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RESEARCH

The effect of estradiol add-back: a longitudinal MRI study in prostate cancer patients

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Abstract

We investigated the effect of estradiol add-back therapy (EAT) on brain activation related to cognitive function and affect in addition to putative changes in gray and white matter volume in testosterone depleted participants with prostate cancer. We conducted a randomized controlled, double-blinded trial in which 40 patients received 0.9 mg of transdermal estradiol per day for 6 months or matched placebo. Anatomical MRI and three functional MRI (fMRI) scans were obtained for the emotion recognition task, verbal memory task, and visuospatial memory task. Activation in corresponding cognitive and affective brain networks was demonstrated for all tasks. Longitudinally, there was no difference in brain activation, reaction time, or accuracy in response to the fMRI tasks between the EAT group and placebo group at 6 months. In addition, there was no detectable change in whole-brain gray or white matter volume or in hippocampal volume between the two groups after 6 months. This study supports earlier findings that EAT does not improve verbal memory or affect and has no immediate effect on hippocampal volume in testosterone depleted patients with prostate cancer.

Keywords: prostate cancer; MRI; androgen deprivation; estradiol

Introduction

Androgen deprivation therapy (ADT) using gonadotropin-releasing hormone (GnRH) agonists is one of the mainstay therapies for prostate cancer (1). This therapy reduces circulating testosterone to castrate concentrations. Testosterone, and its derivatives, dihydrotestosterone (DHT) and estradiol (E2), have diverse functions in mammalian physiology (2). Androgen receptors and estradiol receptors are distributed in cortical and subcortical brain regions responsible for cognitive function and emotional regulation (3, 4, 5).

Despite conflicting reports, there is ample evidence to support an association of ADT on cognitive function and affect (6, 7). In one of the largest cohort studies, changes in serum androgens were significantly associated with cognitive decline on the Mini-Mental State Examination (MMSE) in older men after 2 years and 5 years (8). In a prospective randomized controlled trial in 106 hypogonadal men, exogenous androgen add-back therapy was associated with significant improvement in cognitive and depressive symptoms after 8 months of testosterone replacement (9).

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However, similar improvement was not found with testosterone add-back after 1 year in a larger randomized-controlled trial (10).

The evidence supporting the effect of ADT on emotional regulation and affect however, is less clear (11). In a randomized controlled trial there was no difference in depressive symptoms between hypogonadal patients receiving testosterone and patients receiving placebo after 12 months (12). Other studies have shown significantly lower levels of testosterone in depressed men and depressive symptoms improved with discontinuation of ADT (13) as well as with testosterone add-back therapy (14).

Beyond these associations, the evidence supporting direct causation by altered plasma androgens is much less certain (11). These contradictory findings are likely contributed to by methodological variation, inconsistency on which domains are studied, and conflicting approaches to analysis, with a recent review calling for improved prospective study designs and for improved tools such as functional brain imaging to enhance future prospective studies (15, 16, 17).

Neuroimaging studies improved our understanding of the neurobiological effect of ADT on cognition by revealing areas of modified cerebral perfusion, activation patterns, and morphometric changes in the human brain. In a prospective cohort study of patients with prostate cancer, ADT was associated with reduced brain activation in the medial prefrontal cortex during cognitive control in a working memory task after 6 months (18). A similar effect was detected in the posterior parietal area during a visuospatial and mental rotation task in a smaller pilot study after 9 months of ADT (19). Another study reported changes in cerebral metabolism in several areas, including reduced metabolism in the posterior cingulate and left inferior parietal lobule correlating with reduced performance on a mental rotation task (20). Reduced gray matter volume in the dorsolateral prefrontal cortex in ADT patients was also reported, an effect that was associated with longer reaction time on a working memory task (21). It is noted that these studies all utilized healthy participants for control subjects or prostate cancer patients that did not receive ADT.

An accumulating body of evidence points towards an important role for E2 in mediating the effect of androgens on cognitive function and mood. E2 is believed to exert a direct effect on hippocampal synapse density (22) and to influence memory function by controlling attention and rate of learning (23). This notion is supported by studies that show improved verbal memory with E2 replacement in prostate cancer patients on ADT (24), though a recent randomized controlled trial by our group did not confirm this effect (25). Furthermore, estradiol has an established role in regulating affect as well as cognitive function, primarily verbal and working memory, through its receptors in the temporal lobe including the hippocampus (26).

Several studies in women reported reversal of the cognitive effects of reduced androgen levels with E2 add-back therapy (EAT) across varied contexts. In one of the earlier studies in pre-menopausal women undergoing ovarian suppression, EAT restored cerebral activation to the cognitive brain network including the prefrontal cortex. Notably, activation in the hippocampus was restored with EAT but not with progesterone add-back, albeit with no significant improvement in performance (27). A similar effect of increased activation in the cognitive brain network with increasing E2 serum levels was seen in premenopausal women in the late phase of their menstrual cycle (28), and in postmenopausal women on combined menopausal hormone therapy who performed better on visuospatial and working memory tasks compared to controls (29, 30).

