doi:10.1093/scan/nsq071 SCAN (2011) 6, 612–620

Changes in functioning of mesolimbic incentive processing circuits during the premenstrual phase

Lindsey Ossewaarde,[1] Guido A. van Wingen,[1,2] Sabine C. Kooijman,[1] Torbjörn Bäckström,[3] Guillén Fernández,[1,2] and Erno J. Hermans[1,2]

[1]Radboud University Nijmegen, Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Nijmegen, The Netherlands, [2]Radboud University Nijmegen Medical Centre, Department of Neurology, Nijmegen, The Netherlands, and [3]Umeå Neurosteroid Research Center, Department of Clinical Science, Obstetrics and Gynecology, Norrlands University Hospital, Umeå, Sweden

The premenstrual phase of the menstrual cycle is associated with marked changes in normal and abnormal motivated behaviors. Animal studies suggest that such effects may result from actions of gonadal hormones on the mesolimbic dopamine (DA) system. We therefore investigated premenstrual changes in reward-related neural activity in terminal regions of the DA system in humans. Twenty-eight healthy young women underwent functional magnetic resonance imaging on 2 days during the menstrual cycle, once during the late follicular phase and once during the premenstrual phase, in counterbalanced order. Using a modified version of the monetary incentive delay task, we assessed responsiveness of the ventral striatum to reward anticipation. Our results show enhanced ventral striatal responses during the premenstrual as compared to the follicular phase. Moreover, this effect was most pronounced in women reporting more premenstrual symptoms. These findings provide support for the notion that changes in functioning of mesolimbic incentive processing circuits may underlie premenstrual changes in motivated behaviors. Notably, increases in reward-cue responsiveness have previously been associated with DA withdrawal states. Our findings therefore suggest that the sharp decline of gonadal hormone levels in the premenstrual phase may trigger a similar withdrawal-like state.

Keywords: menstrual cycle; motivated behavior; reward anticipation; ventral striatum; fMRI

INTRODUCTION

The premenstrual phase of the menstrual cycle, when progesterone and estradiol levels decline after a relatively stable period with high levels, is associated with marked changes in normal and abnormal motivated behaviors. For example, increases have been shown of food cravings (Tomelleri and Grunewald, 1987; Dye *et al.*, 1995) and excessive cleaning (Dillon and Brooks, 1992) in healthy women during the premenstrual phase. Such symptoms are aggravated in women with premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) (York *et al.*, 1989; Reed *et al.*, 2008). Moreover, women with obsessive compulsive disorder (OCD) and nicotine and cocaine abusers show an exacerbation of compulsive and craving symptoms, respectively, in the premenstrual phase (Allen *et al.*, 2000; Evans *et al.*, 2002; Franklin *et al.*, 2004; Vulink *et al.*, 2006). Because such motivated behaviors are associated with dopaminergic (DA) functioning, these behavioral changes are thought to arise from effects of gonadal hormone fluctuations on the mesolimbic DA system (Chen *et al.*, 1996; Thompson and Moss, 1997; Reddy and Kulkarni, 1998, 1999). Previous human neuroimaging studies have shown menstrual cycle dependent (Fernández *et al.*, 2003; Goldstein *et al.*, 2005; Protopopescu *et al.*, 2005; Dreher *et al.*, 2007; Weis *et al.*, 2008) and exogenous gonadal hormone related changes in brain function (Hermans *et al.*, 2008; van Wingen *et al.*, 2008 and 2009; Hermans *et al.*, 2010). However, neural mechanisms in the premenstrual phase potentially underlying the change in motivated behaviors during this phase have never been investigated in humans.

A substantial body of animal research has shown that gonadal hormones exert actions on the mesolimbic DA system (Kuppers *et al.*, 2000; Mani, 2006). Estrogen and progesteron receptors are expressed in the nucleus accumbens (NAcc), a terminal region of the mesolimbic DA system (Roy *et al.*, 1986; Shughrue *et al.*, 1998; McEwen, 2002; Mani, 2008). Both progesterone and estrogens have been shown to increase DA turnover in this region (Di Paolo *et al.*, 1985; Bazzett and Becker, 1994; Di Paolo, 1994; Thompson and Moss, 1994; Petitclerc *et al.*, 1995; Le Saux *et al.*, 2006; Larson and Carroll, 2007). The striatum moreover exhibits high accumulations of neuroactive metabolites of progesterone such as allopregnanolone, which are allosteric modulators of the γ-aminobutyric acid type A (GABA-A) receptor (Bixo *et al.*, 1986; Wang *et al.*, 1995). The decline in gonadal hormone levels in the premenstrual phase may therefore explain alterations in DA-related motivated behaviors. In support of this notion, animal studies have found that experimentally induced hormone fluctuations can result in

Received 9 April 2010; Accepted 28 June 2010
Advance Access publication 3 September 2010
This work was supported by grant numbers 918.66.613 and 451.07.019 from the Netherlands Organization for Scientific Research (NWO) and grant no. 14x-11198 from the Swedish research council.

Correspondence should be addressed to Lindsey Ossewaarde, Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Radboud University Nijmegen, P.O. Box 9101 6500 HB Nijmegen, The Netherlands. E-mail: lindsey.ossewaarde@donders.ru.nl

concomitant increases in DA-related motivated behaviors such as compulsions (Flaisher-Grinberg *et al.*, 2009).

