all scans/subjects (including both HT and placebo scans).

## 3. Results

### 3.1. Estradiol levels and clinical ratings

On the day of inclusion in the study (i.e. before HT or placebo treatment), plasma estradiol level was  $28 \pm 22.3$  pmol/L, confirming women's perimenopausal status (Burger et al., 1999). This baseline level was significantly lower than under HT ( $173 \pm 36.4$  pmol/L) ( $t = 3.93, P < 0.005$ ) and did not differ from placebo level ( $20.6 \pm 0.7$  pmol/L) ( $P = 0.22$ ). As expected, when directly comparing estradiol concentrations on the two scanning days, HT increased plasma estradiol levels under HT ( $173 \pm 36.4$  pmol/L) as

compared to placebo ( $20.6 \pm 0.7$  pmol/L) (paired *t*-tests,  $P < 0.001$ ) (Fig. 3A).

We also compared the scores obtained in the nine domains assessed by the Women Health Questionnaire after 8 weeks under HT as compared to 8 weeks under placebo (Fig. 3B). These scores did not significantly differ between HT and placebo (paired *t*-test, all  $P > 0.05$ ), suggesting that larger samples neuropsychological studies are needed to ensure sufficient power to observe differences between treatment types.

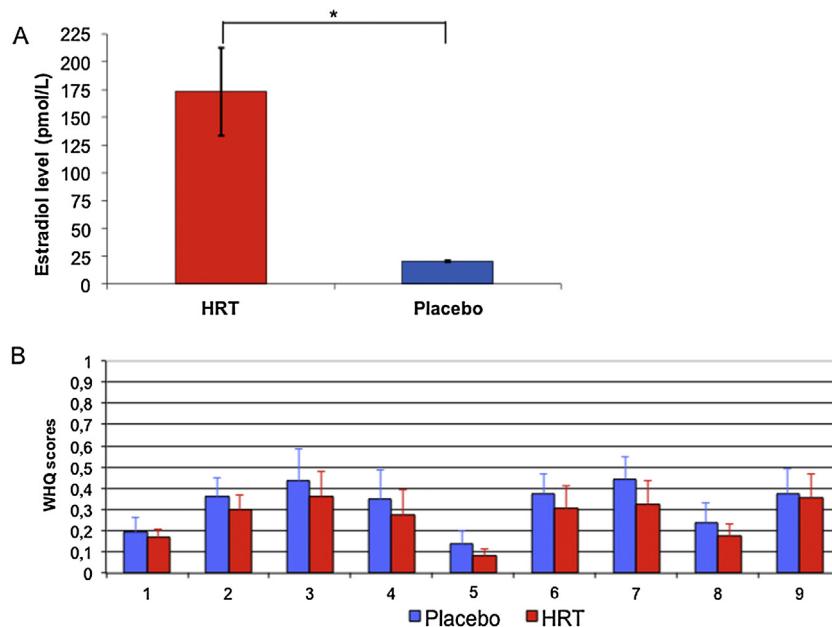
### 3.2. Behavioral results

Response times (RT) at the cue presentation were analyzed using a  $2 \times 4$  repeated-measures ANOVA that included the type of treatment (HT and Placebo) and the four types of slot machines (Supp. Fig. 1A). There was no significant RT difference between type of treatment ( $F_{(1,12)} = 1.58, P = 0.23$ ), nor between types of slot machines ( $F_{(3,36)} = 2.27, P = 0.09$ ). There was no interaction between these two factors ( $F_{(3,36)} = 0.62, P = 0.61$ ).

