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Selective Estrogen Receptor Modulation Increases Hippocampal Activity during Probabilistic Association Learning in Schizophrenia

Jochen Kindler[1,2,3], Cynthia Shannon Weickert[1,2,4], Ashley J Skilleter[1,2,4], Stanley V Catts[5], Rhoshel Lenroot[1,2,4] and Thomas W Weickert*[,1,2,4]

[1]School of Psychiatry, University of New South Wales, Randwick, NSW, Australia; [2]Neuroscience Research Australia, Randwick, NSW, Australia; [3]Department of Psychiatric Neurophysiology, University of Bern, Bern, Switzerland; [4]Schizophrenia Research Institute, Darlinghurst, NSW, Australia; [5]School of Medical Science, University of Queensland, Brisbane, QLD, Australia

People with schizophrenia show probabilistic association learning impairment in conjunction with abnormal neural activity. The selective estrogen receptor modulator (SERM) raloxifene preserves neural activity during memory in healthy older men and improves memory in schizophrenia. Here, we tested the extent to which raloxifene modifies neural activity during learning in schizophrenia. Nineteen people with schizophrenia participated in a twelve-week randomized, double-blind, placebo-controlled, cross-over adjunctive treatment trial of the SERM raloxifene administered orally at 120 mg daily to assess brain activity during probabilistic association learning using functional magnetic resonance imaging (fMRI). Raloxifene improved probabilistic association learning and significantly increased fMRI BOLD activity in the hippocampus and parahippocampal gyrus relative to placebo. A separate region of interest confirmatory analysis in 21 patients *vs* 36 healthy controls showed a positive association between parahippocampal neural activity and learning in patients, but no such relationship in the parahippocampal gyrus of healthy controls. Thus, selective estrogen receptor modulation by raloxifene concurrently increases activity in the parahippocampal gyrus and improves probabilistic association learning in schizophrenia. These results support a role for estrogen receptor modulation of mesial temporal lobe neural activity in the remediation of learning disabilities in both men and women with schizophrenia.

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INTRODUCTION

Probabilistic association learning requires gradual learning of probabilistic-based cue–outcome associations that is dependent on frontal–parietal–striatal neural activity in healthy adults (Fera *et al*, 2005; Poldrack *et al*, 1999). Probability estimation (ie, determining the likelihood that a particular event will occur) is a form of inductive reasoning that is related to categorization (Smith, 1989), integral to normal thought processing, and central to daily function, for example, when determining whether or not to prepare for a rainy day on the basis of dark clouds in the sky or when determining how to respond appropriately on the basis of social cues displayed by other people (Behrens *et al*, 2008; Poldrack *et al*, 1999; Weickert *et al*, 2009). People with schizophrenia display impaired probabilistic association learning concurrent with a reduction in frontal–parietal–striatal neural activity (Weickert *et al*, 2009, 2010). However, people with schizophrenia who are capable of learning the probabilistic associations at levels comparable to healthy controls display increased activity in an alternate neural network that includes rostral prefrontal cortex, anterior cingulate, and the parahippocampal gyrus (Weickert *et al*, 2009). Identification of ways to increase activity in nodes of this alternative neural network may bring about learning benefits in schizophrenia. As sex hormones can have potent effects on cognition and brain physiology (Li and Singh, 2014; Sherwin, 2003), we predicted that treatment with a selective estrogen receptor modulator (SERM) could increase learning and memory-related brain activity in men and women with schizophrenia.

Many convergent, indirect lines of evidence from clinical behavioral data to molecular neuropathology suggest that estrogen signaling may be altered in the brains of people with schizophrenia. First, the onset of schizophrenia typically occurs in close proximity to puberty during late adolescence (Lieberman *et al*, 2001), suggesting that sex steroid-triggered maturational changes may unmask vulnerability. Additionally, gender differences in symptoms of psychosis (Goldstein and Link, 1988), age of onset, and course of schizophrenia have been well documented (Gur *et al*, 1996; Hafner, 2003). Low estrogen/testosterone levels in females/males with

*Correspondence: Dr TW Weickert, School of Psychiatry, University of New South Wales, Neuroscience Research Australia, Barker Street, Randwick, NSW 2031, Australia, Tel: +61 2 9399 1730, Fax: +61 2 9399 1034, E-mail: t.weickert@unsw.edu.au
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schizophrenia, respectively, are related to worse negative symptoms and cognition (Ko *et al*, 2006; Moore *et al*, 2013). Significant genetic associations of estrogen receptor alpha (ESR1) polymorphisms with schizophrenia and changes in ESR1 mRNA in the brains of people with schizophrenia have also been reported (Perlman *et al*, 2005, 2004; Weickert *et al*, 2008). Taken together, these findings suggest that there may be blunted sex steroid responses in the brains of people with schizophrenia.