Together these findings indicate that ADT may have suppressive effects on cognitive function and affect and, if so established, EAT holds promise for mitigating this effect. To our knowledge, the study reported herein is the first to investigate the effect of EAT on brain activation to cognitive demands and affect in prostate cancer patients receiving ADT. We conducted a randomized controlled trial of transdermal EAT over 6 months on verbal memory, visuospatial and affective fMRI tasks. We hypothesized increased activation in cognitive and affective brain networks in response to EAT and a corresponding improvement in cognitive performance and emotion recognition. We also hypothesized that this effect is likely to be associated with a protective effect of EAT on gray matter and hippocampal morphology.

Materials and methods

Participants

The study was conducted at Austin Health, a tertiary referral hospital affiliated with the University of Melbourne. The trial was approved by the Austin Health Human Research Ethics Committee (HREC/16/Austin/98). Each participant provided written informed consent and the trial was registered with the Australian New Zealand Clinical Trials Registry (identifier No. ACTRN12616000373471). The CONSORT checklist was followed in reporting this randomized trial (Supplementary Materials, see the section on supplementary materials given at the end of this article).

Methods have been reported previously (31, 32). Briefly, participants were recruited from outpatient clinics between November 2017 and February 2020. Participants were cisgender men diagnosed with prostate cancer and receiving GnRH agonist or antagonist therapy for a minimum of 4 weeks.

Exclusion criteria included: impaired performance status (Eastern Cooperative Oncology Group Performance Status (ECOG) >2); history of venous

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thromboembolism (VTE); breast cancer; systolic blood pressure >160 or diastolic blood pressure >100; New York Heart Association class 3 or 4 angina or heart failure; stroke, transient ischemic attack, myocardial infarction, or angina within 12 months; current oral glucocorticoid treatment; prior chemotherapy; alcohol or illicit drug abuse.

Participants were randomly allocated, in a double-blinded fashion, to one of two intervention groups: (i) EAT group that received an E2 transdermal gel 1 mL (0.9 mg) per day for 6 months and (ii) matching placebo group that received placebo gel 1 mL per day. Randomization occurred by stratifying participants by ADT duration (≤3 or >3 months) and then by eligibility to undergo brain MRI scanning. Participants were then allocated by restricted randomization, using a computer-generated randomization scheme operated by clinical pharmacists independent of trial investigators, in blocks of sizes of 4, to E2 or placebo in a ratio of 1:1. The E2 gel brand used was Sandrena™ 1 mg/g estradiol (Aspen Pharmacare) and the placebo gel was a-gel™ (Fresenius Kabi) that matched the E2 gel for color, smell, and consistency. E2 and placebo gels were repackaged into identical 10 mL syringes by pharmacy. We previously showed that 28 days of treatment with 1mg of transdermal E2 patches in testosterone deplete men with prostate cancer was sufficient to restore serum E2 concentrations into the reference range reported for healthy men (33).

Power analysis

Based on our previous investigation in this group (32) and results from other observational studies investigating changes in fat mass after 6 months of ADT (34), we used a conservative difference of 4.3% and S.D. of 7.1% that yielded 43 participants per group to provide 80% power at a threshold of 0.05 for significance. We aimed to recruit 54 participants per group to allow for 20%.

Neuroimaging acquisition and preprocessing

MRI was performed for each participant in a single session with a 3T Siemens Skyra whole-body scanner equipped with 32-channel head coil at the Melbourne Brain Centre, Heidelberg Victoria, Australia. Anatomical scans were obtained first followed by fMRI scans in a total scanning time of 30 min. Foam padding was used to immobilize the participants' heads. Participants were instructed to remain still throughout the scan, to attempt all trials with minimal movement, to respond as quickly and accurately as possible and to not dwell on previous responses or mistakes.

Structural T1-weighted gradient echo images were obtained for each subject that contained 192 slices with voxel resolution of 0.9 mm^3, in-plane resolution of 256, TE 2.49 ms, TR 1900 ms and flip angle of 9°. This was followed by functional T2*-weighted MRI scans that were performed using single-shot gradient echo EPI with a TE 30 ms, TR 3000 ms, and flip angle of 90° providing a voxel size of 3 mm^3. Interleaved acquisition with 44 slices per volume was used to obtain time series of 140 volumes for the mental rotation task, 161 volumes for the verbal memory task and 134 volumes for the emotion recognition task. The fMRI experiment was performed using Presentation® software (Version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA, USA; www.neurobs.com). Reaction time and responses made by participants inside the scanner were logged for statistical analyses.