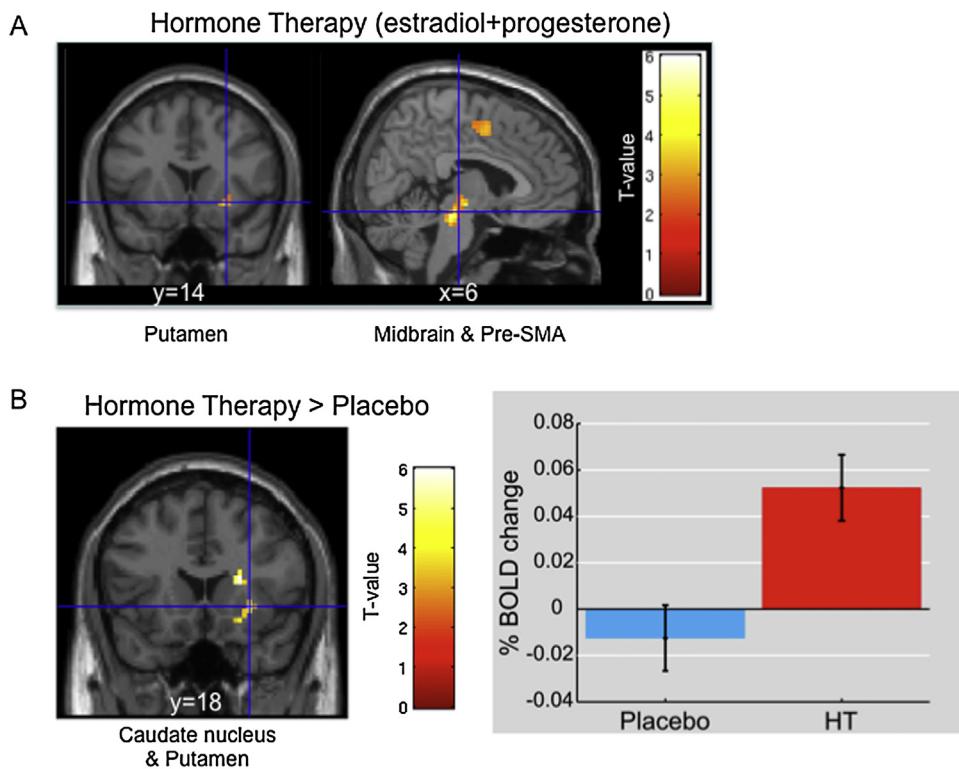
RTs at the outcome were analyzed using a  $2 \times 3 \times 2$  repeated-measures ANOVA which included the type of treatment (HT and placebo), the 3 types of potentially rewarded slot machines and their two possible outcomes (reward/no reward) (Supp. Fig. 1B). Again, there was no significant RT difference between type of treatment ( $F_{(1,10)} = 1.01, P = 0.34$ ), between types of slot machines ( $F_{(2,20)} = 0.06, P = 0.94$ ), nor between outcomes types ( $F_{(1,10)} = 0.42, P = 0.53$ ). There was no interaction between type of treatment and type of slot machines ( $F_{(2,20)} = 2.63, P = 0.09$ ), no interaction between type of slot machines and outcome ( $F_{(2,20)} = 0.24, P = 0.78$ ). However, there was an interaction between the type of treatment and outcome ( $F_{(1,10)} = 5.96, P < 0.05$ ), indicating that women were faster to respond for rewarded outcome under HT than under placebo. There was also an interaction between the 3 factors ( $F_{(2,20)} = 4.17, P < 0.05$ ), showing that the previous effect was more pronounced for slot machines with lower reward probability (i.e. when winning was less expected). These results reflect increased motivational or hedonic processing, as indexed by RTs, for less likely rewards under HT.

### 3.3. fMRI results

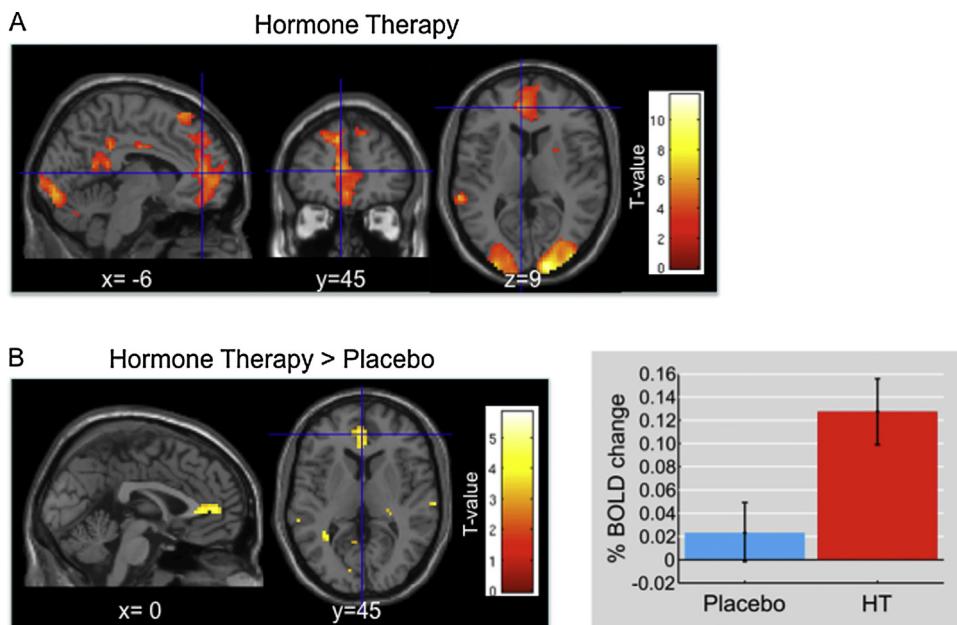
To distinguish brain response to anticipation of potential monetary rewards from the one at the time of rewarded