In humans, circulating estrogen is a potent regulator of emotional responses (Amin *et al*, 2005), working memory (Keenan *et al*, 2001), and frontal cortex activity (Berman *et al*, 1997), all of which are altered in major mental illness. Estrogen therapy has been shown to improve recovery from acute psychotic symptoms by reducing both positive and general anxiety symptoms of schizophrenia primarily in older females, and also in women of childbearing age (Kulkarni *et al*, 2014). In general, estrogen has been shown to improve cognitive function in non-psychotic elderly women (Hogervorst *et al*, 2000; Matthews *et al*, 1999), particularly when postmenopausal treatment is initiated early (Anderson *et al*, 2004). However, the usefulness of hormone-based treatments to improve cognition in premenopausal women with schizophrenia and in men with schizophrenia is unknown.

Raloxifene is an SERM approved for use as an osteoporosis preventative at a daily oral dose of 60 mg. A daily dose of 120 mg raloxifene has also been shown to be effective in preserving brain activity and maintaining cognitive function in older men and women (Goekoop *et al*, 2006; Yaffe *et al*, 2005). Raloxifene supplementation has also been shown to reduce positive and negative psychotic symptom severity in postmenopausal women with schizophrenia (Kulkarni *et al*, 2010; Usall *et al*, 2011). Moreover, a recent study reported beneficial effects of raloxifene on memory and executive function in postmenopausal women with schizophrenia (Huerta-Ramos *et al*, 2014). Additionally, we have found that 120 mg per day of adjunctive raloxifene administration improved both verbal memory and attention in men and women with schizophrenia (Weickert *et al*, 2015).

In the present study, adjunctive raloxifene was administered to men and women with schizophrenia at a daily oral dose of 120 mg using a randomized, double-blind, placebo-controlled, cross-over design functional magnetic resonance imaging (fMRI) study during probabilistic association learning. Our hypothesis was that administration of the SERM raloxifene would improve probabilistic association learning and reverse abnormal brain activity in people with schizophrenia. An additional confirmatory analysis in a larger sample of people with schizophrenia and healthy controls was performed to show that altered regional brain activity identified during raloxifene treatment was related to learning in schizophrenia.

PARTICIPANTS AND METHODS

Treatment Trial: Effects of Raloxifene on Neural Activity in Schizophrenia

Participants. Twenty-five chronically ill adults with a diagnosis of schizophrenia or schizoaffective disorder were recruited into the study. All patients met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for schizophrenia or schizoaffective disorder on the basis of the Structured Clinical Interview for DSM-IV Axis 1 disorders (SCID) (First, 2007). All participants were screened for exclusion criteria, which included a concurrent DSM-IV Axis I diagnosis other than schizophrenia or schizoaffective disorder, history of uncontrolled diabetes or cardiovascular disease including hypertension, recent alcohol/substance abuse (within the past 5 years), head injury with loss of consciousness, epileptic seizures, structural brain abnormalities, developmental disorders, mental retardation, and/or central nervous system infection. A four subtest version of the Wechsler Adult Intelligence Scale, 3rd Edition (WAIS-III) (Wechsler, 1997) and the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001) were administered to all participants to obtain estimates of current IQ and premorbid IQ in schizophrenia. All people with schizophrenia were receiving antipsychotic medication (95% receiving second-generation antipsychotics) for at least 1 year before participation. All participants had normal vision or their vision was corrected to normal with MRI-compatible lenses.

Mean daily dose of antipsychotic medication for each person with schizophrenia was converted to approximate daily mean chlorpromazine (CPZ) equivalents dose using standard guidelines (Leucht *et al*, 2003). Symptom severity in people with schizophrenia was assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay *et al*, 1987) by a psychologist or psychometrician trained in administration and scoring. All procedures were approved by the University of New South Wales and the South Eastern Sydney and Illawarra Area Health Service Human Research Ethic Committees. The procedure was explained and written informed consent was obtained from each participant before entry into the study.

Treatment trial design. In a 13-week, randomized, placebo-controlled, double-blind, cross-over trial, with a 1-week 'wash-out' period, patients received 120 mg encapsulated raloxifene (2×60 mg, oral administration) daily in the active condition and oral administration of two placebo (lactose) capsules daily in the placebo condition as an adjunctive treatment to their current antipsychotic medication. Following treatment in the first 6-week phase, patients entered a 1-week wash-out followed by the second 6-week phase consisting of the alternate treatment (raloxifene or placebo). See Figure 1 for the study flow diagram. This study assessed a subset of patients who received the fMRI scan from the larger clinical trial of raloxifene (Weickert *et al*, 2015).

Participants were monitored on a weekly basis to assess potential adverse events and self-reported compliance (compliance was also determined by returned pill counts). Additional methods for monitoring treatment compliance were achieved through the collection of blood samples at baseline and at the 6- and 13-week assessments for circulating hormones and clotting factors affected by treatment. The trial was registered before initiation with the Australian and New Zealand Clinical Trials Registry.

