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A study of visuospatial working memory pre- and post-Gonadotropin Hormone Releasing Hormone agonists (GnRHa) in young women

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Abstract

Gonadotropin Hormone Releasing Hormone agonists (GnRHa) produce an acute decline in ovarian hormone production leading to a 'pseudo' menopause. This is therapeutically useful in the management of a variety of gynaecological conditions but also serves as a powerful model to study the effects of ovarian hormones on cognition. Animal and human behavioral studies report that memory is particularly sensitive to the effects ovarian hormone suppression (e.g. post GnRHa). Further, it has recently been reported that ovariectomy in young women increases the risk of cognitive impairment in later life. However, the underlying brain networks and/or stages of memory processing that might be modulated by acute ovarian hormone suppression remain poorly understood. We used event-related fMRI to examine the effect of GnRHa on visual working memory (VWM). Neuroimaging outcomes from 17 pre-menopausal healthy women were assessed at baseline and 8 weeks after GnRHa treatment. Seventeen matched wait-listed volunteers served as the control group and were assessed at similar intervals during the late follicular phase of the menstrual cycle. We report GnRHa was associated with attenuation of left parahippocampal (BA 35) and middle temporal gyri (BA 21 ,22, 39) activation, with a significant group-by-time interaction at left precuneus (BA 7) and posterior cingulate cortex (PCC) (BA 31) at encoding, and with cerebellar activation at recognition in the context of unimpaired behavioral responses. Our study suggests that acute ovarian hormone withdrawal following GnRHa, and perhaps at other times, (e.g. following surgical menopause and postpartum) alters the neural circuitry underlying performance of VWM.

Keywords: Visuospatial memory; Working memory; Ovarian suppression; Estrogen; Functional brain imaging

Introduction

Gonadotropin Hormone Releasing Hormone agonists (GnRHa) down-regulate pituitary gonadotropin-releasing hormone receptors to produce a decline in ovarian hormone production. GnRHa subsequently produces a temporary 'pseudo' menopause which is useful in the management of a variety of gynaecological conditions (Craig et al., 2007). This medically-induced menopausal state also provides a powerful model to study the early effects of ovarian hormone suppression on the brain. This is an important period to investigate as a number of studies suggest that it represents a critical period of brain aging. It has recently been reported, for example, that young women (≤45 years old) who undergo ovariectomy have an increased risk of cognitive impairment or dementia when they are older compared to matched controls (Rocca et al., 2007). It has also been reported that women who take hormone therapy (HT; i.e. estrogen±progesterone) in the period immediately following menopause may have a reduced risk of cognitive impairment and

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dementia in later life (Zandi et al., 2002). However, the biological mechanisms underlying the effects of ovarian hormone loss in younger women on brain regions involved in cognitive domains such as memory remain poorly understood.

Functional magnetic resonance imaging (fMRI) is a technique that has facilitated investigation into the effects of various medical conditions and drugs on brain function. We have recently used a verbal episodic memory task to study the effects of GnRHa on brain function in young women (≤47 years old) (Craig et al., 2007). Our findings suggested that GnRHa administration led to a change in the typical pattern of prefrontal activation during successful encoding, with decreased activation in left prefrontal cortex, anterior cingulate, and medial frontal gyrus. There have been no studies to date that have used a visual working memory (VWM) task as a probe to study the effects of GnRHa. However, studies into the effects of HT on visual and working memory suggest that brain networks involved in processing VWM tasks may be sensitive to the effects of ovarian hormone suppression (Duff and Hampson, 2000; Resnick et al., 2006; Resnick et al., 1997; Smith et al., 2006).

We therefore sought to analyse the effects of GnRHa on the encoding and retrieval components of VWM using the delayed match to sample (DMTS) task. Prior work has reported widespread prefrontal and parietal regions with visual object encoding as well as parahippocampus, cingulate, and the inferior temporal cortex (Brewer et al., 1998; Pessoa et al., 2002). Previous studies also report that regions activated during the visual recognition include prefrontal cortical regions, anterior cingulate, insula, precentral gyrus, inferior temporal gyrus, extrastriate regions and cerebellum (Buckner et al., 1998; Buckner et al., 1996; Pessoa et al., 2002; Picchioni et al., 2007). Hence we sought to determine if GnRHa modulates the response of these brain regions during this widely used VWM task.

Methods

Subjects

We included thirty-four right-handed healthy pre-menopausal women with benign *leiomyomata uteri* (i.e. 'fibroids'). All women were prescribed GnRHa (two Zoladex® 3.6 mg implants) as part of their routine clinical management and provided informed consent as per the Ethics Committee Guidelines.

Subject assessment

All the women were recruited from the gynaecology clinics of two London teaching hospitals and screened to exclude past or present psychiatric disorders using the Structured Clinical Interview for DSM-IV Axis I and II Disorders (SCID-I and SCID-II) (First et al., 1997a; First et al., 1997b). Scores on the Beck Depression and Anxiety Inventories (BDI and BAI respectively) (Beck et al., 1988; Beck et al., 1996) were also obtained to quantify sub-clinical symptoms of depression and anxiety. General intelligence and cognitive function were measured using the Wechsler Abbreviated Scale of Intelligence (WASI) (1999) and the Mini Mental State Examination (MMSE) (Folstein et al., 1983). Handedness was determined by the Annett handedness scale (Annett, 1970), and menopausal symptoms were assessed using the Greene Climacteric Scale (GCS) (Greene, 1998) at each visit. Women were excluded if they had an IQ less than 70, a MMSE score less than 27, alcohol/drug abuse, significant medical/neurological problems affecting brain function, or if they were taking regular prescribed medication, were left handed or if they did not have regular menstrual cycles. All subjects also underwent routine blood testing to measure their haematological profile and hepatic, renal, thyroid, and ovarian function.

Delayed Matching to Sample (DMTS) task

Stimulus presentation

Subjects wore MRI-compatible air-conducting headphones and visual stimuli were back-projected by an LCD projector (Proxima Desktop Projector 5500) onto a screen 2.5 m from the subject's head and were viewed via a prism mounted on the head coil. The paradigms were programmed in Microsoft Visual Basic Professional 6.0 and presented on a PC running MS Windows XP.

Stimulus materials

Our version of the Delayed Matching to Sample (DMTS) test was adapted from the Cambridge Neuropsychological Test Automated Battery (CANTAB). Each trial consisted of four phases.

Phase 1: During the initial 'encoding' phase, subjects were presented with a complex abstract pattern (the sample) for 5000 ms in the centre of the screen. Subjects were instructed to remember the sample as they would be asked to identify it later.

Phase 2: The next 'maintenance' phase involved a variable delay during which the subject was instructed to hold the sample in memory while maintaining fixation on a central cross. The duration of the maintenance delay varied across trials. Simultaneous trials involved no delay (i.e. the sample and choice patterns were shown together at recognition). In the other trials there was a delay of 4000 ms or 12,000 ms between encoding and recognition, and only the choice patterns were displayed.

Phase 3: In the 'recognition' phase, subjects were shown four patterns in a North, South, East, and West distribution around the central location for 6000 ms and were asked to identify the sample by pressing a joystick in the corresponding direction with their right hand. Each pattern was made up of four sub-elements, each of a different colour. One of the choice patterns was identical to the sample, one was a novel distracter pattern, one had the shape of the sample and the colours of the distracter, and the fourth had the colours of the sample and the shape of the distracter. To discourage strategies based on encoding single quadrants, all four choice patterns had one quadrant in common.