Human studies using blood oxygenation level dependent functional magnetic resonance imaging (BOLD-fMRI) have repeatedly demonstrated reward-related neural activity in a set of regions that are innervated by DA projections from the mesolimbic pathway (Knutson and Cooper, 2005; McBride *et al.*, 2006). BOLD responses in the ventral striatum (the approximate location of the NAcc) have been shown to be particularly strong during reward anticipation (Knutson *et al.*, 2001a and b), and when instrumental responses are required to attain rewards (Bjork and Hommer, 2007). Moreover, responses in this region to food-related stimuli have been shown to be increased when participants are in a state of hunger (LaBar *et al.*, 2001). Increased ventral striatal BOLD responses to drug-related cues have been associated with craving and drug withdrawal (Kilts *et al.*, 2001, 2004; Rolls and McCabe, 2007; Wrase *et al.*, 2007), although opposite effects have also been observed for non-drug related reward cues (Wrase *et al.*, 2007; van Hell *et al.*, 2010). Based on behavioral findings described above, we expected to find a more generally increased reward sensitivity in women in the premenstrual phase, and therefore predicted that ventral striatal activation during anticipation of monetary rewards would be enhanced in the premenstrual phase. In addition, we explored whether cycle-dependent changes in ventral striatal activity would be associated with inter-individual differences in neuroactive steroid fluctuations and self-report measures of premenstrual symptoms.

To address these questions, 28 healthy women underwent fMRI once in the premenstrual phase and once in the late follicular phase of the menstrual cycle in a counterbalanced crossover design. Women were scanned in these particular menstrual cycle phases because PMS and PMDD patients are most symptomatic in the premenstrual phase and are least symptomatic in the late follicular phase (Sveindottir and Bäckström, 2000). In the MRI scanner, participants performed a modified version of the monetary incentive delay (MID) task (Knutson *et al.*, 2001a) in which cues were presented that either signaled trials that were potentially rewarding or non-rewarding. Interindividual differences in premenstrual symptoms were assessed using the Moos Menstrual Distress Questionnaire (MDQ; Moos, 1968). Additionally, we collected saliva for measuring neuroactive steroid levels (allopregnanolone). For menstrual cycle phase verification, we used ovulation predictor kits and obtained self-reports of the onset of menstruation before and after test sessions.

MATERIALS AND METHODS

Participants

Twenty-eight healthy right-handed women (mean age: 22.8 years, range: 18–38 years) participated in this study. None of the women had used hormonal contraceptives for the previous 3 months and all had regular menstrual cycles (26–32 days over the past year). Women were excluded when they consumed more than three alcoholic beverages per day on average and smoked more than 20 cigarettes per week. In addition, they were asked not to consume alcohol 24 h prior to the experiment or to use psychoactive substances for 72 h. They had no history of psychiatric disorders (as determined with the Mini International Neuropsychiatric Interview, M.I.N.I) (Sheehan *et al.*, 1998) and were additionally screened specifically for PMDD or PMS using the Dutch version of the MDQ (Moos, 1968). All women were physically healthy and reported no MRI contraindications. Data of one woman were excluded from data analysis due to excessive head movement during scanning. The study was approved by the local ethical review board (CMO Region Arnhem-Nijmegen, The Netherlands) and all women provided written informed consent.

Design and procedure

Women were tested in a crossover design with the counterbalanced factors menstrual cycle phase (late follicular *vs* premenstrual) and condition (potentially rewarding *vs* non-rewarding) as within subject factors. During a screening day prior to scanning sessions, subjects completed the MINI (Sheehan *et al.*, 1998) and the MDQ (Moos, 1968). Each woman was scanned in the afternoon or evening once during the late follicular phase and once during the premenstrual phase. Time of day was kept equal for both test sessions (within participants). Timing of test sessions within the menstrual cycle phase was ascertained as follows. For the late follicular phase, test sessions were planned between Days 8 and 12 (mean time point of test session: Day 10, s.d.: 1 day) with respect to the first day of the menstrual cycle (i.e. start of menstruation). To determine the premenstrual phase, two time points were used. First, participants contacted the experimenter when they had a positive result from their LH-peak assessments (as determined using commercially available ovulation predictor kits; Wondfo Biotech, Guangzhou, China). Subsequently, an appointment was made for premenstrual scanning ~14 days after the start of ovulation. To ascertain that this test session took place in the premenstrual phase, participants were asked to contact the experimenter again when their next menstruation started (mean time point of test session: 2 days before menstruation started, s.d.: 1 day). Menstrual cycle phases could further be verified retrospectively using salivary neuroactive steroid concentrations (see below).

On testing days, participants arrived between 2 and 7 pm. Approximately 1.5 h before scanning, subjects practiced the MID task outside the scanner. In order to measure salivary concentrations of allopregnanolone, 10 ml of saliva was collected at the beginning of each test session (5 min after arrival). Saliva was collected in plastic tubes and kept frozen at −20°C. Participants lay in the scanner in a supine position. During and between preparation scans, participants performed a training run of the MID.

MID task

This task was based on the MID task developed by Knutson *et al.* (2001) and consisted of 25 potentially rewarding trials, 25 non-rewarding trials and 25 periods of low-level fixation with a mean duration equal to trials. In total, trials lasted between 8.5 and 11.5 s (mean 10 s). Thus, the total duration of the task was 12.5 min. At the beginning of each trial, a cue (cue duration: 3.5–8.5 s; mean: 6 s) was presented signaling a potentially rewarding (red square) or non-rewarding (green square) trial. Following this cue, a target was presented to which subjects had to respond as fast as possible (by pressing a button) irrespective of the cue type. When the button was pushed within the presentation time of the circle, the target remained on the screen, thus providing the participant with feedback that the target was hit. Otherwise, it disappeared. When the target was hit in a rewarding trial, they earned one euro. After disappearance of the target (duration: 1.2–5.3 s; mean 3.25 s), short feedback was provided (500 ms) of the current cumulative gain. To ascertain that reward outcome was similar across participants and sessions, the target duration was variable (150–500 ms) and shortened with 20 ms for the subsequent trial when the previous target was hit. The target duration was lengthened with 10 ms in the subsequent trial when the previous target was missed. This procedure results in a hit rate of ~33% on average, ensuring that all participants won approximately the same amount of money (between 8 and 11 euros). Prior to the experiment, practice trials were presented outside and inside the scanner to familiarize the participants with the task. They were required to hit the target in five and three potentially rewarding trials, respectively, before procedures continued. This task was performed twice, once during the late follicular phase and once during the premenstrual phase.