Probabilistic association learning 'weather prediction' test. Participants completed a probabilistic association learning 'weather prediction' test in the MRI scanner that alternates the experimental and perceptual–motor control tests as

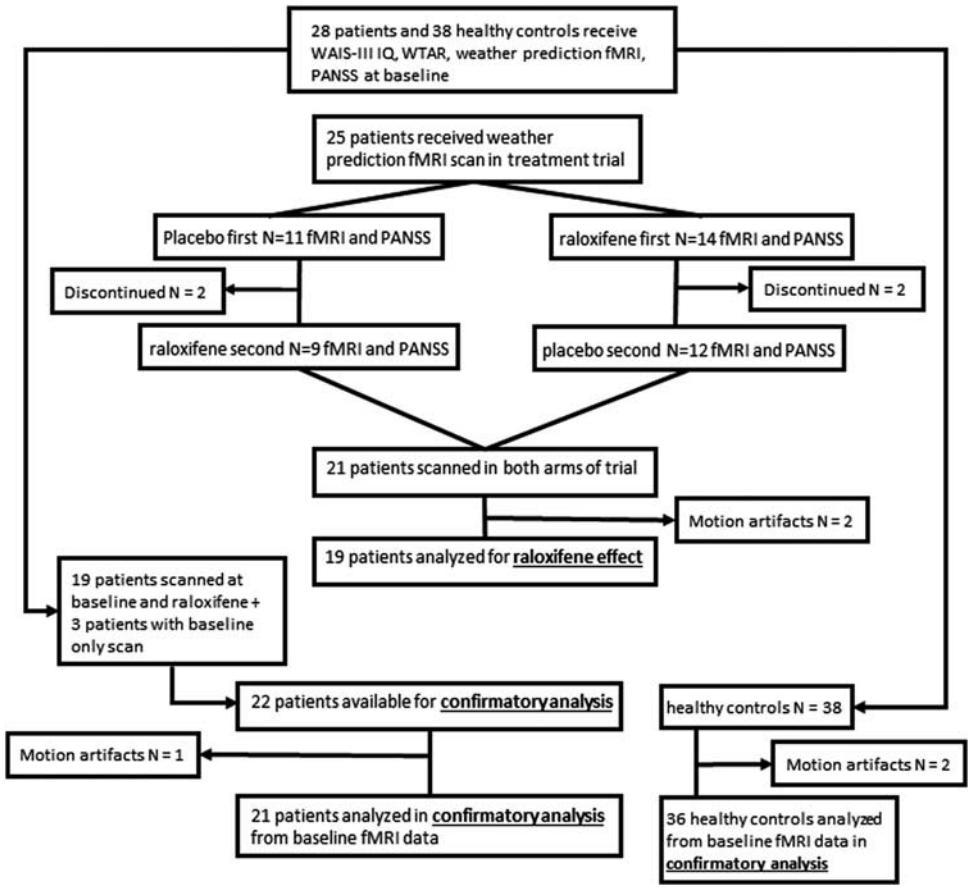

Figure 1 Flow diagram of raloxifene treatment trial and confirmatory analysis.

described in detail previously (Fera *et al*, 2005; Poldrack *et al*, 1999; Weickert *et al*, 2009). The task used was identical to that used in these previous studies with the exception that the probability schedule for the task was modified for use in repeated fMRI sessions. Before the functional MRI scan all participants were instructed that the test would require them to predict an outcome of rain or shine on the basis of four cue cards composed of simple geometric shapes that were presented individually or in combinations of up to 3 cards. They were told that they should guess at first but they would gradually improve at determining which cue card combinations predicted a particular outcome. The relationships between cue card combinations and their outcomes ('rain' or 'shine') were predetermined on a probabilistic basis. Card combinations were presented pseudorandomly with the constraint that identical cue combinations would not appear consecutively and identical outcomes were limited to five consecutive occurrences. For the perceptual–motor control test, participants were told that they would be required to determine whether or not two of four unique and identical cue cards were presented during each trial (responding 'two' or 'not two'), with the cue cards being presented either individually or in combinations of up to three cards. For additional details, see Supplementary Methods.

Scanning procedure. A 3 Tesla Phillips Achieva MRI scanner with a 8-channel birdcage head coil at Neuroscience Research Australia (Randwick, NSW, Australia) was used to acquire 162 whole-brain EPI images, TR/TE = 3000/30; 45 interleaved slices, thickness = 3 mm, gap = 0.3 mm; voxel size $2.14 \times 2.14 \times 3$ mm^3; flip angle = 90°; FOV = 240 mm. A T1-weighted high-resolution anatomical scan was obtained for each participant for registration purposes, TR/TE = 5.3/2.4; 180 slices, thickness = 1 mm, no gap; voxel size $1 \times 1 \times 1$ mm^3; FOV = 256 mm. Scans comparing active and placebo conditions were performed at weeks 6 and 13. The weather prediction task scanning procedure followed previously described methods (Weickert *et al*, 2009). The experimental paradigm consisted of 16 blocks of 30 s each in which weather prediction blocks (6 trials/block) alternated with the control task (6 trials/block). There were 48 weather prediction and 48 perceptual–motor control trials in total, equating to 8 blocks of each condition. One hundred and sixty-two scans were collected in a total scan time of 8.25 min. Missed trials were not included in the scoring.

Statistical analyses

Demographics and behavior. Fisher's exact test, paired *t*-tests, and two-sample *t*-tests were calculated, as appropriate. For the behavior analysis, a linear mixed model was used with restricted maximum-likelihood estimation and a covariance type of heterogeneous first-order autoregressive with percent correct at each trial block as dependent variables. Model selection was based on Schwarz's Bayesian criterion. Results were Sidak adjusted for multiple testing.