Phase 4: In the final 'delay' phase of the trial subjects were simply instructed to maintain visual fixation on a central cross. This delay was designed to equalise the inter-trial (encoding) interval to 27,000 ms across trials, while randomly varying the inter-stimulus (recognition) interval, with the length of the delay dependent upon the duration of the preceding maintenance phase.

Subjects were trained on 7 practice trials. In the experimental task there were 42 trials, 14 for each maintenance delay (simultaneous, 4000 ms and 12,000 ms), presented in a pseudorandom order in two runs of approximately 10 min each.

Behavioral data

All behavioral data, response accuracy and response latency, were recorded on a personal computer using Visual Basic (Microsoft Corp., Redmond, USA) and analysed in SPSS Version 13.0 (SPSS Inc., Chicago, USA).

Experimental paradigm

The overall study design was an observational, repeated measure, wait-list control design. Consecutive women put on a 6-month waiting list to receive 8 weeks continuous pre-operative GnRHa who met eligibility criteria and agreed to take part in the study were randomly divided into two groups. (The waiting list for surgery enabled randomisation of women into the two groups without delaying treatment, in either group, for purposes of this study). Women in the 'experimental' group ($n=17$) were initially scanned (Time 1) before GnRHa was administered between days 9–13 of their menstrual cycle (i.e. when they were predicted to be in the latter part of the follicular phase associated with high estrogen/low progesterone levels, as estrogen, not progesterone, has been most clearly associated with improving memory). They subsequently received GnRHa and had a repeat scan 8 weeks later (Time 2) when still taking GnRHa. The protocol for the wait-list 'control' group ($n=17$) was identical. However, they

were subsequently re-scanned 8 weeks later (during the same phase of their menstrual cycle) without having received GnRHa during the intervening 8 weeks.

Blood collection and endocrine determinants

Blood was collected into tubes without anticoagulant and allowed to clot. Samples were centrifuged for 15 min at 1500 g and serum separated within 4 h. Estradiol and progesterone concentrations were quantified using a competitive immunoassays (supplied by Bayer Advia Centaur) using direct chemiluminometric technology [*estradiol intra-assay precision*: level 1 (mean concentration = 242.2 pmol/l), CV = 12.1%, level 3 (mean concentration 642.3 pmol/l) CV = 4%, level 5 (mean concentration = 2377.1 pmol/l, CV = 6.3%); *progesterone intra-assay precision*: level 1 (mean concentration = 12.4 pmol/l), CV = 12.4%, level 3 (mean concentration 22.9 pmol/l) CV = 3.7%, level 6 (mean concentration = 154.9pmol/l, CV = 2.5%)]. Luteinising Hormone (LH) and Follicular Stimulating Hormone (FSH) were quantified using two-site sandwich immunoassays (supplied by Bayer Diagnostics Europe) using direct chemiluminometric technology [*LH intra-assay precision*: level 1 (mean concentration = 4.2 IU/l), CV = 2.3%, level 3 (mean concentration 16.4 IU/l) CV = 2.3%, level 6 (mean concentration = 118.2 IU/l, CV = 2.6; *FSH intra-assay precision*: level 1 (mean concentration = 6.9 IU/l), CV=2.7%, level 3 (mean concentration 23.4 IU/l) CV = 21.2%, level 6 (mean concentration = 144.3 IU/l, CV = 1.2%)].

MRI scanning methods

Gradient echo echoplanar imaging (EPI) data were acquired on a neuro-optimized GE Signa 1.5 Tesla system (General Electric, Milwaukee WI, USA) at the Maudsley Hospital, London. Consistent image quality was ensured by a semi-automated quality control procedure. A quadrature birdcage headcoil was used for radio frequency transmission and reception.

324 T2*-weighted images depicting blood oxygen level-dependent (BOLD) contrast were acquired during each run at each of 22 near-axial non-contiguous 7 mm thick planes parallel to the intercommissural plane (TE 40 ms, TR 2 s, in-plane resolution 7 mm, interslice gap 0.7 mm).

The first four images were discarded to allow the magnetization to reach equilibrium amplitude. A jittered acquisition sequence optimised sampling of the BOLD response.

At the same time, brain activation maps were co-registered to a high-resolution gradient echo image of the whole brain. This was acquired in the intercommissural plane consisting of 43 slices (slice thickness = 3 mm; gap = 0.3 mm; TR = 3 s; flip angle = 90°; matrix = 128 × 128). This EPI data set provided almost complete brain coverage.

Analysis of fMRI data

Data were analyzed with XBAM v3.4 (Institute of Psychiatry, London, UK) on a Sun Workstation (Sun Microsystems Inc. Santa Clara, USA.)[1].

[1] *f*MRI analysis, in its most common forms, requires fitting of a time-series model at many thousands of intracerebral voxels in a number of individuals. Most assessments of the significance of the resulting model fits in common use in *f*MRI analysis use normal theory and the validity of the normality assumption is not often tested. Our 'in house' method of analysis, XBAM v3.4 (Institute of Psychiatry, London, U.K.), makes no such assumptions. Instead, it uses median statistics to control outlier effects and permutation rather than normal theory based inference. Furthermore our most common test statistic is computed by standardising for individual difference in residual noise before embarking on second level, multi-subject, testing using robust permutation-based methods. This allows a mixed effects approach to analysis. A recent paper by Thirion et al. (2007) has conducted a detailed analysis of the validity and impact of normal theory based inference in fMRI in large number of subjects. They have found substantial deviations from normality in a significant number (22%) of intracerebral voxels using the most common measure of response size (unstandardised beta) used in fMRI analysis. They recommend a mixed effects rather than simple random effects analysis, permutation-based inference and cluster or parcel level rather than voxel level inference.

Individual analysis

Images were first realigned (Bullmore et al., 1999a,b) to minimise motion related artefacts and smoothed using a Gaussian filter (5 mm). Responses to the experimental paradigms were detected by time-series analysis using gamma variate functions to model the BOLD response.

Our model consisted of correctly and incorrectly encoded and recognised stimuli and maintenance. The experimental conditions of interest were correctly encoded and correctly recognised responses (after 4000 ms and 12,000 ms delays) contrasted with baseline (visual fixation on the central cross). We compared the effects of delay (i.e. 4000 ms or 12,000 ms) following GnRHa but found no significant differences in the accuracy or brain regions activated and we therefore combined the 4000 ms and 12,000 ms conditions in order to increase the statistical power of our analysis.

In order to exclude colinearity for the covariates of encoding and recognition from incorrectly performed trials the design model explicitly incorporated these events as separate conditions, as part of a single stage linear model.

The analysis was implemented as follows. First, each experimental condition was convolved separately with the 4000 and 8000 ms gamma functions to yield two models of the expected haemodynamic response to that condition. The weighted sum of these two convolutions that gave the best fit to the time series at each voxel was then computed. This weighted sum effectively allows voxel-wise variability in time to peak haemodynamic response.

In order to constrain the possible range of fits of physiologically plausible BOLD responses, a constrained fitting procedure (Friman et al., 2003) was used. Following this fitting operation, a goodness of fit statistic was computed at each voxel. This was the ratio of the sum of squares of deviations from the mean intensity value due to the model (fitted time series) divided by the sum of squares due to the residuals (original time series minus model time series). This statistic is called the SSQratio. The percentage change in the BOLD signal at each voxel was also calculated. This was $[(\mathrm{fit}_{max} - \mathrm{fit}_{min})/\text{mean signal intensity}] \times 100$, where fit_{max} and fit_{min} were the maximum and minimum values of the fitted response for the time series in question.