MR data acquisition

MRI scans were collected using a Siemens (Erlangen, Germany) TIM Trio 3.0 T MRI scanner equipped with an eight channel phased array head coil. The following scans were obtained: two runs of 402 T2* weighted BOLD images each (gradient echo EPI, TE/TR: 25/1890 ms, flip angle 80°, FOV: 212 × 212 mm, matrix 64 × 64, 3-mm slice thickness, 0.3 mm slice gap, 37 ascending slices. To reduce signal drop-out and geometric distortions, we used a short TE, an oblique axial angulation (de Zwart, 2006), and reduced echo-train length by means of factor 2 accelerated GRAPPA (Griswold *et al.*, 2002). Structural scans were obtained using an MP-RAGE sequence (TE/TR: 2.96/2300 ms, flip angle: 8°, FOV: 256 × 256 × 192 mm, voxel size: 1 mm isotropic, GRAPPA acceleration factor 2).

Analysis of behavioral performance

For each participant, reaction time (RT) data of responses to targets during the modified MID task were filtered by removing values outside the 150–1000 ms range. In addition, those RTs exceeding 3 s.d's from the mean were removed. Furthermore, one participant, who had mean RTs > 3 s.d. from the group average, was excluded from further RT analyses. RTs were analysed using a repeated measures ANOVA with menstrual cycle phase and reward condition as within-subject factors. Alpha was set at 0.05. As a result of the adaptive procedure adjusting the target presentation time (see above), the total amount of monetary gain in the MID task does not reflect performance.

Analysis of fMRI data

fMRI data were analysed using Statistical Parametric Mapping software (SPM5; Wellcome Department of Imaging Neuroscience, London). To allow for T1 equilibration, the first five EPI-volumes of each run were discarded. The remaining images were realigned to the first volume, slice time corrected, coregistered to the structural MR image, spatially normalized to standard Montreal Neurological Institute (MNI) 152 coordinate space, resampled into $2 \times 2 \times 2\,mm^3$ voxels, and smoothed with an isotropic 8-mm full-width half maximum Gaussian kernel.

Statistical analysis was performed within the framework of the general linear model. For each of the two runs, the rewarding and non-rewarding trials were modeled as separate regressors in an event-related manner for the duration of the anticipation cue (i.e. between 3.5 and 8.5 s for both the red and the green square). Subsequently, these regressors were convolved with the canonical hemodynamic response function implemented in SPM5. The six parameters corresponding with head movement obtained from the realignment procedure were also included in the model for both sessions, as well as a high-pass filter with a 1/128 Hz cut-off frequency. We applied proportional global signal scaling to reduce effects due to global signal variations between scan sessions. The single subject parameter estimates of each session and condition obtained from the first level analysis were included into subsequent second level analyses treating subjects as random variables. A repeated measures ANOVA was used, with menstrual cycle phase (late follicular *vs* late premenstrual) and reward condition (potentially rewarding *vs* non-rewarding) as within-subject factors, with non-sphericity corrections for repeated measures. For our main region of interest (NAcc), a small volume correction (SVC) was used that was based on an anatomical mask of this region. This mask was created as follows: bilateral NAcc was delineated in T1 weighted scans of 60 separate individuals (21 males, 39 females; mean age: 21.9 years, age range: 18–38 years) using an automated segmentation procedure as implemented in FSL FIRST (see http://www.fmrib.ox.ac.uk/fsl/first/index.html). Subsequently, all T1-weighted images were normalized into MNI152 space using SPM5 as described earlier, and the same transformation was applied to all segmented images. After visual inspection, all segmented images (boolean maps) were averaged, resulting in a probability mask. This mask was thresholded at a

probability of 0.75 (total volume: 2179 mm^3). Given the uncertainties in exact localization of a small area such as the NAcc, we refer to the region covered by this ROI mask with the more cautious term ventral striatum. A FWE correction for multiple comparisons across the entire brain was used for all other brain regions.

For additional correlation analyses with allopregnanolone changes across the menstrual cycle and MDQ scores, contrast estimates reflecting the premenstrual increase in reward-related responses were extracted from the largest ventral striatal cluster (consisting of 16 voxels in the left ventral striatum) in the interaction effect (peak voxel at −6, 20, −4; 'Results' section). The MDQ consists of eight different subscales (pain, water resistance, autonomic reactions, negative affect, impaired concentration, behavioral changes, arousal and control), and for every subscale a score for three cycle phases (during menstruation, premenstrual and rest of the cycle) was obtained. For every subscale, we calculated a difference score reflecting premenstrual symptoms (premenstrual *vs* rest of the cycle). Subsequently, by taking the sum across all subscales, a total MDQ difference score was calculated, which was used for the correlational analysis. A similar analysis was performed with the difference in allopregnanolone levels between the late follicular and the premenstrual phase.