**Figure 3** Circulating serum estradiol levels and evaluation of women's quality of life. (A) Estradiol level (pmol/L) measured on the two days of the scanning sessions under HT and placebo. (B) Scores of the nine parameters ((1) depressed mood, (2) somatic symptoms, (3) memory/concentration, (4) vasomotor symptoms, (5) anxiety/fears, (6) sexual behavior, (7) sleep problems, (8) menstrual symptoms, (9) attractiveness) of the Women's Health Quality of life questionnaires obtained before the beginning of the study, and all along the placebo and HT period reported for all subjects after averaging their individual scores. The higher the score, the worse women felt.



**Figure 4** Significant changes in BOLD response after hormone therapy (HT) and significant difference between HT and placebo in striatal activity during reward anticipation. (A) Significant changes in putamen and midbrain BOLD responses during reward anticipation relative to sure knowledge of no-reward delivery after hormone therapy. (B) Between treatment differences (HT > placebo) in putamen and caudate nucleus activity during anticipation of potentially rewarded slot machines. The color bar indicates t-values. The graph shows the average percent signal change in a 10 mm-radius sphere centered on the peak of putamen activity during reward anticipation. For interpretation of the references to color in this figure, the reader is referred to the web version of the article. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Figure 5** Significant changes in BOLD response after hormone therapy and significant difference between HT and placebo in ventromedial prefrontal cortex activity at the time of rewarded outcome. (A) HT induced higher activity in a network including the anterior medial prefrontal cortex at the time of rewarded outcome. No significant BOLD change was observed at rewarded outcome in the anterior medial PFC after placebo administration. (B) Higher BOLD responses in the anterior medial PFC at the time of rewarded outcome after direct statistical comparison between HT and placebo. The graph shows the average percent BOLD change in a 10 mm-radius sphere centered on the peak of anterior medial PFC. For interpretation of the references to color in this figure, the reader is referred to the web version of the article. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

outcome, we measured the BOLD signal during the reward anticipation phase where "slot machine" stimuli were presented and at the time of rewarded outcome. We found that hormonal substitution increased reward-related activity in perimenopausal women. Under HT administration, reward anticipation evoked increased BOLD signal in the putamen, midbrain and pre-supplementary motor area (Fig. 4A, Table 1). In sharp contrast, under placebo, no significant putamen or midbrain activation was elicited during reward anticipation ( $P > 0.01$ , uncorrected). Direct statistical comparison between treatment types showed that HT, as compared to placebo, elicited higher putamen ( $x, y, z = 30, 18, -3$ ,  $t = 5.19$ ,  $P < 0.05$  FDR corrected) and caudate nucleus ( $x, y, z = 24, 18, 15$ ,  $t = 5.47$ ,  $P < 0.05$  FDR corrected) activities during the reward anticipation phase (Table 2). No voxel survived direct statistical comparison in the opposite comparison placebo > HT ( $P > 0.01$ ).