In order to sample the distribution of SSQ ratio under the null hypothesis that observed values of the SSQ ratio were not determined by experimental design (with minimal assumptions), the time series at each voxel was permuted using a wavelet-based resampling method (Breakspear et al., 2003). This process was repeated 20 times at each voxel and the data combined over all voxels, resulting in 20 permuted parametric maps of SSQ ratio at each plane for each subject. The same permutation strategy was applied at each voxel to preserve spatial correlational structure in the data during randomisation. Combining the randomised data over all voxels yields the distribution of SSQ ratio under the null hypothesis. A test that any given voxel is activated at any required type I error can then be carried out by obtaining the appropriate critical value of SSQ ratio from the null distribution. For example, SSQ ratio values in the observed data lying above the 99th percentile of the null distribution have a probability under the null hypothesis of ≤ 0.01. We have shown that this permutation method gives very good type I error control with minimal distributional assumptions (Breakspear et al., 2003; Bullmore et al., 2001).

Group mapping

In order to extend inference for each condition of interest to the group level, the observed and randomised SSQ ratio maps for each subject were transformed into standard space by a two stage process involving first a rigid body transformation of the fMRI data into the gradient echo image of the same subject, followed by an affine transformation onto a Talairach template (Brammer et al., 1997). By applying the two spatial transformations computed above for each subject to the statistic maps obtained by analysing the observed and wavelet-randomised data, a generic brain activation map (GBAM) was produced for each experimental condition, firstly across the two runs for each subject, then across subjects. The median observed SSQ ratio over all subjects at each voxel (median values were used to minimise outlier effects) can then be tested at each intracerebral voxel in standard space (Schmahmann et al., 1999; Talairach and Tournoux, 1988) against a critical value of the permutation distribution for median SSQ ratio ascertained from the spatially transformed wavelet-permuted data (Brammer et al., 1997). In order to increase sensitivity and reduce the multiple comparison problem encountered in fMRI, hypothesis testing was

Table 1
Results of baseline screening tests

	Control group (SEM)	Experiment group (SEM)	*P*	95% Interval of	Confidence difference
Age	41 (1)	39 (2)	0.27	−1.75	6.09
Full scale IQ	104 (8)	102 (4)	0.38	−16.38	19.88
MMSE	30 (0.3)	29 (0.1)	0.81	−0.52	0.66
BDI	4 (1)	4 (1)	0.84	−3.92	3.2
BAI	5 (2)	6 (2)	0.82	−6.05	4.82

This table illustrates the absence of significant differences between the two groups at baseline.

carried out at the cluster level using method developed by Bullmore et al. (Bullmore et al., 1999a,b), shown to give excellent cluster-wise type I error control in both structural and functional fMRI analysis. When applied to fMRI data, this method estimates the probability of occurrence of clusters under the null hypothesis using the distribution of median SSQ ratios computed from spatially transformed data obtained from wavelet permutation of the time series at each voxel. Image-wise expectation of the number of false positive clusters under the null hypothesis is set for each analysis at less than one.

Between group testing

Analysis of variance was carried out on the effect size maps in standard space by first computing the difference in median SSQ ratio between groups at each voxel. Subsequent inference of the probability of this difference under the null hypothesis was made by reference to the null distribution obtained by repeated random permutation of group membership and recomputation of the difference in median SSQ ratios between the two groups obtained from the resampling process. As with the generic brain activation map, cluster-level maps were then obtained with the cluster-wise probability equivalent to less than one false positive cluster per image. We set an image-wide voxel-wise p value of 0.05 and a cluster-wise *p* value of 0.005 (for the encoding task) and 0.0025 (for the recognition task), to ensure that the expected total number of false positive clusters per brain was not greater than one. In our experience, expressing the type I error level (alpha) at which inference is performed in terms of the resulting expected number of error voxels or clusters is intuitively easier for users of our software to understand than a p value which can be difficult to interpret at cluster level.

Contrasts

In order to determine whether any group differences in brain function were confounded by between group differences in menopausal symptoms we carried out *post hoc* correlation analyses between GCS and SSQs at brain regions where significant group differences were observed.

We performed a series of planned contrasts to determine the following effects. These are set out in detail below but, in brief, we initially explored group differences prior to any experimental manipulation (here, we anticipated that there should be no group differences since the groups were matched), we then explored group differences following GnRHa treatment and, critically, we established the presence of a group-by-time interaction. Discussion will be confined to those regions in which there was a group difference following medication that was also seen as a group-by-time (treatment) interaction:

Encoding task

(i) *Between group contrast of activity associated with correct encoding at Time 1*: In this contrast we compared the encoding trials (that were subsequently correctly recognized) at Time 1 (baseline) in the control group with the experimental group. This contrast explored the hypothesis that the two groups were matched at the study outset for neural response at encoding.
(ii) *Between group contrast of activity associated with correct encoding at Time 2*: In this contrast we compared the encoding trials (that were subsequently correctly recognized) at Time 2 in the experimental group (8 weeks post ovarian suppression) with the control group. This contrast explored the neural effects of ovarian suppression on successful visual encoding.
(iii) *Between group analysis of correct encoding at Time 1 vs Time 2*: In this contrast we compared neural activity at successful encoding at Time 1 with Time 2 in the experimental group with the same comparison in the control group. This planned contrast therefore explored the interaction of ovarian suppression and time on successful encoding between the two groups.

Recognition task

We performed a series of planned contrasts to determine the following effects:

(i) *Between group analysis of correct recognition (following 4 s and 12 s delay, analysed together) at Time 1*: In this contrast we compared correctly recognized trials at Time 1 in the control group versus the experimental group. This contrast explored the hypothesis that the two groups were matched at the study outset for neural response at recognition.
(ii) *Between group analysis of correct recognition (following 4 s and 12 s delay, analysed together) at Time 2*: In this contrast we compared correctly recognized trials at Time 2 in the experimental group (8 weeks post ovarian suppression) with the control group. This contrast explored the neural effects of ovarian suppression on successful visual recognition.

Table 2
Plasma hormone concentrations in both groups at baseline (Time 1) and 8 weeks later (Time 2)

	Time 1	Time 2	*z* score	*p*
Control group	Median (25%, 75% quartile)	Median (25%, 75% quartile)		
Estradiol (pmol/l)	650 (180, 1015)	332 (201, 992)	−0.722	0.470
Progesterone (nmol/l)	5 (5, 37.75)	17 (5, 38.25)	−0.628	0.530
Luteinising hormone (u/l)	6.15 (4.27,8.12)	5.1 (3.225,7.775)	−.471	0.638
Follicular stimulating hormone (u/l)	3.8 (2.82,6.65)	4.1 (2.55, 5.37)	−.350	0.727
Experimental group	Median (25%, 75% quartile)	Median (25%, 75% quartile)		
Estradiol (pmol/l)	382 (231.75, 696.5)	36 (30,88)	−3.413	0.001
Progesterone (nmol/l)	5 (5,16)	5 (5,5)	−2.201	0.018
Luteinising hormone (u/l)	5.1 (3.225, 7.775)	0.8(.8,.8)	−3.464	0.001
Follicular stimulating hormone (u/l)	4.6 (1.7, 6.3)	2.75 (2.17, 5.70)	−1.591	0.112

The data expressed in this table highlights that the median estradiol, progesterone, LH and FSH concentrations did not vary significantly in the control group over an 8 week period. However in the experimental group, following 8 weeks of GnRHa, there was a significant reduction in the median estradiol, progesterone and LH concentrations. GnRHa only suppresses FSH to 'follicular phase levels' (AstraZeneca., 2005). Therefore the absence of a statistically significant difference between FSH levels across time in the experimental group does not suggest a failure of GnRHa to suppress ovarian function. Further, in light of the fact that we attempted to assess *all* women at the follicular phase of the cycle this finding would have been predicted at the onset. The high 75% quartile estradiol score in the control group was attributable to an outlier. The fMRI/behavioral results of the study did not alter significantly when she was excluded from the analysis and they were therefore retained in the final analysis.