Gonadal steroid analysis

Saliva samples were collected to assess levels of neuroactive steroids (allopregnanolone). The sampling procedure and assay of salivary allopregnanolone has been detailed previously (Ossewaarde *et al.*, 2010). The samples were thawed and allopregnanolone extracted with diethyl ether. A separation of cross reacting steroids was made with high performance liquid chromatography and thereafter a radioimmunoassay (RIA) was made using an allopregnanolone antiserum kindly provided by Dr R.H. Purdy, Department of Physiology and Pharmacology, the Scripps Research Institute, La Jolla, CA, USA (Purdy *et al.*, 1990) and [11,12] ^{3}H-allopregnanolone (Perkin Elmer Life Sciences, Boston, USA). The sensitivity of the assay was 19 pg. The recovery of allopregnanolone was calculated by using spiked saliva samples and was mean 76.2% range (62–92%) and the results are compensated for recovery. The intraassay coefficient of variation was 21% in these saliva samples. Due to the experimental nature and novelty of the allopregnanolone assessment based on saliva, the entire samples were used for this analysis. Note, however, that progesterone and allopregnanolone levels are strongly positively correlated with each (Wang *et al.*, 1996).

RESULTS

Allopregnanolone assessment

The saliva concentrations of allopregnanolone were higher in the premenstrual phase (mean ± s.d., 0.050 nmol/l ± 0.016) than the late follicular phase (mean ± s.d., 0.035 nmol/l ± 0.010) ($t_{25} = 4.67$, $P < 0.001$). Kolmogorov–Smirnov tests confirmed that the distributions of allopregnanolone concentrations in the two cycle phases did not deviate from a normal distribution (both $Z < 1$). Moreover, distributions contained no outliers. Note that levels of allopregnanolone are tightly linked to levels of progesterone (Wang *et al.*, 1996). Although estradiol and/or progesterone levels are more commonly used for confirmation of menstrual cycle phase, these data therefore do indicate that participants were tested during the intended menstrual cycle phase.

MID Task, RTs

A repeated-measures ANOVA with reward condition (potentially rewarding *vs* non-rewarding) and menstrual cycle phase (late follicular *vs* premenstrual) as within subject factors revealed a main effect of reward condition [$F(1,25) = 61.50$, $P < 0.001$], indicating that responses to the target during potentially rewarding trials were faster than to non-rewarding trials (mean ± s.d. in ms, premenstrual phase: potentially rewarding 227 ± 20; non-rewarding 261 ± 30, late follicular phase: reward 228 ± 21; non-reward 259 ± 27). There was neither a main effect of menstrual cycle phase [$F(1,25) = 0.56$, *ns*], nor an interaction between menstrual cycle phase and reward condition [$F(1,25) = 0.21$, *ns*]. The adaptive reinforcement schedule indeed resulted in a rewarding outcome in ~33% of potentially rewarding trials: observed mean percentages of hits (and s.d.) were 39.2% (4.9%) and 35.9% (6.4%) for potentially rewarding and non-rewarding conditions, respectively.

Functional MRI results

fMRI data were analysed with a second-level repeated measures ANOVA with reward condition (potentially rewarding *vs* non-rewarding) and menstrual cycle phase (late follicular *vs* premenstrual) as within subject factors, using SVCs for our main region of interest: the ventral striatum ('Materials and methods' section). The main effect of reward condition showed expected activations in ventral and dorsal striatum, midbrain, parietal regions, insula and anterior cingulate gyrus, confirming previous observations (Knutson *et al.*, 2001a, b) (Table 1). Next, we investigated whether reward condition effects differed between the two menstrual cycle phases. A significant interaction between reward condition and menstrual cycle phase was observed in the ventral striatum ($P = 0.015$, SVC), with larger reward condition effects in the premenstrual phase (Figure 1A, Table 1). Separate tests for the premenstrual and late follicular phases nonetheless showed robust reward condition effects in the ventral striatum in both menstrual cycle phases (both $P < 0.001$, SVC; Figure 1B and C, Table 1). No main effects were found of the factor menstrual cycle phase in the ventral striatum ($P > 0.05$, SVC) or other reward-related brain areas

Table 1 Local maxima for significant areas of activation for the main and simple effects, and menstrual cycle phase × reward interaction

	Side	*x*	*y*	*z*	*T*-value
Main effect of reward condition: reward > non-reward					
Ventral striatum	R	8	8	−4	7.64**
Ventral striatum	L	−8	6	−4	9.01**
Supplementary motor area	R/L	4	6	54	9.35***
Cerebellum	L	−38	−54	−30	8.87***
Cerebellum	R	30	−58	−26	6.95***
Insula	R	46	20	−8	6.92***
Insula	L	−32	28	0	6.76***
Primary motor cortex	R	52	−2	50	5.58***
Primary motor cortex	L	−46	−6	58	5.87***
Parahippocampal gyrus	R	14	−24	−12	5.67***
Midbrain	R/L	0	−18	−18	5.54***
Mid-cingulate gyrus	R	16	−28	44	5.44***
Hippocampus	L	−22	−24	−10	5.00***
Middle frontal gyrus	R	34	42	26	5.13***
Middle frontal gyrus	L	−34	46	24	4.95***
Menstrual cycle phase × reward interaction: premenstrual > late follicular					
Ventral striatum	R	8	20	−2	3.54*
Ventral striatum	L	−8	22	−2	3.48*
Premenstrual reward > premenstrual non-reward					
Ventral striatum	R	10	14	−6	6.97**
Ventral striatum	L	−8	8	−4	7.46**
Supplementary motor area	R/L	4	8	50	7.51***
Cerebellum	L	−38	−54	−30	7.76***
Thalamus	L	−6	−4	6	8.02***
Hippocampus	R	16	4	−14	5.43***
Hippocampus	L	−16	−6	−10	5.47***
Insula	R	46	20	−6	6.07***
Insula	L	−32	28	−2	5.79***
Middle frontal gyrus	R	34	40	26	5.01***
Mid-cingulate gyrus	R	16	−30	46	4.97***
Follicular reward > follicular non-reward					
Ventral striatum	R	8	8	−4	5.94**
Ventral striatum	L	−10	6	−6	7.28**
Supplementary motor area	R/L	4	6	54	7.63***
Cerebellum	R	32	−56	−28	5.16***
Cerebellum	L	−36	−52	−32	7.20***
Insula	R	32	24	−8	6.13***
Insula	L	46	20	−6	6.07***
Midbrain	R/L	2	−18	−18	5.75***
Parahippocampal gyrus	R	12	−22	−14	5.46***
Temporal pole	R	60	10	2	5.01***

*$P < 0.05$, FWE rate corrected for the reduced search volume (ventral striatum).
**$P < 0.001$, FWE rate corrected for the reduced search volume (ventral striatum).
***$P < 0.05$, FWE rate corrected for the entire brain.
R, right; L, left.