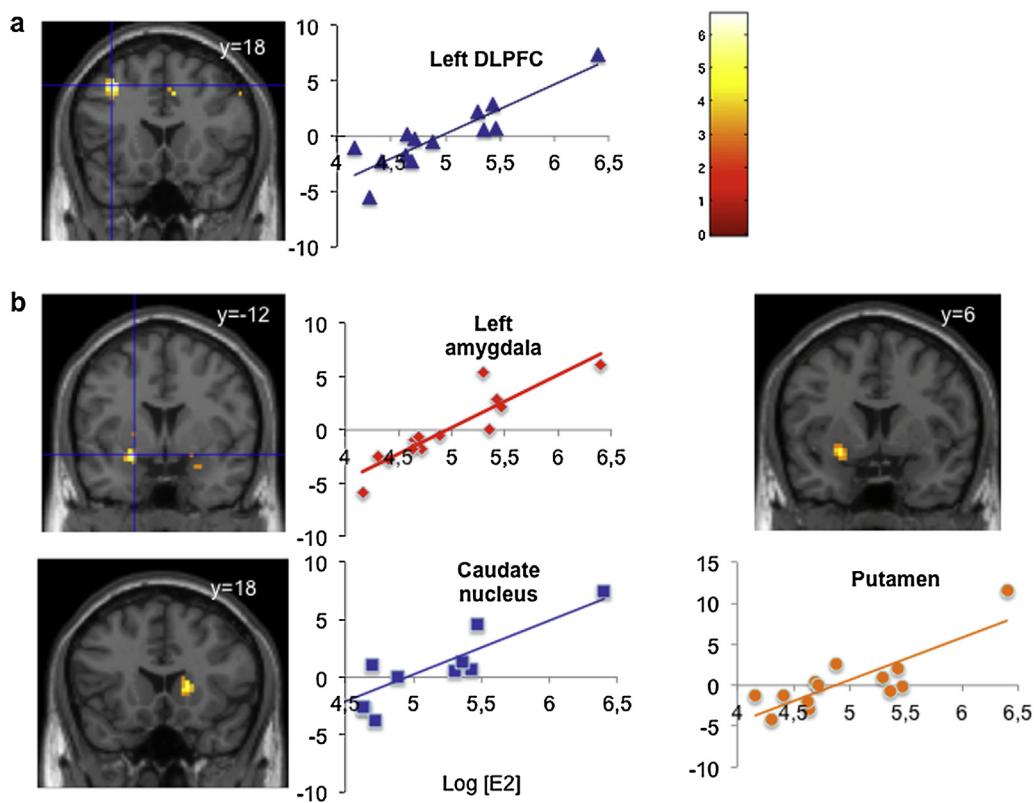
At the time of rewarded outcome, an increased BOLD response was observed in the vmPFC under HT administration (Fig. 5A, Table 1). In contrast, under placebo, rewarded outcome did not elicit significant vmPFC activation ( $P > 0.01$ , uncorrected). Direct statistical comparison between treatment types confirmed that HT, as compared to placebo, elicited higher vmPFC activity at rewarded outcome ( $x, y, z = 0, 45, 6$ ,  $t = 4.44$ ,  $P < 0.05$  FDR corrected), while no brain region was observed in the opposite comparison (Fig. 5B, Table 2).

It could be argued that the results of increased BOLD signal with HT are not specific to areas of the brain involved in reward-related activity but that there is a global increase in

brain activity because estrogen may play a vasodilator role. However, if there were a global increased brain activity due to HT, we would have observed higher BOLD response in multiple brain regions, outside of the reward system, which was not the case (see Table 2). To address this point directly, we performed a new fMRI analysis focusing on the time of the motor response (response-button held in left hand). As expected, all women engaged a network including the right motor cortex. When directly comparing HT > placebo at the time of this motor response, no motor cortex activity was observed, even at a very lenient threshold ( $P < 0.01$ , uncorrected). These results demonstrate that motor-related activations remain unaltered by the hormonal treatment in our experiment and directly indicate that our findings are not due to a general vasodilator function of estrogen, but, rather, are more specifically related to the reward circuit recruited by our task.

### 3.3.1. Correlation to estradiol levels

Finally, to pinpoint specific effects of estradiol levels on brain regions engaged during reward anticipation and at the time of rewarded outcome, we investigated the relationships between brain activation and estradiol levels in women under HT. A number of correlations were noted in regions reported in prior studies to be modulated by ovarian steroids during the menstrual cycle. During reward anticipation, positive correlation with estradiol was found in the left lateral prefrontal cortex. At time of reward delivery, a positive correlation with estradiol levels was found in the bilateral amygdalo-hippocampal complex, the



**Figure 6** Regression analysis between brain activity and estradiol levels during HT. (a) During anticipation of potential rewards, estradiol level correlated positively with activity of the left dorsolateral prefrontal cortex. (b) At the time of the rewarded outcome, estradiol level correlated positively with activity of the bilateral amygdalo-hippocampal complex, left putamen and right caudate nucleus. The graphs show these positive correlations between log estradiol levels and parameter estimates in these brain regions. For interpretation of the references to color in this figure, the reader is referred to the web version of the article. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 3** BOLD response correlating with estradiol level in the group of women under HT during reward anticipation and at the time of rewarded outcome. Abbreviations: L: left; R: right. All correlations are reported at  $P < 0.001$ , uncorrected, except \* which was only present at  $P < 0.005$ , uncorrected.