Table 3
Behavioral performance in both groups at baseline (Time 1) and 8 weeks later (Time 2) with respect to accuracy and response time

Group	Time	% Accuracy (SEM)	Response time (s) (SEM)
Control	1	74.02 (3.59)	2.59 (.09)
	2	75.78 (2.90)	2.49 (.07)
Experimental	1	77.08 (3.39)	2.77 (.08)
	2	77.95 (2.73)	2.61 (.06)

The data in this table illustrates the accuracy and response time scores of both groups across time. These scores did not differ significantly either between or within groups.

(iii) *Between group analysis of correct recognition (following 4 s and 12 s delay, analysed together) at Time 1 vs. Time 2*: In this contrast we compared neural activity during correctly recognized trials at Time 1 with Time 2 in the experimental group with the same comparison in the control group. This planned contrast explored the interaction of ovarian suppression and time on successful recognition between the two groups.

Additional statistical analyses: Simple t-tests were used to compare age, full scale IQ, MMSE, BDI and BAI between groups at baseline. Due to a low detectable limit of the hormone assays, Wilcoxon rank tests were used in the group comparisons of plasma hormone levels across time. In order to compare accuracy of encoding and recognition between the two groups over time we used mixed repeated measure ANOVAs.

Results

Baseline screening tests

At baseline the two groups of women did not differ on scores on the Mini-Mental State Examination (MMSE), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), age or IQ (Table 1).

Plasma hormone levels

There were no significant differences at baseline (Time 1) in plasma ovarian steroid concentrations but these were significantly reduced in the experimental group post GnRHa (Zoladex) administration (Table 2). Despite our attempts to scan women in the follicular phase of the cycle (i.e. between days 9–13) the results of blood tests suggested that at baseline

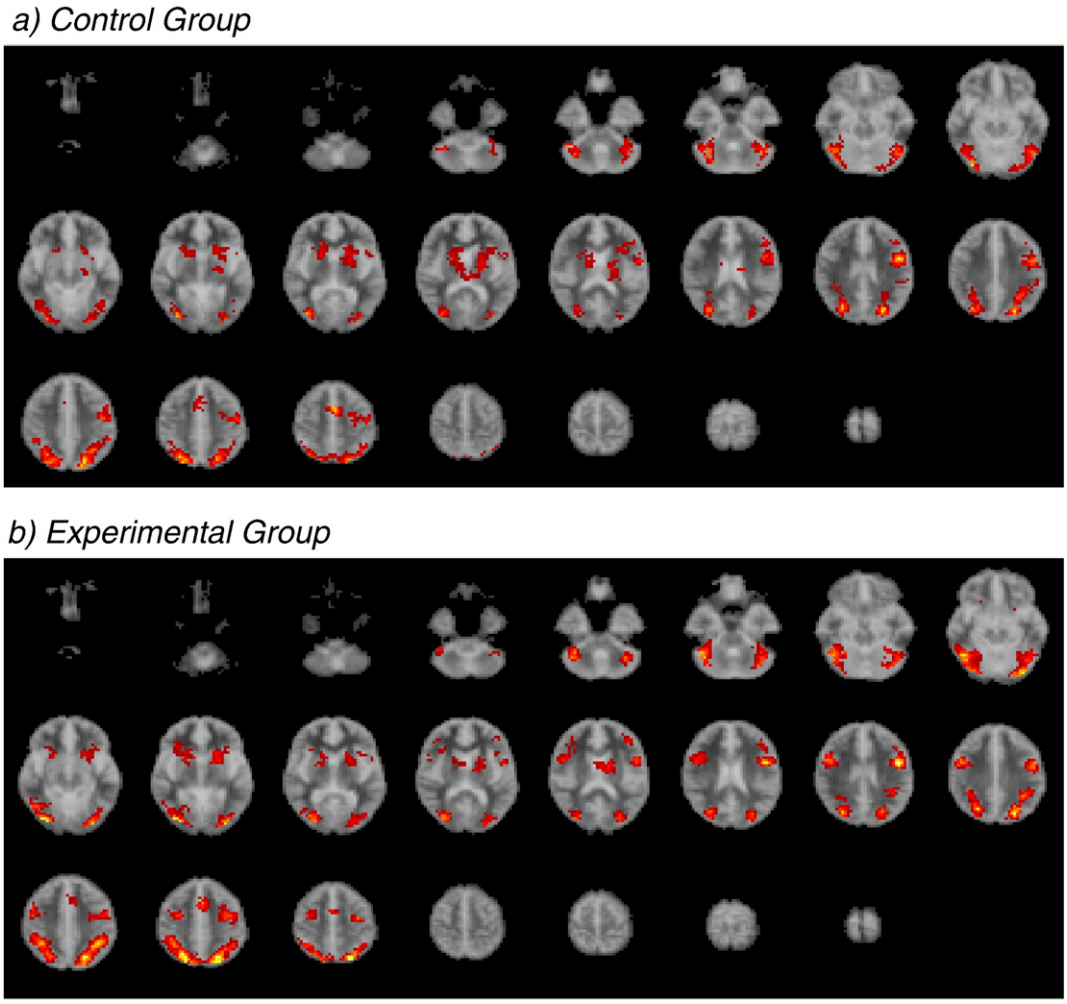

Fig. 1. Brain regions activated during trials that were successfully encoded (i.e. subsequently correctly recognized) at baseline (Time 1). The brain regions activated in both groups during successful encoding at baseline are displayed in a and b. These regions included prefrontal and parietal regions, parahippocampus, cingulate, and the inferior temporal cortex. There were no significant between group differences at baseline within any brain regions.

in both groups and in the control group at Time 2 some women had post ovulatory hormone profiles. However, our results were not significantly different when these women were excluded and they were therefore included in the final analysis.

Greene climacteric scale

The interaction of Green Climacteric Scale (GCS) score between time and group was significant $F(1,32)=5.902$, $p=0.021$.