(all $P > 0.001$, uncorrected). Thus, these findings support our hypothesis of enhanced ventral striatal reward-related activity in the premenstrual phase.

Additionally, we investigated the relationship between menstrual cycle related changes in ventral striatal activity and individual differences in the sensitivity to gonadal hormone changes as reflected by allopregnanolone changes and the total MDQ difference score (displaying premenstrual symptoms as compared to symptoms during the rest of the cycle). Reward-related activation (potentially rewarding *vs* non-rewarding activity) in the ventral striatum during the late follicular phase was subtracted from activation during the premenstrual phase, yielding an estimate of the interaction effect described earlier. Although there was no significant correlation with menstrual cycle related changes in allopregnanolone levels ($P > 0.05$), results showed a positive correlation between the interaction effect and total MDQ difference scores [$r(27) = 0.46$, $P < 0.05$]. This indicates that women with the highest total MDQ difference scores had the largest premenstrual increase in ventral striatal activity. When exploring the different subscales, negative affect [$r(27) = 0.42$, $P < 0.05$], behavioral changes [$r(27) = 0.45$, $P < 0.05$] and control [$r(27) = 0.54$, $P < 0.01$] showed a significant positive association with the menstrual cycle related

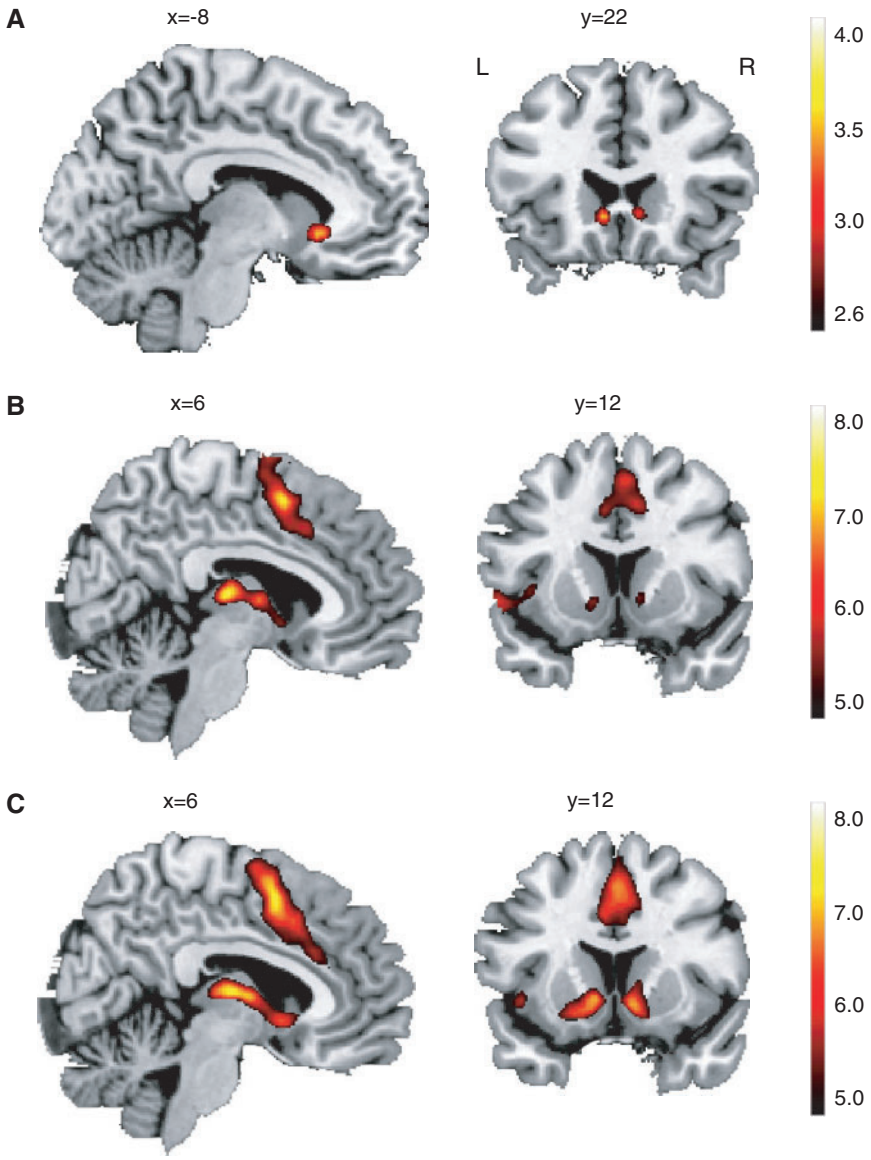

Fig. 1 Statistical brain activation T-maps showing menstrual cycle phase by reward interaction, and simple effects. (**A**) Menstrual cycle phase by reward interaction ($P < 0.001$, uncorrected, for visualization purposes). (**B**) Reward activity during the late follicular phase ($P < 0.05$, whole brain FWE corrected). (**C**) Reward activity during the premenstrual phase ($P < 0.05$, whole brain FWE corrected). R, right side of the brain; L, left side of the brain; FWE, family wise error.

change in ventral striatal reward activity. The subscales pain, water resistance, autonomic reactions and arousal did not reach the statistical threshold (all $P > 0.05$).