Significance level was set at $p<0.05$. Statistics were performed with SPSS 22.0.

fMRI processing and first-level analysis. Preprocessing was performed with SPM8 (Wellcome Trust Centre for Neuroimaging), running under MATLAB version 2010b. Functional images were realigned to the first image in the sequence, coregistered to the T1 anatomical scan, and normalized. Three dummy scans were obtained before each fMRI data acquisition to allow for the equilibration of the MRI signal. Images were smoothed with an 8 mm FWHM Gaussian Kernel. Anatomical scans were also screened for structural abnormalities by a radiologist. All data sets were screened for artifacts, excessive movement (>3 mm along x, y, or z axes), and unsuccessful normalization.

Data were analyzed from 19 of the 25 patients who completed both treatment conditions (4 did not complete both conditions) and displayed no imaging artifacts (2 displayed excessive motion artifacts). In the first-level analysis, whole brain voxel-wise analyses were performed for each subject using a t-statistic, producing a statistical image for the contrast of weather prediction minus perceptual–motor control to arrive at the relative activation specific to probabilistic association learning. Movement parameters created during preprocessing were applied as regressor covariates. These individual contrast images were then used in a second-level random-effects model that accounts for both scan-to-scan and subject-to-subject variability.

fMRI second-level analysis. For the main analysis, a paired *t*-test was performed comparing patients receiving raloxifene treatment with the same patients receiving placebo, whole brain, voxel-wise analysis, FWE corrected ($p<0.05$), with performance as a covariate. Therefore, difference maps of the first-level contrasts (raloxifene–placebo) using the difference of overall performance (raloxifene–placebo) as covariate was entered in the second-level one-sample *t*-test analysis.

Confirmatory Analysis: Relationship Between Hippocampal Activity and Probabilistic Association Learning in People with Schizophrenia and Healthy Controls

Participants. Twenty-two people with schizophrenia or schizoaffective disorder and 38 healthy controls were scanned to perform a confirmatory analysis of the relationship between hippocampal activity and probabilistic association learning in schizophrenia. Baseline data from 19 of the patients in the treatment trial were used in the analysis of patients *vs* healthy controls. Imaging data from an additional three patients who did not participate in the treatment trial were also included in this analysis. Patient inclusion/exclusion criteria for this analysis followed the same procedures as those provided for the treatment trial. All people with schizophrenia were receiving antipsychotic medication (86% receiving second-generation antipsychotics) for at least 1 year before participation. Healthy controls were screened for exclusion criteria, which consisted of any DSM-IV Axis I disorder, having a first-degree relative with a diagnosis of schizophrenia and the other exclusions listed for patients in this study. All participants had normal vision or their vision was corrected to normal with MRI-compatible lenses.

Behavioral task, imaging acquisition, and processing. The behavioral task, imaging acquisition, and processing were performed as described for the treatment trial. After quality assessment, three participants (two healthy controls and one patient) were excluded from the fMRI analysis owing to artifacts leaving a total of 21 patients and 36 healthy controls for the analyses.

Statistical analyses

Demographic and behavioral analyses. The demographic and behavioral analyses were similar to those described for the clinical trial with the exception that group comparisons were between patients and healthy controls and in addition to percent correct at each trial block, the slope (percent correct at trial block 8 minus percent correct at trial block 1) was also used as a measure of learning.

fMRI analyses. First-level fMRI analyses were similar to those described for the clinical trial. Initially, whole brain one-sample *t*-tests were performed separately for healthy controls and people with schizophrenia. Given the increased activity in the parahippocampal gyrus with raloxifene treatment (see Results), a region of interest (ROI) analysis using the parahippocampal gyrus as per WFU PickAtlas (Maldjian *et al*, 2003) was performed comparing patients and controls in a two-sample *t*-test, with age and behavior as covariates to determine the diagnostic difference in this brain region. The confirmatory analyses used linear regressions of the BOLD signal during probabilistic association learning with the learning slope used as a covariate of interest, in the patient and control groups separately to determine the relationship between activity and learning in each diagnostic group. Following up the results of the treatment trial showing increased parahippocampal and hippocampal activity with raloxifene administration in schizophrenia and on the basis of previous findings (Weickert *et al*, 2009), we predicted a positive association of neuronal activity in the parahippocampal ROI with cognitive performance in the people with schizophrenia. The peak activation (x, y, z = 27, −16, −24) in the raloxifene treatment trial was used as an ROI with a sphere of 15 mm for small volume correction of the results for the confirmatory regression analysis. Finally, the correlation strengths between groups were compared directly using a Fisher *r*-to-*z* transformation after extracting β-estimate parameters for the parahippocampal gyrus ROI and calculating Pearson's correlations between activation and learning slope.