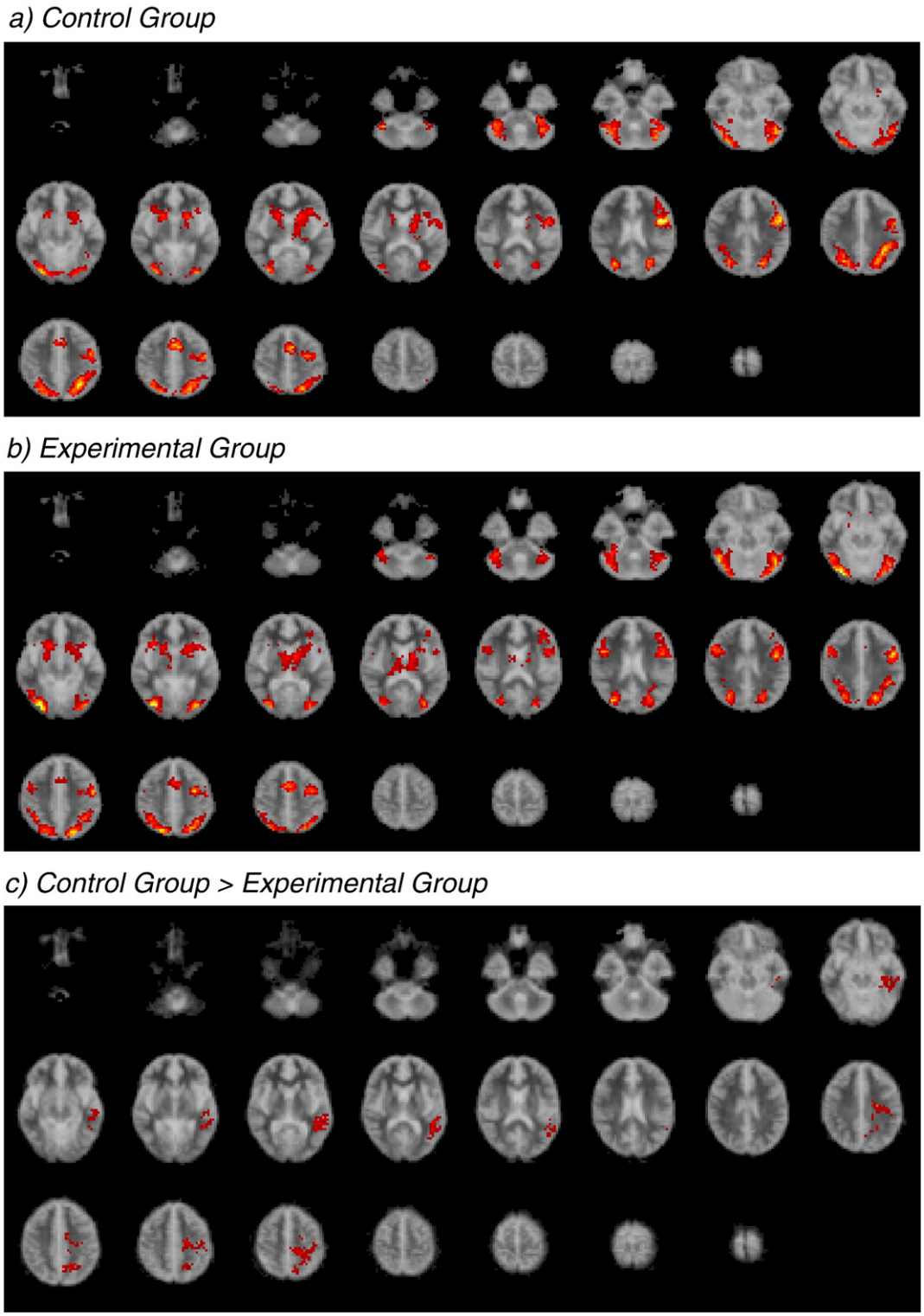

Fig. 2. Brain regions activated during successfully encoded trials (i.e. subsequently correctly recognized) 8 weeks after baseline scan (i.e. Time 2). The brain regions activated in both groups at Time 2 are displayed in a and b. These regions are similar to 1a and b above. Subpanel c illustrates the increase in brain activation in the control group, compared to the experimental (GnRHa) group at Time 2 (see Table 4 for precise regions). There is no evidence of a reciprocal increase in other brain regions in the experimental group.

This confirmed that women randomized to receive GnRHa had more menopausal symptoms than women in the control group.

Behavioral results/task performance

A mixed repeated measurement ANOVA revealed that there were no significant main effect of time (Time 1 vs. Time 2) ($F(1,32)=0.275$, $p=0.604$), group ($F(1,32)=0.493$, $p=0.488$) or a significant interaction between time and group ($F(1,32)=0.032$, $p=.86$) on recognition accuracy (Table 3).

A mixed repeated measurement ANOVA of recognition response latency revealed a significant main effect of time ($F(1,32)=8.95$, $p=0.005$) with response time reducing between Time 1 and Time 2 in both groups. However, there was no main effect of group ($F(1,32)=2.169$, $p=.151$) nor a significant interaction between time and group ($F(1,32)=.414$, $p=.524$) (Table 3).

fMRI results

Post-hoc analyses indicated that the correlation between GCS and SSQs was statistically insignificant and therefore we did not covary for GCS in the results below.

a) Phase 1. Encoding task

(i) *Between group analysis of correct encoding at Time 1*: There were no significant between group differences in brain activation at baseline. This established that the two groups were matched at the outset of the study in terms of brain responses. Furthermore brain activation was evident in regions predicted to be activated during successful visual encoding including prefrontal and parietal regions, parahippocampus, cingulate, and the inferior temporal cortex bilaterally (Fig. 1).

(ii) *Between group analysis of correct encoding at Time 2*: This analysis of variance (ANOVA) showed significant group differences in the left superior parietal cortex (BA 5), anterior cingulate (BA 24), posterior cingulate (BA 31), precuneus (BA 7), parahippocampus (BA 35) and middle temporal gyri (BA 21, 22, 39) (Fig. 2, Table 4).

(iii) *Between group analysis of correct encoding at Time 1 vs. Time 2*: This ANOVA suggested a significant group-by-time interaction between brain activation during correctly encoded trials in the control group (Time 1 vs. Time 2) versus the same comparison in the experimental group. A group-by-time interaction was observed in left cerebellum, precuneus (BA 7), posterior cingulate (BA 31) and fusiform gyrus (BA 37) (Fig. 3, Table 5).

In summary, these planned analyses suggest that, at the outset, the two randomly allocated groups did not differ in behavioral performance or neural activity during successful encoding. Following ovarian suppression we detected a significant reduction in brain activation in the experimental group in the left medial parietal cortex (precuneus), posterior cingulate, paracentral lobule, parahippocampus, middle temporal gyrus. In the time-by-group interaction we detected significant reduction in brain activation of left precuneus and posterior cingulate cortex.

Table 4
Brain regions associated with increased activation in the control group versus the experimental group at Time 2 (i.e. after the experimental group had received 8 weeks of GnRHa)

	Area	Brain region	Side	Tal(x)	Tal(y)	Tal(z)	BA	Cluster-wise probability	SSQ ratio
Encoding	Frontal	Paracentral lobule	L	−14	−33	48	5		
	Limbic	Parahippocampus	L	−36	−26	−13	35		
		Cingulate (anterior)	L	−14	−11	42	24		
			L	−7	−4	37	24		
		Cingulate (posterior)	L	−18	−48	37	31		
	Parietal	Precuneus	L	−18	−56	42	7		
			L	−7	−59	48	7		
	Temporal	Middle temporal gyrus	L	−47	−59	15	39	0.002419	0.02877
			L	−54	−33	4	22		
			L	−43	−48	9	21		
			L	−51	−26	−7	21		
			L	−43	−44	−2	21		
			L	−40	−30	−2	21		
Recognition	Cerebellum		R	7	−48	−18	71	0.001387	0.008142
			R	0	−56	−29	71		
			R	11	−63	−13	71		
			L	−14	−70	−24	71		
	Brain stem		R	4	−52	−40	70		
			L	−4	−41	−35	70		

This table illustrates brain regions in the control group associated with increased activation, during successful encoding and recognition, compared to the experimental group at Time 2 (i.e. after the experimental had received 8 weeks of GnRHa). Talairach coordinates in bold print indicate the most activated voxel within a cluster. Other Talairach coordinates represent other active areas in clusters (derived from decomposition of each cluster into contiguous slices, 5.72 mm diameter in the *z* dimension).