DISCUSSION

The aim of the present study was to identify potential neural mechanisms underlying changes in motivated behaviors across the menstrual cycle. As hypothesized, we observed premenstrual increases in reward-related BOLD responses in the ventral striatum, a target region of the mesolimbic DA system. Notably, this premenstrual increase in ventral striatal activity was most pronounced in women who reported more premenstrual symptoms. Together, these findings support the notion that dysregulations of dopaminergic incentive processing circuits underlie premenstrual symptoms related to changes in motivated behaviors.

The observed increase of reward-related responses in the ventral striatum during the premenstrual phase can be explained by two possible neurobiological mechanisms. First, the observed findings may be caused by a delayed effect of changes in absolute levels of gonadal hormones across the menstrual cycle. In line with this notion, serum progesterone concentrations robustly predict negative symptom severity in women with PMS with a delay of ~3–4 days (Wang *et al.*, 1996). These patients moreover exhibit recurrent food cravings which are related to the steroid and mood state fluctuations across the menstrual cycle (Dye *et al.*, 1995; Dye and Blundell, 1997). Furthermore, it has been shown that especially hedonic intake of palatable food is increased by

administration of gonadal steroids, suggesting direct effects on reward seeking (Chen *et al*., 1996; Reddy and Kulkarni, 1998, 1999). Second, premenstrual increases in ventral striatal responsiveness may be caused by the 'decline' of gonadal hormone levels during the premenstrual phase. Because gonadal hormones are known to potentiate DA release (Di Paolo *et al*., 1985; Bazzett and Becker, 1994; Di Paolo, 1994; Thompson and Moss, 1994; Petitclerc *et al*., 1995; Le Saux *et al*., 2006; Larson and Carroll, 2007), their decline in the premenstrual phase after a period of increased levels may prompt a down-regulation of endogenous DA activity and thus mimic a withdrawal state.

Previous studies have shown that dopaminergic withdrawal states are associated with enhanced cue-induced DA release, which is thought to attribute 'incentive salience', or motivational value, to drug or reward associated stimuli (Robinson and Berridge, 1993). Notably, in humans, such withdrawal states appear to prompt changes in motivated behavior and its neural substrates that are analogous to the findings reported here, with enhanced ventral striatal responsiveness to food or drug cues (Kilts *et al*., 2001; Rolls and McCabe, 2007). Therefore, increased ventral striatal responsiveness in the premenstrual phase may result from a similar DA withdrawal state that is caused by the abrupt decline in gonadal hormone levels. To corroborate this notion, future research in humans should investigate whether the present findings generalize to other periods of gonadal hormone decline, such as postpartum or (peri) menopausal periods. Furthermore, effects of controlled hormone withdrawal can be investigated either through termination of hormone replacement therapy or hormonal contraception, or through pharmacological gonadal suppression using administration of gonadotropin-releasing hormone agonists such as leuprolide. Such studies may shed new light on potential gonadal hormone withdrawal effects both on neuronal and behavioral levels.

An important limitation of the present study is that the BOLD-fMRI signal in the ventral striatum cannot provide direct information about dopaminergic neurotransmission. However, a recent fMRI study has shown that BOLD responses during reward anticipation in the ventral striatum correlate positively with DA release (Schott *et al*., 2008). It has been suggested that the ventral striatal BOLD signal reflects changes in postsynaptic D1-receptor agonism (Knutson and Gibbs, 2007). Pharmacological fMRI studies have indeed shown that DA agonists induce ventral striatal BOLD responses (Breiter *et al*., 1997; Vollm *et al*., 2004). Although this suggests that the ventral striatal BOLD signal is related to DA function, our findings do not necessarily represent elevated DA release specifically. Moreover, conclusions regarding the specificity of our finding to reward anticipation are limited by the fact that our task only included trials with anticipated rewards and non-rewarding control trials.

In conclusion, the present study demonstrated enhanced reward-related neural responses in the ventral striatum during the premenstrual phase. These findings provide support for the notion that changes in functioning of mesolimbic incentive processing circuits may underlie premenstrual increases in normal and abnormal motivated behaviors such as food and drug cravings. These effects are likely caused by the sudden decrease in gonadal hormone levels during the premenstrual phase and may thus reflect a withdrawal-like state. The results of the present study may generalize to other periods of instability in the gonadal hormone milieu and may therefore provide novel insights into the pathophysiology of psychiatric disorders associated with gonadal hormonal fluctuations.

REFERENCES

Allen, S.S., Hatsukami, D., Christianson, D., Brown, S. (2000). Effects of transdermal nicotine on craving, withdrawal and premenstrual symptomatology in short-term smoking abstinence during different phases of the menstrual cycle. *Nicotine Tobacco Research*, *2*, 231–41.

Bazzett, T.J., Becker, J.B. (1994). Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. *Brain Research*, *637*, 163–72.

Bixo, M., Bäckström, T., Winblad, B., Selstam, G., Andersson, A. (1986). Comparison between pre- and postovulatory distributions of oestradiol and progesterone in the brain of the PMSG-treated rat. *Acta Physiologica Scandinavica*, *128*, 241–6.

Bjork, J.M., Hommer, D.W. (2007). Anticipating instrumentally obtained and passively-received rewards: a factorial fMRI investigation. *Behavioural Brain Research*, *177*, 165–70.

Breiter, H.C., Gollub, R.L., Weisskoff, R.M., et al. (1997). Acute effects of cocaine on human brain activity and emotion. *Neuron*, *19*, 591–611.