Anatomical structure	Reward anticipation (correlation with estradiol)				Rewarded outcome (correlation with estradiol)			
	MNI coordinates			Z-value	MNI coordinates			Z-value
	x	y	z		x	y	z	
Left DLPFC	-36	18	45	4.12				
Left amygdalo-hippocampal complex					-24	-12	-12	4.26
L putamen					-24	6	-12	3.52
R caudate					18	18	6	3.78
vmPFC					0	51	-6	3.32*

ventromedial prefrontal cortex, the right caudate nucleus and the left ventral putamen (Fig. 6 and Table 3).

#### 4. Discussion

The present study is the first to investigate the effects of sequential estradiol plus progesterone administration on the reward system at the end of the menopause transition. It

bridges research preclinical studies on the role of estradiol on the dopaminergic system with women epidemiological studies of HT on cognition. Taking an experimental cognitive neuroscience approach is necessary and important to understand the impact of sex-steroid hormones substitution on specific neural systems. One critical advantage of using the current crossover placebo-controlled design is that we controlled for a number of factors that have contributed to the discrepancies in the estrogen–cognition literature.

These factors include differences in the estrogen compounds used, their route of administration, HT duration, age at HT initiation, inclusion of both past and current users HT and cyclic versus continuous regimens (Shafir et al., 2012). In contrast, in our study each crossover woman serves as her own control using the exact same dose, duration and mode of administration of HT, thereby reducing the influence of these confounding factors.

Our results directly demonstrate that when HT starts early at the end of perimenopause, it increases the response of distinct components of the reward system. More specifically, we found a relative increased activity of the striatum during reward anticipation and of the anterior medial PFC at the time of rewarded outcome when HT was administrated less than a year since the last menses. This increased activity in the reward system with HT extends basic preclinical behavioral and neurochemical data demonstrating neuroregulatory effects of both estrogen and progesterone on the reward dopaminergic system (Becker et al., 2001; Jackson et al., 2006). A mechanistic account of our HT findings may be based on estrogens effects on striatal dopamine release, neuronal firing or on maintained levels of specific estradiol receptors expression at different sites (Daniel, 2013). These possible mechanisms may underlie the modulatory role of estradiol on the nigrostriatal dopaminergic system that we observed.

At the time of reward delivery, higher vmPFC activity was observed when women were taking HT as compared to placebo. This activation was associated with faster responses time (Fig. S1), possibly reflecting higher motivational or hedonic reward value coding. The vmPFC has been reported to encode the hedonic value or the experienced reward at the time of outcome (Sescousse et al., 2010). These results extend to the reward domain and the role of the vmPFC subregion, previous reports in the working memory domain showing that the dorsolateral part of the prefrontal cortex is a key structure of estrogen's effects on cognition (Berent-Spillson et al., 2010; Berman et al., 1997). In ovariectomized monkeys, estradiol treatment is associated with changes in dorsolateral prefrontal cortex structural plasticity, and reverses age-related impairment in prefrontal cognitive function (Hao et al., 2006).

Previous studies reported sex steroid hormone influences on the reward system during the menstrual cycle (Dreher et al., 2007; Ossewaarde et al., 2011; Alonso-Alonso et al., 2011; Bayer et al., 2013). Using the same reward task, we observed increased activity in the caudate nuclei and the amygdala during the midfollicular phase as compared to the luteal phase, when estradiol is unopposed by progesterone (Dreher et al., 2007). The current study extends this finding by directly showing that the absence of ovarian steroids reduces, while HT restores, reward-evoked neural activity in older women. In particular, our current study indicates that the same brain regions, including the amygdala and caudate nucleus, engaged at the time of rewarded outcome during the midfollicular phase, show positive correlations with estradiol levels in perimenopausal women under HT. These results parallel recent fMRI reports of a modulation of emotion processing circuit, including brain regions such as the amygdala, during passive viewing of negative stimuli by the hormonal cycle in young women (Protopopescu

et al., 2005; Goldstein et al., 2005; Andreano and Cahill, 2010), after progesterone administration in young women (van Wingen et al., 2008) and by hormonal treatment in postmenopausal women (Shafir et al., 2012). Thus, HT may modulate similar brain networks processing arousing stimuli around the menopause transition and during the menstrual cycle.