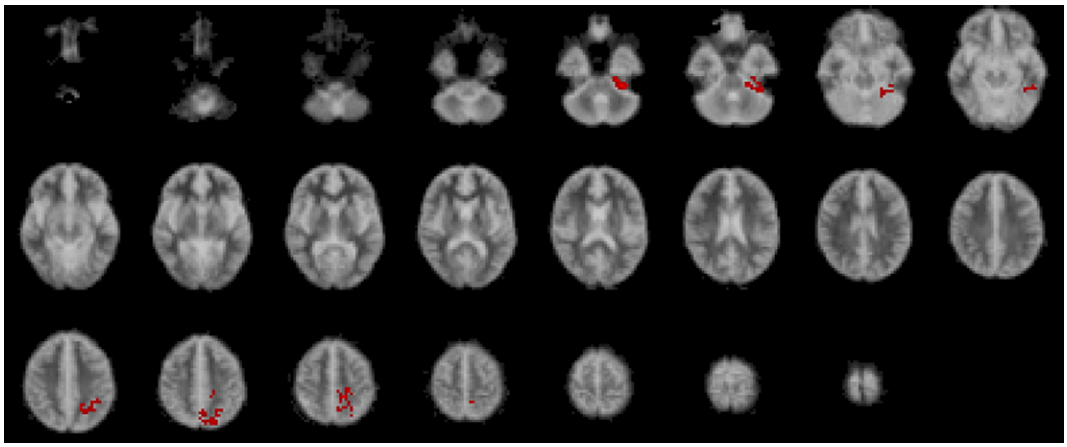

Fig. 3. Between group analysis of brain regions activated during correct encoding at baseline (Time 1) versus 8 weeks later (Time 2). This figure illustrates the difference between brain activation during correctly encoded trials in the control group (Time 1 vs. Time 2) versus the same comparison in the experimental group. It highlights a significant group-by-time interaction in left cerebellum, precuneus, posterior cingulate and fusiform gyrus (see Table 5 for further detail).

b) Phase 3. Recognition task

(i) *Between group analysis of correct recognition at Time 1 (following 4 s and 12 s delay)*: There were no significant between group differences in brain activation at baseline establishing that the two groups were matched at the outset of the study in terms of brain responses. Furthermore brain activation was evident in regions predicted to be activated during successful visual recogniton including prefrontal cortical regions, anterior cingulate, insula, precentral gyrus, inferior temporal gyrus, extrastriate regions and cerebellum (Fig. 4).

(ii) *Between group analysis of correct recognition at Time 2 (following 4 s and 12 s delay combined)*: This analysis of variance (ANOVA) suggested a significant difference between groups with a reduced activation at cerebellum and brainstem in the experimental group following ovarian suppression relative to the control group (Fig. 5, Table 4).

(iii) *Between group analysis of correct recognition (following 4 s and 12 s delay combined) at Time 1 vs Time 2*: An analysis ANOVA suggested a significant group-by-time interaction between brain activation during correctly recognized trials in the control group (Time 1 vs. Time 2) versus the same comparison in the experimental group. This interaction was observed bilaterally in the cerebellum and fusiform gyrus and at the left inferior temporal gyrus (Fig. 6, Table 5).

In summary these analyses of the recognition task suggest that there were no group differences in neural activity at successful recognition at baseline. Following ovarian suppression however we detected a significant reduction in brain activation in the cerebellum (and brainstem) in the experimental group. In the time-by-group interaction we also detected a significant reduction in brain activation in the cerebellum.

Discussion

In summary we identified significant modulatory effects of GnRHa on the brain activation responses to a widely used VWM task (i.e., the Delayed Match to Sample (DMTS) task). We report that GnRHa is associated with attenuation of left precuneus (BA 7) and posterior cingulate cortex (PCC) (BA 31)

Table 5
Brain regions in the control group associated with increased activation across time, during successful encoding and recognition, compared to the experimental group

	Area	Brain Region	Side	Tal(x)	Tal(y)	Tal(z)	BA	Cluster-wise probability	SSQ ratio
Encoding	Limbic	Cingulate (posterior)	L	−14	−30	42	31		
	Parietal	Precuneus	L	−14	−67	42	7	0.003908	0.039197
	Temporal	Fusiform gyrus	L	−43	−41	−13	37		
	Cerebellum		L	−29	−37	−29		0.004461	0.033853
			L	−32	−37	−18			
Recognition	Cerebellum		L	−36	−52	−24		0.001927	0.020745
			R	−25	−52	−18		0.000609	0.023329
	Temporal	Fusiform gyrus	L	−43	−52	−13	37		
			R	4	−59	−13	37		
		Inferior temporal gyrus	L	−47	−52	−7	37		

This table illustrates brain regions in the control group associated with increased activation across time, during successful encoding and recognition, compared to the experimental group. Voxel-wise significance $p=0.05$; Cluster-wise significance = 0.005 (encoding), 0.0025 (recognition). This combination of voxel and cluster-wise p values leads to an expected number of type I error clusters of < 1 per whole brain. Talairach coordinates in bold print indicate the most activated voxel within a cluster. Other Talairach coordinates represent other active areas in clusters (derived from decomposition of each cluster into contiguous slices, 5.72 mm diameter in the *z* dimension).