Chen, S.W., Rodriguez, L., Davies, M.F., Loew, G.H. (1996). The hyperphagic effect of 3 alpha-hydroxylated pregnane steroids in male rats. *Pharmacology, Biochemistry and Behavior*, *53*, 777–82.

de Zwart, J.A., van Gelderen, P., Golay, X., Ikonomidou, V.N., Duyn, J.H (2006). Accelerated parallel imaging for functional imaging of the human brain. *NMR Biomed*, *19*, 342–51.

Di Paolo, T. (1994). Modulation of brain dopamine transmission by sex steroids. *Reviews in the Neurosciences*, *5*, 27–41.

Di Paolo, T., Rouillard, C., Bedard, P. (1985). 17 beta-Estradiol at a physiological dose acutely increases dopamine turnover in rat brain. *European Journal of Pharmacology*, *117*, 197–203.

Dillon, K.M., Brooks, D. (1992). Unusual cleaning behavior in the luteal phase. *Psychological Reports*, *70*, 35–9.

Dreher, J.C., Schmidt, P.J., Kohn, P., et al. (2007). Menstrual cycle phase modulates reward-related neural function in women. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 2465–70.

Dye, L., Blundell, J.E. (1997). Menstrual cycle and appetite control: implications for weight regulation. *Human Reproduction*, *12*, 1142–51.

Dye, L., Warner, P., Bancroft, J. (1995). Food craving during the menstrual cycle and its relationship to stress, happiness of relationship and depression; a preliminary enquiry. *Journal of Affective Disorders*, *34*, 157–64.

Evans, S.M., Haney, M., Foltin, R.W. (2002). The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology*, *159*, 397–406.

Fernández, G., Weis, S., Stoffel-Wagner, B., et al. (2003). Menstrual cycle-dependent neural plasticity in the adult human brain is hormone, task, and region specific. *Journal of Neuroscience*, *23*, 3790–5.

Flaisher-Grinberg, S., Albelda, N., Gitter, L., et al. (2009). Ovarian hormones modulate 'compulsive' lever-pressing in female rats. *Hormones and Behavior*, *55*, 356–65.

Franklin, T.R., Napier, K., Ehrman, R., et al. (2004). Retrospective study: influence of menstrual cycle on cue-induced cigarette craving. *Nicotine Tobacco Research*, *6*, 171–5.

Goldstein, J.M., Jerram, M., Poldrack, R., et al. (2005). Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. *Journal of Neuroscience*, *25*, 9309–16.

Griswold, M.A., Jakob, P., Heidemann, R., et al. (2002). Generalized autocalibrating partially parallel acquisitions (GRAPPA). *Magnetic Resonance in Medicine*, *47*, 1202–10.

Hermans, E.J., Ramsey, N.F., van Honk, J. (2008). Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. *Biological Psychiatry*, *63*, 263–70.

Hermans, E.J., Bos, P.A., Ossewaarde, L., et al. (2010). Effects of exogenous testosterone on the ventral striatal BOLD response during reward anticipation in healthy women. *Neuroimage*, *52*, 277–83.

Kilts, C.D., Gross, R.E., Ely, T.D., Drexler, K.P. (2004). The neural correlates of cue-induced craving in cocaine-dependent women. *American Journal of Psychiatry*, *161*, 233–41.

Kilts, C.D., Schweitzer, J.B., Quinn, C.K., et al. (2001). Neural activity related to drug craving in cocaine addiction. *Archives of General Psychiatry*, *58*, 334–41.

Knutson, B., Adams, C.M., Fong, G.W., Hommer, D. (2001a). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *Journal of Neuroscience*, *21*, RC159.

Knutson, B., Cooper, J.C. (2005). Functional magnetic resonance imaging of reward prediction. *Current Opinion in Neurology*, *18*, 411–7.

Knutson, B., Fong, G.W., Adams, C.M., Varner, J.L., Hommer, D. (2001b). Dissociation of reward anticipation and outcome with event-related fMRI. *Neuroreport*, *12*, 3683–7.

Knutson, B., Gibbs, S.E. (2007). Linking nucleus accumbens dopamine and blood oxygenation. *Psychopharmacology*, *191*, 813–22.

Kuppers, E., Ivanova, T., Karolczak, M., Beyer, C. (2000). Estrogen: a multifunctional messenger to nigrostriatal dopaminergic neurons. *Journal of Neurocytology*, *29*, 375–85.

LaBar, K.S., Gitelman, D.R., Parrish, T.B., et al. (2001). Hunger selectively modulates corticolimbic activation to food stimuli in humans. *Behavioral Neuroscience*, *115*, 493–500.

Larson, E.B., Carroll, M.E. (2007). Estrogen receptor beta, but not alpha, mediates estrogen's effect on cocaine-induced reinstatement of extinguished cocaine-seeking behavior in ovariectomized female rats. *Neuropsychopharmacology*, *32*, 1334–45.

Le Saux, M., Morissette, M., Di Paolo, T. (2006). ERbeta mediates the estradiol increase of D2 receptors in rat striatum and nucleus accumbens. *Neuropharmacology*, *50*, 451–7.

Mani, S. (2008). Progestin receptor subtypes in the brain: the known and the unknown. *Endocrinology*, *149*, 2750–6.

Mani, S.K. (2006). Signaling mechanisms in progesterone-neurotransmitter interactions. *Neuroscience*, *138*, 773–81.

McBride, D., Barrett, S.P., Kelly, J.T., Aw, A., Dagher, A. (2006). Effects of expectancy and abstinence on the neural response to smoking cues in cigarette smokers: an fMRI study. *Neuropsychopharmacology*, *31*, 2728–38.

McEwen, B. (2002). Estrogen actions throughout the brain. *Recent Progress in Hormone Research*, *57*, 357–84.