RESULTS

Treatment Trial: Effects of Raloxifene on Neural Activity in Schizophrenia

Behavior analysis, compliance, and adverse events. For demographics of the patients in the raloxifene treatment trial, see Table 1a. All patients were chronically ill, treated with antipsychotic medication (95% receiving second-generation antipsychotics), and displayed mild-to-moderate symptom severity based on their PANSS scores. In relation to probabilistic association learning (see Figure 2a), there was a significant main effect of treatment with patients showing significantly better overall performance during raloxifene (mean = 59.4; SD = 2.1) relative to placebo

Table 1a Demographic Summary of Patients in Raloxifene Treatment Trial

	Schizophrenia, $n=19$ baseline	Placebo treatment	Raloxifene treatment	D.f.	t-Value	*P-value*
Age (years)	37.6 (7.6)	—	—	—	—	—
Gender	12M/7f	—	—	—	—	—
Education (years)	13.2 (2.3)	—	—	—	—	—
WAIS-III IQ	90.5 (11.1)	—	—	—	—	—
WTAR	103.4 (6.3)	—	—	—	—	—
Age of onset (years)	23.9 (6.4)	—	—	—	—	—
Illness duration (years)	13.8 (6.4)	—	—	—	—	—
CPZ	675.5 (597.1)	683.2 (590.8)	653.4 (578.8)	18	0.156	0.878
PANSS total	60.9 (17.2)	58.0 (14.6)	57.7 (15.1)	18	0.060	0.953
PANSS positive	14.6 (5.6)	14.6 (6.0)	13.7 (5.10)	18	0.457	0.653
PANSS negative	15.1 (6.1)	14.7 (4.6)	15.0 (5.8)	18	−0.145	0.886

Notes: Means ± SDs within parentheses.
CPZ, mean daily chlorpromazine equivalent dose; PANSS, Positive and Negative Syndrome Scale; WAIS-III, Wechsler Adult Intelligence Scale, 3rd Edition; WTAR, Wechsler Test of Adult Reading.

(mean = 53.0; SD = 2.9), $F_{1,102.0}=8.0$, $p=0.006$; however, there was no trial block by treatment condition interaction ($F_{7,59.7}=0.96$, $p=0.47$). Additionally, there was no significant difference between placebo and raloxifene treatment groups in relation to PANSS positive, negative, or total symptom severity scores or mean daily CPZ dose (see Table 1a).

Based on the pill counts, compliance was 94.6% in the placebo condition of the trial and 96.9% in the raloxifene condition for this fMRI study. Raloxifene was associated with a statistically significant difference in follicle-stimulating hormone based on a treatment by sex analysis and a trend towards significant differences in prothrombin time and anti-thrombin III (see Supplementary Table S1 for details); however, these statistically significant or near-significant changes with raloxifene treatment were determined to not be clinically relevant. There were no significant differences between the frequencies of adverse events in patients during raloxifene *vs* placebo conditions. For a detailed table of adverse events, see Supplementary Table S2.

fMRI. The comparison of raloxifene with placebo treatment conditions demonstrated increased fMRI BOLD activity during the raloxifene condition in the mesial temporal lobe including the right parahippocampal gyrus/hippocampus (x, y, z = 27, −16, −24, $T=8.29$, $z=5.18$, FWE corrected (whole brain), $p=0.009$; see Figure 2b). Supplementary Table S3 shows details of significant activation changes elicited by raloxifene administration relative to placebo in brain regions surviving the FWE correction for multiple comparisons.

Confirmatory Analysis: Relationship Between Hippocampal Activity and Probabilistic Association Learning in People with Schizophrenia and Healthy Controls

Demographics and behavior. Demographics of the patients and healthy controls for this confirmatory analysis are provided in Table 1b. There were significant differences between groups in relation to age, education, and IQ. During probabilistic association learning, there was a significant main effect of group in relation to performance with patients showing worse overall performance than controls ($F_{1,57.5}=4.0$, $p=0.05$), but there was no significant diagnostic group by trial block interaction ($F_{7,188.2}=1.2$, $p=0.28$). The mean learning slopes in patients and healthy controls were 3.1 ± 6.4% (SEM) and 5.9 ± 4.2% (SEM), respectively, NS, $p=0.71$.

fMRI. Results of the one-sample *t*-tests for healthy controls *vs* patients are shown in Figure 3 and Supplementary Table S4. During probabilistic association learning healthy controls predominantly activated the dorsolateral prefrontal cortex, the occipital cortex, the parietal cortex, and the basal ganglia (striatum). Deactivations in the healthy control group were detected in the lateral and medial temporal lobe, the hippocampus, and the medial frontal lobe. Patients activated the dorsolateral prefrontal cortex, the occipital cortex, and the parietal cortex to a lesser extent, whereas no significant activations were detected in the basal ganglia/striatum.

The two-sample *t*-test analysis comparing brain activity in healthy controls and people with schizophrenia within the ROI yielded significantly increased BOLD activity in people with schizophrenia in the bilateral parahippocampal gyrus ($T=2.5$, $p=0.008$, small volume corrected; see Supplementary Figure S1 and Supplementary Table S5). The regression analysis based on the slope of the probabilistic association learning curve and parameter estimates from the parahippocampal gyrus ROI showed a significant relationship in patients ($T=1.86$, $p=0.04$, $n=21$) but not in healthy controls ($T=-0.63$, $p=0.73$, $n=36$) (see Supplementary Figure S2 for the Pearson's correlation analysis results). The peak voxel activation based on the regression analysis was detected in the right parahippocampal gyrus in the patients, which was in close proximity and slightly overlapping with the area reported in the treatment trial (x, y, z = 27, −9, −17, $T=4.25$, $Z=3.52$, small volume FWE corrected, $p<0.05$; see Figure 4). Results of the Fisher *r*-to-*z* transformation analysis showed that the correlations between learning and brain activity were significantly different between the two diagnostic groups ($z=1.81$, $p=0.04$).