FMRI tasks

Verbal memory task

The verbal memory task (VMT) is a short-term verbal memory task that is based on word recall strategy (35). A block design was used in which four conditions were presented consecutively (Supplementary Fig. 1). The task began with a REST period in which a fixation cross appeared on the screen for 12 s. Subjects were instructed to make no response and to not think of anything in particular. This was followed by a MEMORIZE condition in which 12 single words were presented consecutively (Word List 1), one word at a time with each word lasting for 2 s giving an overall presentation time of 24 s. Words were written in 48-pt white block letters on a black background. Subjects were instructed to remember the words that appeared on the screen but to make no overt responses. A REHEARSE condition followed in which the word REHEARSE appeared on the screen for 12 s. Subjects were instructed to rehearse the words they had memorized in their heads without making overt responses or moving their mouths. Finally, a RECALL condition followed in which 12 words were presented consecutively for 24 s in total (Word List 2). Six words were distractor words and six were from the preceding MEMORIZE condition (Word List 1). Subjects were instructed to press the left button if they had seen the word in the preceding MEMORIZE condition and to do nothing if the word was new. Different words were presented in each block for a total of eight blocks lasting for 8 min and 30 s.

Emotion recognition task

A modified version of the original emotion face-matching task (36) that was published previously by others (37) was used in this study. Two active conditions representing faces with fearful and sad affect were presented with faces that showed neutral affect as the distractor. Participants were instructed to press the button corresponding to the face (right or left) with the affect that matched the affect of the target face

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(top center; Supplementary Fig. 2). Each active condition was presented in three blocks of six trials that lasted 5 s each. Each trial contained a target emotion (sad or fearful) and two probe faces: one showing the same affect as the target face and another face showing neutral affect. Active blocks were interspersed with six control blocks of 6 trials of shapes (circles and ovals) that lasted 5 s each with similar arrangement to the faces (target shape and two probes). Subjects were instructed to press the button corresponding to the shape that matched the target shape (top center; Supplementary Fig. 2). The task took 6 min and 14 s to complete.

Mental rotation task

The mental rotation task (MRT) is a visuospatial memory task that follows the original ten cube-shaped structures of Shepard and Metzler (38). The cubes are attached face-to-face to form rigid arm-like objects (Supplementary Fig. 3). Two objects were presented at different angles of rotation and participants were required to determine whether the presented objects were identical or mirror images of each other. This could be achieved by rotating the objects mentally to achieve congruence or incongruence with the other object. Participants were instructed to press the left button if the two objects were identical and to press the right button if the objects were mirror images of each other. Eight active blocks of five trials each were presented with each trial lasting 5 s. The active blocks were interspersed by seven passive rest blocks in which two identical squares inside two circles with the same degree of rotation were presented for 15 s each (Supplementary Fig. 3). Participants were instructed to do nothing during the rest blocks. The task took 6 min and 30 s to complete.

MRI analysis

Image preprocessing was performed using Statistical Parametric Mapping (SPM12; http://www.fil.ion.ucl.ac.uk/spm/) software running in Matlab v.9.5 (MathWorks, Matick, MA, USA). The steps included motion correction by affine transformation to the first image in the time series and co-registration of fMRI images with participants' structural T1 scans. Structural scans were concurrently normalized to the SPM-T1 template and the resulting transformation matrix was applied to the functional data to achieve accurate spatial normalization across participants. Structural scans were then segmented using the SPM intensity-based segmentation algorithm. The resultant combined white matter and cerebrospinal fluid (CSF) mask was subtracted from the gray matter mask with a stringent threshold of 1% so that voxels containing more than 1% white matter or CSF were removed. The resultant combined white matter and CSF mask was used to extract spurious signal from the data and was subjected to principal component analyses using a CompCor method (39). The first five components were retained from each analysis. A linear regression model that included these ten component signals and the six head motion parameters (three rotation, three translation) estimated during the head motion correction procedure and the first-order derivatives of all 16 signals were fitted on a voxel-wise basis (40).

The noise-corrected data were high-pass filtered (f > 0.08 Hz) and spatially smoothed with a Gaussian kernel of 8 mm full-width at half-maximum. All image sequences were routinely inspected for registration and normalization errors.

First-level modeling

The general linear model (GLM), as implemented in SPM12 was used to conduct a whole-brain voxel-wise analysis to define the task-activated brain regions. The blocks from each of the conditions of the three tasks (Shapes, Sad, and Fearful conditions for the ERT; Fixation, Memorize, Rehearse, and Recall conditions for the VMT and Rest; Congruent and Incongruent conditions for the MRT) for each time point were coded as individual regressors, convolved with a box-car hemodynamic response function and incorporated as covariates in a GLM that was fitted on a voxel-wise basis to the measured BOLD signal. For each participant, the activated brain network was defined as regions showing statistically significant activation during emotional faces compared to Shapes for the ERT, Memorize and Recall trials compared to Fixation for the VMT, and Incongruent trials compared to Congruent trials for the MRT.