Moos, R.H. (1968). The development of a menstrual distress questionnaire. *Psychosomatic Medicine*, *30*, 853–67.

Ossewaarde, L., Hermans, E.J., van Wingen, G.A., et al. (2010). Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle. *Psychoneuroendocrinology*, *35*, 47–55.

Petitclerc, M., Bedard, P.J., Di Paolo, T. (1995). Progesterone releases dopamine in male and female rat striatum: a behavioral and microdialysis study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *19*, 491–7.

Protopopescu, X., Pan, H., Altemus, M., et al. (2005). Orbitofrontal cortex activity related to emotional processing changes across the menstrual cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 16060–5.

Purdy, R.H., Moore, P.H. Jr, Rao, P.N., et al. (1990). Radioimmunoassay of 3 alpha-hydroxy-5 alpha-pregnan-20-one in rat and human plasma. *Steroids*, *55*, 290–6.

Reddy, D.S., Kulkarni, S.K. (1998). The role of GABA-A and mitochondrial diazepam-binding inhibitor receptors on the effects of neurosteroids on food intake in mice. *Psychopharmacology*, *137*, 391–400.

Reddy, D.S., Kulkarni, S.K. (1999). Sex and estrous cycle-dependent changes in neurosteroid and benzodiazepine effects on food consumption and plus-maze learning behaviors in rats. *Pharmacology, Biochemistry and Behavior*, *62*, 53–60.

Reed, S.C., Levin, F.R., Evans, S.M. (2008). Changes in mood, cognitive performance and appetite in the late luteal and follicular phases of the menstrual cycle in women with and without PMDD (premenstrual dysphoric disorder). *Hormones and Behavior*, *54*, 185–93.

Robinson, T.E., Berridge, K.C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research Brain Research Reviews*, *18*, 247–91.

Rolls, E.T., McCabe, C. (2007). Enhanced affective brain representations of chocolate in cravers vs. non-cravers. *European Journal of Neuroscience*, *26*, 1067–76.

Roy, E.J., Wilson, M.A., Kelley, D.B. (1986). Estrogen-induced progestin receptors in the brain and pituitary of the South African clawed frog, Xenopus laevis. *Neuroendocrinology*, *42*, 51–6.

Schott, B.H., Minuzzi, L., Krebs, R.M., et al. (2008). Mesolimbic functional magnetic resonance imaging activations during reward anticipation correlate with reward-related ventral striatal dopamine release. *Journal of Neuroscience*, *28*, 14311–19.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*, 22–33.

Shughrue, P.J., Scrimo, P.J., Merchenthaler, I. (1998). Evidence for the colocalization of estrogen receptor-beta mRNA and estrogen receptor-alpha immunoreactivity in neurons of the rat forebrain. *Endocrinology*, *139*, 5267–70.

Sveindottir, H., Bäckström, T. (2000). Prevalence of menstrual cycle symptom cyclicity and premenstrual dysphoric disorder in a random sample of women using and not using oral contraceptives. *Acta Obstetricia et Gynecologica Scandinavica*, *79*, 405–13.

Thompson, T.L., Moss, R.L. (1994). Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. *Journal of Neurochemistry*, *62*, 1750–6.

Thompson, T.L., Moss, R.L. (1997). Modulation of mesolimbic dopaminergic activity over the rat estrous cycle. *Neuroscience Letters*, *229*, 145–8.

Tomelleri, R., Grunewald, K.K. (1987). Menstrual cycle and food cravings in young college women. *Journal of the American Dietetic Association*, *87*, 311–5.

van Hell, H.H., Vink, M., Ossewaarde, L., et al. (2010). Chronic effects of cannabis use on the human reward system: an fMRI study. *European Neuropsychopharmacology*, *20*, 153–63.

van Wingen, G.A., van Broekhoven, F., Verkes, R.J., et al. (2008). Progesterone selectively increases amygdala reactivity in women. *Molecular Psychiatry*, *13*, 325–33.

van Wingen, G.A., Zylicz, S.A., Pieters, S., et al. (2009). Testosterone increases amygdala reactivity in middle-aged women to a young adulthood level. *Neuropsychopharmacology*, *34*, 539–47.

Vollm, B.A., de Araujo, I.E., Cowen, P.J., et al. (2004). Methamphetamine activates reward circuitry in drug naive human subjects. *Neuropsychopharmacology*, *29*, 1715–22.

Vulink, N.C., Denys, D., Bus, L., Westenberg, H.G. (2006). Female hormones affect symptom severity in obsessive-compulsive disorder. *International Clinical Psychopharmacology*, *21*, 171–5.

Wang, M., Seippel, L., Purdy, R.H., Bäckström, T. (1996). Relationship between symptom severity and steroid variation in women with

premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one. *Journal of Clinical Endocrinology and Metabolism*, *81*, 1076–82.

Wang, M.D., Wahlström, G., Gee, K.W., Bäckström, T. (1995). Potency of lipid and protein formulation of 5 alpha-pregnanolone at induction of anaesthesia and the corresponding regional brain distribution. *British Journal of Anaesthesia*, *74*, 553–7.

Weis, S., Hausmann, M., Stoffers, B., et al. (2008). Estradiol modulates functional brain organization during the menstrual cycle: an analysis of interhemispheric inhibition. *Journal of Neuroscience*, *28*, 13401–10.

Wrase, J., Schlagenhauf, F., Kienast, T., et al. (2007). Dysfunction of reward processing correlates with alcohol craving in detoxified alcoholics. *Neuroimage*, *35*, 787–94.

York, R., Freeman, E., Lowery, B., Strauss, J.F. (1989). Characteristics of premenstrual syndrome. *Obstetrics and Gynecology*, *73*, 601–5.