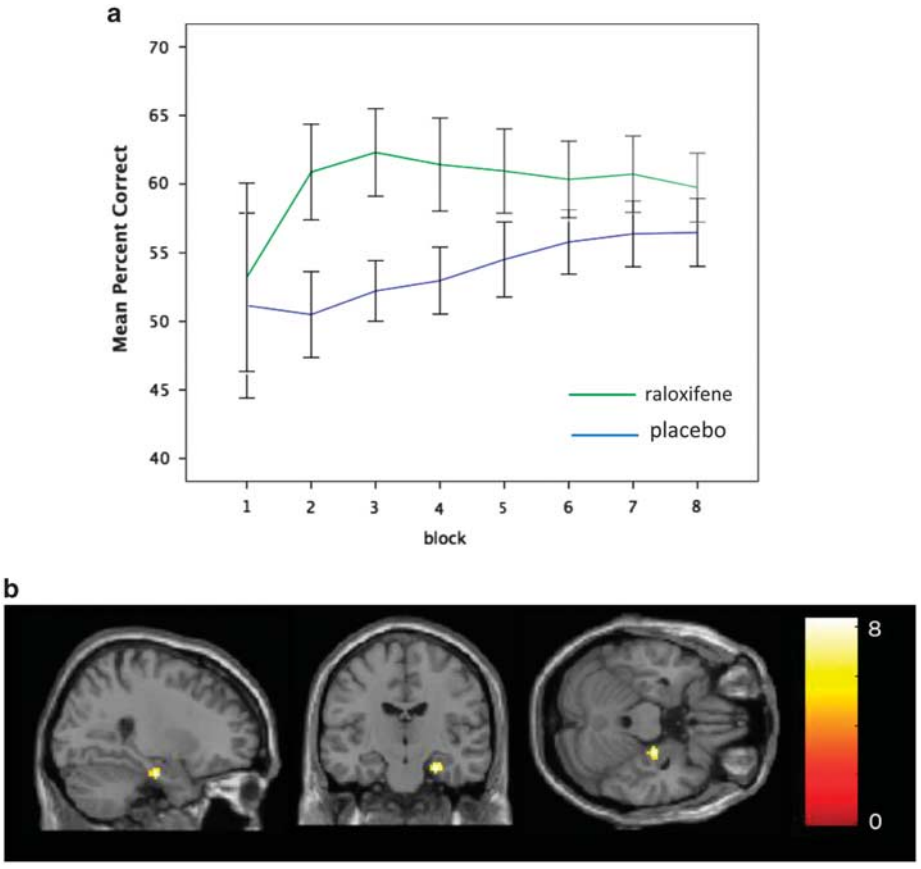

Figure 2 (a) Effects of raloxifene relative to placebo on probabilistic association learning in people with schizophrenia. Cumulative percentage correct at each functional magnetic resonance imaging (fMRI) trial block is shown during probabilistic association learning in the placebo (blue line) and raloxifene (green line) conditions. (b) Effects of raloxifene relative to placebo on neural activity during probabilistic association learning in people with schizophrenia. Raloxifene > placebo, (x, y, z = 27, − 16, − 24), $T = 8.29$, $z = 5.18$, family-wise error rate (FWE) corrected (whole brain), $p = 0.009$, parahippocampal gyrus/hippocampus. Yellow areas show significantly increased blood oxygenation level-dependent (BOLD) signal during raloxifene treatment relative to placebo. The corresponding cluster information is provided in Supplementary Table S1.

Table 1b Demographic Summary of the Schizophrenia and Healthy Control Samples for Confirmatory Analysis

	Schizophrenia, $n = 21$	Healthy controls, $n = 36$	D.f.	t-Value/Fisher's exact	*P-value*
Age (years)	38.9 (8.5)	31.1 (7.0)	55	3.7	0.000
gender	12M/9f	17M/19f	1		0.585
Education (years)	13.7 (2.9)	15.2 (1.8)	55	2.3	0.024
WAIS-III IQ	92.2 (11.6)	105.1 (11.5)	53	4.0	<0.001
WTAR	103.7 (6.8)	109.9 (6.2)	53	3.1	0.003
Age of onset (years)	25.5 (6.9)	—	—	—	—
Illness duration (years)	14.4 (6.7)	—	—	—	—
CPZ	658.2 (567.9)	—	—	—	—
PANSS total	59.38 (15.1)	—	—	—	—
PANSS positive	15.0 (5.7)	—	—	—	—
PANSS negative	14.33 (5.3)	—	—	—	—

Notes: Means ± SDs within parentheses.
CPZ, mean daily chlorpromazine equivalent dose; PANSS, Positive and Negative Syndrome Scale; WAIS-III, Wechsler Adult Intelligence Scale, 3rd Edition; WTAR, Wechsler Test of Adult Reading.