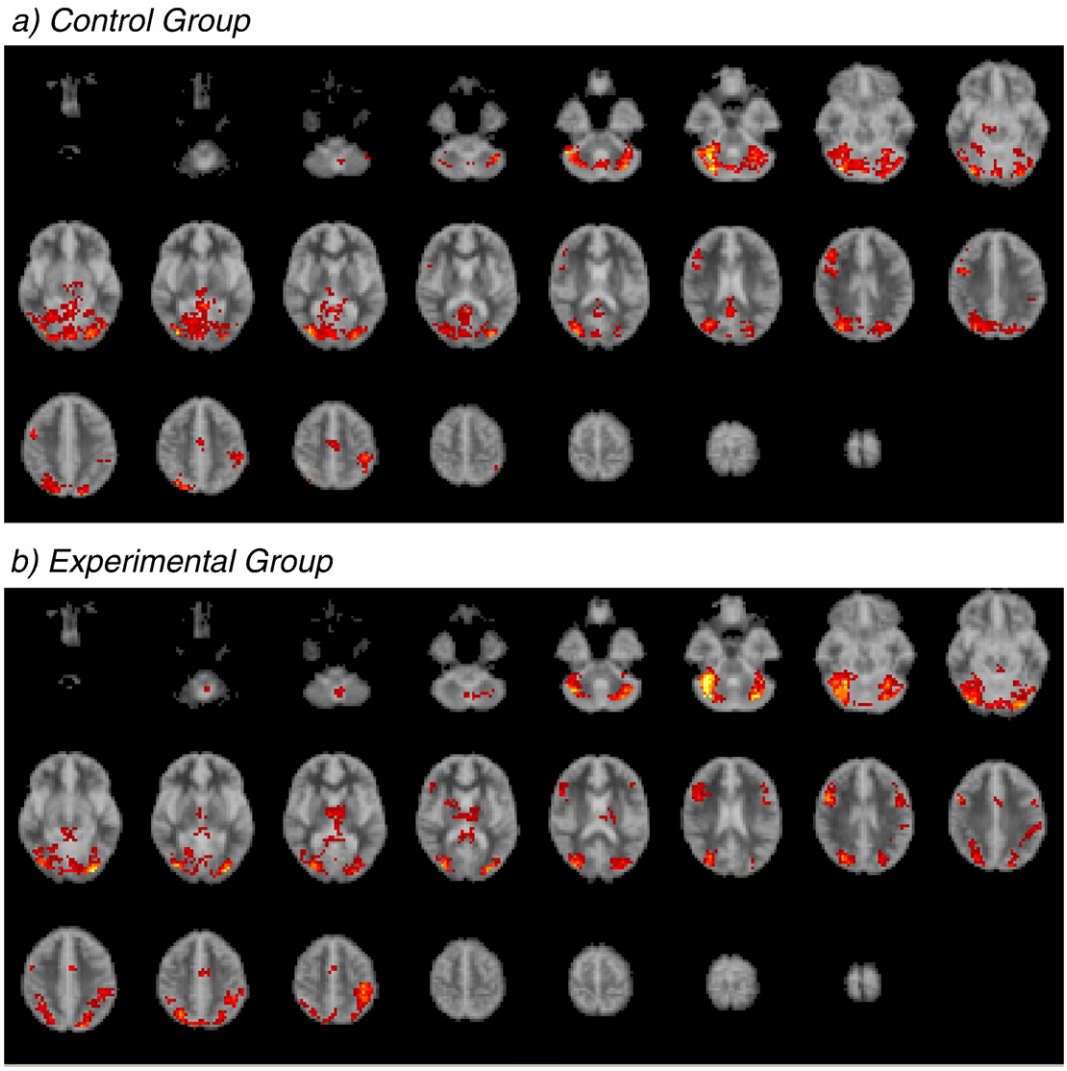

Fig. 4. Brain regions activated during successfully recognized trials at baseline (Time 1). The brain regions activated in both groups during successful recognition at baseline are displayed in a and b. These regions included prefrontal cortical regions, anterior cingulate, insula, precentral gyrus, inferior temporal gyrus, extrastriate regions and cerebellum. There were no significant absence of a between group difference at baseline within any brain regions.

activation at encoding, and cerebellar activation at recognition, in the context of unimpaired behavioral responses.

Analysis of the underlying networks disrupted by GnRHa

The brain regions activated during the DMTS task at baseline (Figures 1 and 4) are characteristic of the brain network typically reported in studies of VWM and consistent with those regions that we had predicted would be activated *a priori* (see above). This suggests that (i) our subjects were engaging in the DMTS task in an appropriate manner and (ii) GnRHa significantly attenuated activation of task dependent brain regions within this network.

The precuneus plays a key role in the encoding of visuospatial material (Knauff et al., 2003; Suchan et al., 2002) and is reported to form a network with other brain regions important to encoding (see (Cavanna and Trimble, 2006)for review). This includes the posterior cingulate cortex (PCC) which animal (Markowska et al., 1989) and human (Rypma and D'Esposito, 1999) studies have reported is important for visual encoding and VWM (see (Nielsen et al., 2005) for review). With respect to the recognition phase of our study GnRHa was associated with reduced brain activation at the cerebellum; which animal (Mandolesi et al., 2001; Mandolesi et al., 2003) and human studies (Ravizza et al., 2006) have reported plays an important role in successful working memory and may be particularly important for attentional processing (Tomasi et al., 2007).

Thus GnRHa was associated with reduced activation in several brain regions that are important to VWM. However in contrast to the findings from this study, several previous investigations, by our group and others, reported that loss of ovarian function (post GnRHa or menopause) is associated with reduced brain activation at the prefrontal cortex (PFC) during tasks that included verbal encoding (Craig et al., 2007), modified Wisconsin Card Sorting (Berman et al., 1997) and visual delayed matching to sample (Smith et al., 2006). Our observation that the DMTS task activated PFC at both time points suggests that the

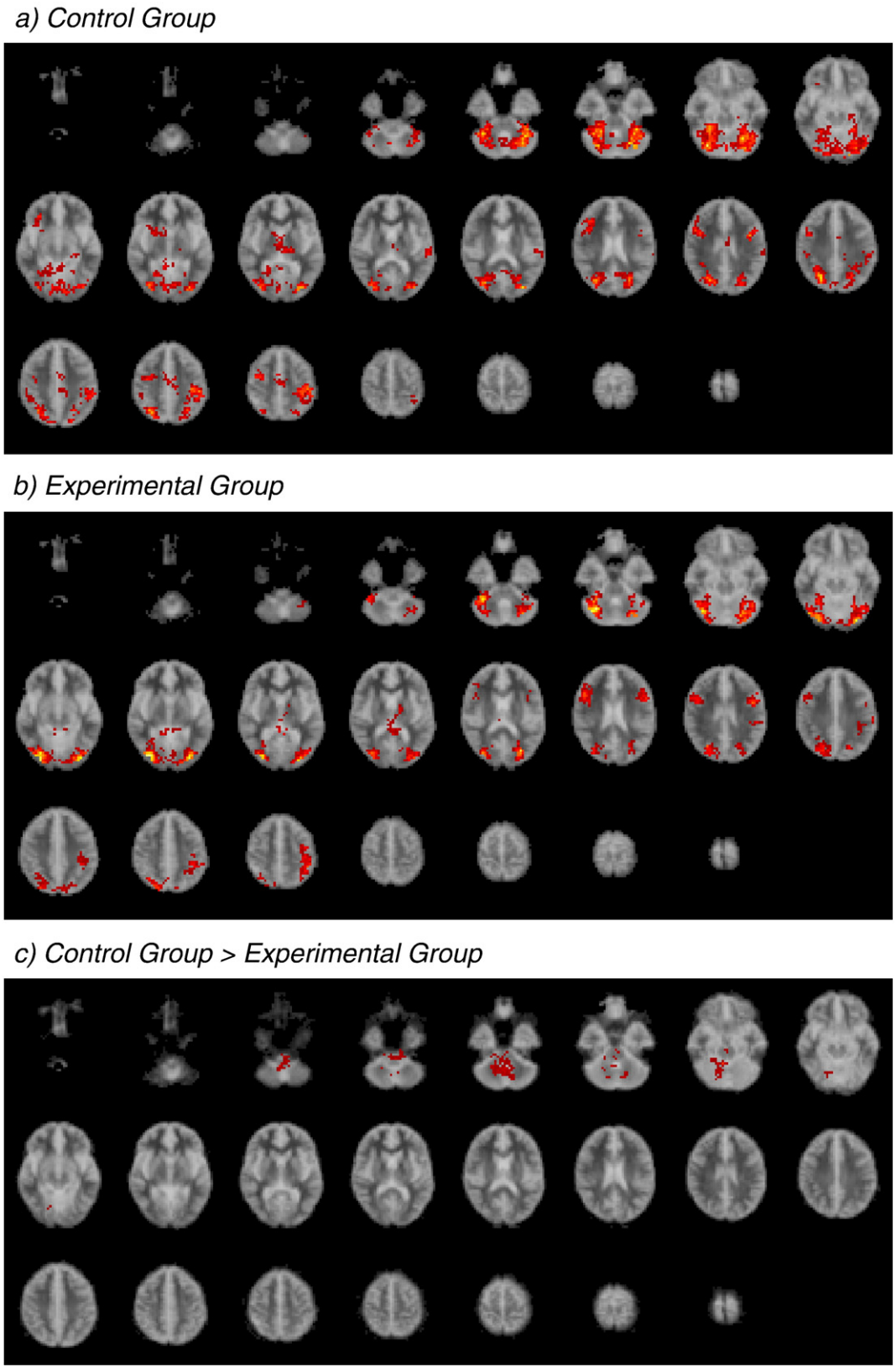

Fig. 5. Brain regions activated during successfully recognized trials 8 weeks after baseline scan (Time 2). The brain regions activated during successful recognition in both groups at Time 2 are displayed in a and b. These regions are similar to 5a and b above. Subpanel c illustrates the increase in brain activation in the control group, compared to the experimental (GnRHa) group at Time 2 (see Table 4 for precise regions). There is no evidence of a reciprocal increase in other brain regions in the experimental group.