Our current results have implications to identify some of the neurobiological mechanisms underlying the beneficial influence of early HT on neurophysiological aspects of cognitive aging. The reward circuit is known to show reduced striatal and vmPFC BOLD response and lower number of dopaminergic receptors with aging (Dreher et al., 2008; Wong et al., 1988; Eppinger et al., 2013). Our findings that HT administrated to perimenopausal women increases reward-related activity, indicate that early initiation of HT opposes to the relative reduction of reward circuit-related activity observed in women under placebo. Our findings also help to understand how HT influences neurological and neuropsychiatric disorders known to engage a deficient dopaminergic reward system. For example, HT has been shown to play a beneficial effect on the risk of Parkinson's disease in women with natural menopause (Popat et al., 2005), and to improve Parkinson's disease symptoms (Saunders-Pullman et al., 1999). Together, our findings suggest that HT beneficial effects may occur through interactions between estradiol and the dopaminergic reward system. It should be noted that the BOLD signal does not provide direct information about dopamine transmission. Yet, recent studies have reported a positive association between genotype-dependent dopamine synaptic availability and increased BOLD signal at prefronto-striatal sites (Dreher et al., 2009; Schott et al., 2008).

Since women were scanned right after estradiol therapy and before progesterone administration, the effects we observed may primarily be attributed to estradiol effects and not to progesterone. However, it is possible that the week washout period between the first month of HT and the start of the second month of HT may not have been sufficient to prevent from interactions with progesterone. Thus, although progesterone was not the primary focus of this study and was only included to replicate natural hormone cycles, we cannot rule out the roles of progesterone and its metabolite allopregnanolone, known to affect neural and cognitive processes (Reddy, 2010). Other possible interactions include interactions between ovarian steroids and serotonin. Preclinical research in nonhuman primates suggests that 17 $\beta$ -estradiol increases tryptophan hydroxylase mRNA and protein content in the raphe (Bethea et al., 2000; Sanchez et al., 2005) and decrease 5-HT<sub>1A</sub> autoreceptor mRNA and protein expression in the raphe (Henderson and Bethea, 2008; Pecins-Thompson and Bethea, 1999). In women, positron emission tomography (PET) studies demonstrated an effect of HT on 5-HT<sub>2A</sub> receptors (Kugaya et al., 2003; Moses-Kolko et al., 2003). Furthermore, estradiol affect neuropeptide synthesis such as oxytocin, and steroid neuropeptide interactions can shift social-emotional responsiveness, especially through modulation of the amygdala (Bos et al., 2012).

Finally, our findings have implications to understand the positive impact of early HT initiation on reward-related motivational processes. There is evidence that primary

rewards (e.g. erotic stimuli) and secondary rewards engage the striatum in common (Sescousse et al., 2010, 2013). A randomized, double-blind, placebo-controlled study evaluating the effects of HT on subjective quality of life reported an increase in sexual interest and thoughts in recently postmenopausal women under HT (Maki et al., 2007). Our results suggest that this increased sex drive due to HT may be mediated by increased activity of the reward system. In the present study, we only observed a trend toward a reduction of sexual difficulties from the WHQ with HT, which may be explained by our relatively small sample size. Yet, our fMRI findings indicate that BOLD changes were more sensitive than these scores alone to detect effects of HT on reward-related brain activity.

To conclude, the current study characterizes the brain structures involved in reward processing that are modulated by early initiation of sequential estradiol plus progesterone at perimenopause. Our findings provide compelling evidence for the key role played by estradiol in the neurobiology of aging in women, indicating that HT taken at the beginning of menopause efficiently improves response of specific components of the reward system. This HT effect may modulate basic reward-related behavioral functions, such as approach behavior during reward anticipation and hedonic behavior at the time of reward delivery (Stavarache et al., 2009).

## Contributors

J.T. performed research (scanning of subjects and behavioral analysis) and wrote a first draft of the manuscript. Author E.M. and J.T. performed the fMRI statistical analysis. Author H.D. performed the hormonal measures. Author J.T. and M.P. recruited the women and M.P. assessed them clinically before inclusion in the study. Author J-C D. designed the study, wrote the protocol, managed the literature searches and wrote the manuscript. All authors contributed to and have approved the final manuscript.

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## Conflict of interest statement

The authors report no conflict of interest and no financial relationship with commercial interests.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2014.08.012>.

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