DISCUSSION

The results from this study suggest that estrogen receptor modulation by raloxifene improves probabilistic association learning in schizophrenia by increasing fMRI BOLD signal in the parahippocampal gyrus. It is possible that raloxifene is having a direct effect on neurons within the hippocampus as the hippocampal/parahippocampal regions have been shown to have a high density of estrogen receptors

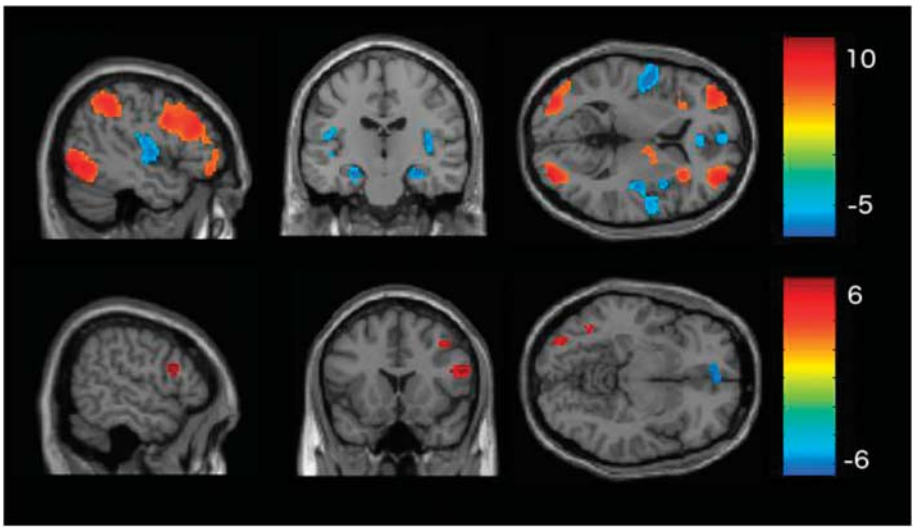

Figure 3 Regions of activation and deactivation during probabilistic association learning in healthy controls and people with schizophrenia. One-sample *t*-tests, healthy controls (upper row) and people with schizophrenia (lower row), $p < 0.05$, family-wise error rate (FWE) corrected. Activation and deactivation during probabilistic association learning—red-orange: activation; blue: deactivation. Upper row: Healthy controls, $T = 5.48$, $n = 36$, cluster size > 20. Lower row: People with schizophrenia, $T = 5.43$, $n = 21$, cluster size > 20.

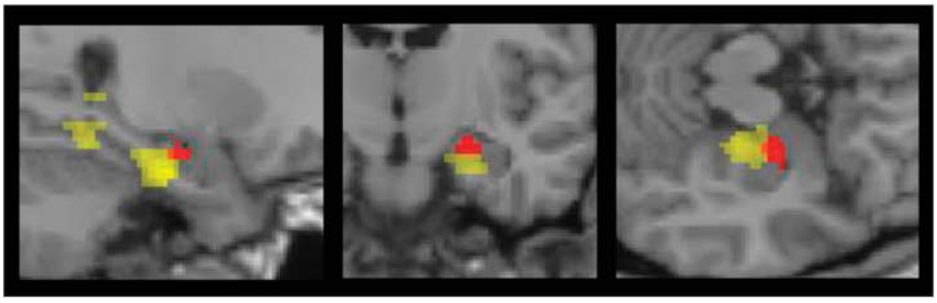

Figure 4 Overlay of the increase of hippocampal/parahippocampal activity in the raloxifene treatment group (x, y, z = 27, − 16, − 24, yellow area, raloxifene treatment) and the peak activation based on the regression of blood oxygenation level-dependent (BOLD) signal during probabilistic association learning on the learning slope in people with schizophrenia relative to healthy controls in the confirmatory analysis, (x, y, z = 27, − 9, − 17, $T = 4.25$, $Z = 3.52$, family-wise error rate (FWE), small volume corrected, sphere 15 mm, at $p < 0.05$, red area, confirmatory analysis), orange represents areas of overlap.

(Montague *et al*, 2008; Sholl and Kim, 1989). At least some of the beneficial effects of estrogen on cognition are due to neurogenesis in the hippocampus, which can be linked to improved memory and attention (Brinton, 2009). Estrogen has also been shown to increase growth factors, such as IGF and BDNF, in the hippocampus (Hao *et al*, 2007; Wang *et al*, 2004; Woolley and McEwen, 1994) and can increase pyramidal neuron spine density (Woolley and McEwen, 1994). We have found reduced estrogen receptor mRNA in the dentate gyrus and reduced BDNF in the hilus of the hippocampus in people with schizophrenia compared with controls with no significant changes of ESR-1 or BDNF in the other subfields (Perlman *et al*, 2005; Ray *et al*, 2014; Thompson Ray *et al*, 2011). Thus, it is possible that raloxifene may increase hippocampal activity by directly stimulating hippocampal neurons and improving the trophic environment in people with schizophrenia. Indeed, previous fMRI treatment trials of raloxifene (Goekoop *et al*, 2006) show increased brain activation in the parahippocampal gyrus with the administration of raloxifene during a recognition memory test in healthy elderly males and it is well known that increased brain activity can increase BDNF production (Dugich-Djordjevic *et al*, 1992; Wetmore *et al*, 1994).