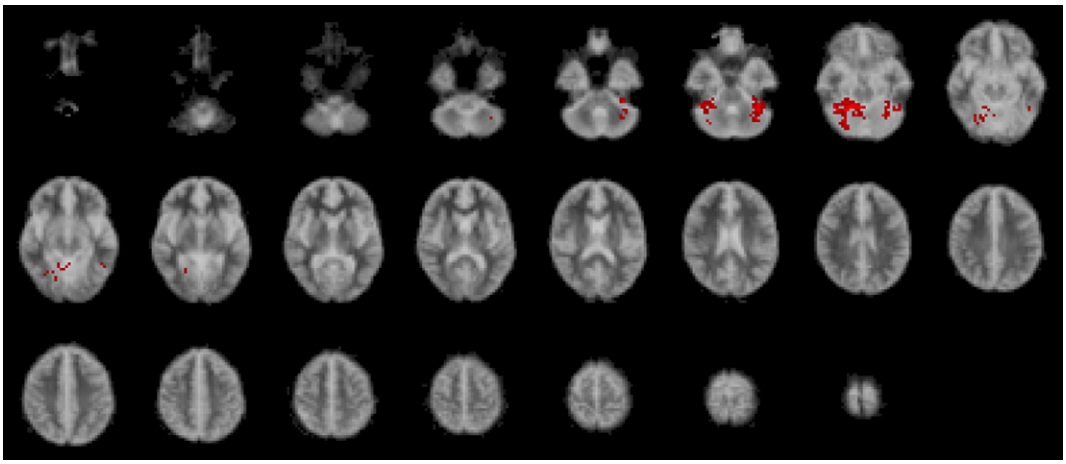

Fig. 6. Between group analysis of brain regions activated during successfully recognized trials at baseline (Time 1) versus 8 weeks later (Time 2). This figure illustrates the difference between brain activation during correctly recognised trials in the control group (Time 1 vs. Time 2) versus the same comparison in the experimental group. It highlights a significant group-by-time interaction in bilateral cerebellum and fusiform gyrus and at the left inferior temporal gyrus (see Table 5 for further detail).

absence of an interaction at this brain region was not due to the failure of our task to activate this area. Although this finding was unexpected there are several possible explanations that may account for the disparity with other studies. Firstly in the current investigation we only included healthy young pre-menopausal women, whereas two previous studies analysed young women with either pre-existing mood disorders (Berman et al., 1997) or older (postmenopausal) women receiving supplemental hormone therapy (Smith et al., 2006). These differences in experimental design are significant as PFC function is attenuated by both low mood (Frangou, 2006) and older age (Pardo et al., 2007). Secondly, we studied the effects of GnRHa following 8 weeks of administration (as opposed to up to the 12 weeks used by others (Berman et al., 1997)) and the effects of GnRHa may be greater after longer periods (Palomba et al., 2004). Thirdly we used an event-related design whereas previous studies employed a block design (Berman et al., 1997; Smith et al., 2006). Hence our design, unlike previous studies, permitted analysis of the effects of GnRHa on brain activation during *successful* recognition independent of other working memory processes and behavioral confounds.

Neuroendocrinological basis of the effect of GnRHa on VWM

Although GnRHa is used to model the effects of early menopause the changes in brain function reported in this study may have been attributable to the *direct* effects of GnRHa or the *indirect* effects of reduced luteinising hormone (LH), follicular stimulating hormone (FSH) and/or ovarian hormones on the brain. Distribution studies in rats report that, under normal circumstances, GnRHa does not cross the blood brain barrier (BBB) (Barnfied et al., 1988). This suggests that our findings are likely to be attributable to the indirect endocrine effects of GnRHa as ovarian hormones (estrogen and progesterone), FSH, LH all cross the BBB. Among these potential candidates, perhaps the strongest case can be made for implicating reduced ovarian hormones, and particularly estrogen, as the key neuroendocrine determinant of memory loss and brain dysfunction. This suggestion is supported by other imaging studies which reported that the development and activity of the precuneus, PCC and cerebellum is modified by sex steroids.

For example, we reported that young women with Turner's syndrome (a sex chromosome aneuploidy characterised by ovarian dysgenesis) have reduced gray matter volume in the precuneus (Cutter et al., 2006) suggesting that this region may be particularly sensitive to ovarian hormones. However, others have also reported that although women with Turner's syndrome (compared to controls) have decreased gray matter they have *increased* precuneus activation during spatial orientation tasks (Kesler et al., 2004). The complexity of this relationship is further highlighted by functional brain imaging studies into the effects of estrogen treatment (ET) on postmenopausal women which have reported both increased (Shaywitz et al., 1999) and decreased (Resnick et al., 1998) precuneus activation during working and episodic memory tasks, respectively. The reasons for the inconsistencies between studies with respect to the modulatory effect of sex steroids on the anatomy and function of the precuneus in women are unclear; but they may include differences in the characteristics of subjects studied (e.g. age and previous ET use) and task-specific features (e.g. type of memory studied and the cognitive demands of the task).

In addition to the reported effects of sex steroids on the precuneus studies into the *acute* effects of ET on VWM in postmenopausal women have also reported increased activation of the PCC in women randomly assigned ET (i.e. compared to placebo) (Joffe et al., 2006; Shaywitz et al., 1999). Further, longer term administration of ET has been associated with increased metabolism (Rasgona et al., 2001) and vesicular acetylcholine transporter (VAChT) binding (a marker of cholinergic function) at the PCC (Smith et al., 2001). This suggests that the PCC is also sensitive to estrogen, and that

modulation of the cholinergic system at this region be one of the biological mechanisms underlying the effects of ET.

Finally, studies in post-menopausal women by our group (Craig et al., 2003) and others (Ghidoni et al., 2006) have also reported reduced cerebellar gray matter volume in women who are ET naïve (as compared to women treated with long term estrogens); suggesting that the anatomy of the cerebellum is also affected by estrogen in adult women. In addition to its effects on brain structure ET has also been reported to increase bilateral cerebellar activation during VWM (Smith et al., 2006).

Limitations of the study

Our study has several limitations (Craig et al., 2007). First, to better understand the neurobiological mechanisms underlying the negative effects of GnRHa on function in precuneus, PCC and cerebellum, it would be helpful to have included an add-back estrogen condition. However, this study was limited by the length of time that women were on a waiting list to receive GnRHa and surgery. Secondly, our study may have benefited from analysing the effects of GnRHa over a longer period of administration. Previous studies suggest, for example, that after 6 months GnRHa may have even more profound negative effects on cognition than we detected leading, for example, to reduced scores on the Mini Mental State Examination (Palomba et al., 2004). However, it was not practically possible to keep otherwise healthy women taking GnRHa for that length of time in this study. Finally, it would have been helpful to have measured the certitude of subjects' responses in the recognition component of the task in order to rule out the possibility that the apparent reduction in brain activity following GnRHa being indirectly due an increased tendency to guess (i.e. as opposed to being directly attributable to an effect of GnRHa on the brain).

Summary

Our study suggests that the memory difficulties reported by some women following GnRHa and at times of acute ovarian hormone withdrawal (e.g. following surgical menopause and postpartum) have a neurobiological basis. This may involve attenuation of activation in precuneus and PCC activity during successful visual encoding and cerebellar activity during recognition / VWM. Further research is required to determine the mechanism(s) through which estrogen may effect this modulation.

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