Symptom severity in people with schizophrenia as assessed by the PANSS has been shown to respond to raloxifene or estrogen in other trials (Kulkarni *et al*, 2010; Usall *et al*, 2011). The present study did not demonstrate significant symptom severity reduction with administration of raloxifene; however, the mean baseline PANSS scores in the present study were of mild-to-moderate severity in general, and thus relatively low by comparison with other studies, which typically use a minimum PANSS cutoff score that selects towards more acutely symptomatic patients. The findings of our present study showing increased parahippocampal activity with raloxifene treatment relative to placebo during learning are consistent with the results of previous studies demonstrating a critical role for the hippocampal

complex in verbal memory (Welsh *et al*, 1991) and the findings from our recently published clinical trial of 80 people with schizophrenia which showed a large, significant effect of raloxifene treatment relative to placebo on verbal memory (Weickert *et al*, 2015).

Beneficial effects of raloxifene treatment on cognition have also been demonstrated in healthy elderly (Goekoop *et al*, 2006) and in dementia (Yaffe *et al*, 2005). Although another large clinical trial found no significant difference between raloxifene and placebo in relation to cognitive benefit of raloxifene in older women (Yaffe *et al*, 2001), secondary analyses showed that there was reduced risk of cognitive impairment in the higher dose group (120 mg) (Yaffe *et al*, 2005), which was the dosage used in our present study. Thus, there is accumulating evidence for beneficial effects of raloxifene treatment on cognitive abilities across a number of human conditions in which the estrogen receptor may have an important role.

Probabilistic association learning has been studied in healthy samples, for example, one study (Poldrack *et al*, 2001) demonstrated that the medial temporal lobe is initially activated during early probabilistic association learning, but it quickly becomes deactivated, whereas striatal (caudate nucleus) activity increases during the later trials of probabilistic association learning in healthy individuals. The one-sample *t*-test (see Figure 3) in healthy controls in our present study clearly shows deactivation of the medial temporal lobe and hippocampus that was not observed in schizophrenia. We have previously shown parahippocampal gyrus activation during probabilistic association learning in an independent cohort of people with schizophrenia (Weickert *et al*, 2009). Given that a subset of patients with schizophrenia showing probabilistic association learning that was equivalent to healthy controls also showed increased parahippocampal gyrus activity, the differential parahippocampal gyrus activation may have been compensatory to produce successful probabilistic association learning in schizophrenia. Several outcomes of the present study support such an interpretation. A positive correlation between learning slope and parahippocampal activity was shown in people with schizophrenia but not in healthy controls. An increase of BOLD signal in the parahippocampal gyrus in the raloxifene treatment group also supports the compensatory hypothesis that increased activity in the medial temporal lobe can support probabilistic association learning when frontal–striatal circuitry is deficient. However, as the hippocampus is an area often associated with neuropathology and tissue reduction in schizophrenia (Heckers and Konradi, 2010), it is not clear if sustained neuronal activity in this alternative region would ultimately be beneficial to people with schizophrenia. In the present study, the increased parahippocampal activity associated with raloxifene treatment appears to be beneficial to learning in schizophrenia and is consistent with compensatory function for the parahippocampal gyrus during probabilistic association learning.

Thus, the results from the present study support the view that people with schizophrenia can engage compensatory brain regions during probabilistic association learning. In the presence of reduced prefrontal–striatal activity during probabilistic association learning, people with schizophrenia appear capable of recruiting the parahippocampus, a region that is particularly involved in the processing of memory in healthy individuals. Estrogen receptor modulation by raloxifene in schizophrenia appears to increase the neural activity in the parahippocampal/hippocampus regions even further.

There are some limitations to the present study. We did not rule out any potential carryover effects in our clinical trial. However, carryover effects would have reduced the effect size rather than support the findings of increased parahippocampal activity with raloxifene treatment. Additionally, we recruited patients who were receiving antipsychotic medications and displayed mild symptom severity; thus, it is possible that the relationship between hippocampal activity and learning that we found in schizophrenia may be due to changes associated with antipsychotic administration and/or symptoms. However, there was no significant difference in mean daily CPZ dose or PANSS symptom severity scores between raloxifene and placebo treatment conditions. Healthy controls were not administered raloxifene. This raises the question as to whether raloxifene would produce similar brain activity changes in healthy controls.

In conclusion, this is the first study to report that adjunctive treatment with the SERM raloxifene improves probabilistic association learning and increases parahippocampal/hippocampal gyrus activity in men and women with schizophrenia. Thus, selective estrogen receptor modulation by raloxifene appears to be able to benefit cognitive processing by facilitating neural activity in a brain region that is estrogen receptor sensitive and capable of compensating for deficient frontal–striatal activity during probabilistic association learning in schizophrenia.

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