Second-level modeling

A second-level random-effects full factorial model was built to examine the main effect of both variables (time and condition) as well as the interaction between them. Age was entered as a variable of no interest. Confirmatory t-tests followed to examine differences in activation over time. To this end, the t-test results were masked by the interaction results to ensure that detected effects are limited to the interaction term. Reaction time and accuracy scores were entered into separate models to investigate associations with activated brain network. Group-level statistical maps were subjected to voxel-wise thresholding of P < 0.001 and then were subjected to cluster-based family-wise error correction (P < 0.001) using Gaussian random field theory as implemented in SPM to correct for multiple comparisons.

Voxel-based morphometric analysis

The voxel-based morphometric (VBM) analysis aimed to detect differences in local concentration of brain tissue at the voxel level over time in response to EAT.

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The VBM analysis was conducted using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) running in Matlab v.9.5 (MathWorks) with the following steps: quality control to ensure images had appropriate scan coverage and orientation; segmentation of structural T1 images to corresponding tissue types, gray matter, white matter, and CSF; modulation to improve intersubject alignment by creating group-average template using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL (41)) followed by normalization to the MNI template and smoothing with an 8 mm FWHM smoothing kernel. Images were routinely checked for segmentation and normalization artifacts. Intracranial volume was calculated from the raw structural T1 images in the subject's native space as the total volume of gray matter, white matter, and cerebrospinal fluid.

VBM statistical analyses

To examine putative changes in gray and white matter volume in response to EAT over time, preprocessed whole-brain gray and white matter segments were entered into separate flexible factorial models with group (control, EAT) and time (baseline, 6 months) as factors and age and intracranial volumes as a covariate of no interest. In this analysis the effect of EAT on longitudinal changes in gray and white matter (treatment × time interaction) was the outcome measure. The Automated Anatomical Labeling atlas (42) was used to create a region-of-interest encompassing the hippocampus to mask the interaction results. Correction for multiple comparisons using small-volume correction as implemented in SPM12 ensued and results that survived a threshold of $P > 0.01$ were considered significant.

Results

Seventy-eight participants were randomized to EAT or placebo as reported previously (25, 32, 33). Twenty-six participants (13 randomized to EAT and 13 randomized to placebo) completed a baseline MRI scan. One participant's data in the placebo group at baseline in the ERT and one participant's data in the EAT group at 6 months in the VMT were excluded due to excessive head motion. Many subjects were asked to repeat the MRT task to ensure adherence to task instructions and due to the restricted scanning time only three subjects in the placebo group and five subjects in the EAT group completed the MRT at the two time points (CONSORT Flowchart and Supplementary Table 1). Due to the low number of subjects the analysis of the MRT was aborted.

Adverse events

There were no episodes of venous thromboembolism and no cardiovascular events in either arm during the trial.

Demographics

There was no difference between the two groups at baseline in age, years of education, serum testosterone and E2, duration of ADT, smoking, and weekly alcohol intake (Table 1).

MRI results

The VMT

Activation in the 'Rehearse' block included the hippocampus and amygdala bilaterally, the anterior cingulate cortex, ventral thalamus and the right parietal lobe (Fig. 1A and Supplementary Table 2). Similarly, the 'Recall' condition showed similar activation pattern but to a lesser extent (Fig. 1B and Supplementary Table 2). The activation pattern was consistent with previous studies showing task-dependent activation in cognitive brain networks (Hugdahl *et al.* 2015). Repeated measures ANOVA for the interaction term (group × time) did not yield a significant difference across both active conditions ($P > 0.05$). There was additionally no significant difference in activation between the placebo and the EAT group at baseline or after 6 months in any of the active conditions of the VMT.

The ERT

Sad affect was associated with activation in the somatosensory cortex, ventromedial prefrontal cortex and medial temporal lobe structures including the hippocampus and the amygdala bilaterally (Fig. 2A and Supplementary Table 3). Fearful affect, on the other hand, was associated with activation in the somatosensory cortex, prefrontal and ventromedial prefrontal cortex (Fig. 2B and Supplementary Table 3). There was no significant longitudinal difference in activation between the two groups across both affects (interaction term $P > 0.05$).

Table 1 Demographics and clinical data.

	Placebo ($n = 13$)	**EAT** ($n = 13$)	***t* stats**	***P***
Age (years) mean (S.D.)	71.4 (5.87)	68.92 (8.25)	0.89	0.38
Testosterone (nmol/L) mean (S.D.)	0.38 (0.27)	0.31 (0.14)	0.68	0.50
ADT duration (months)	2.08 (1.38)	1.77 (1.36)	0.57	0.57
Alcohol (standard drinks)	4.77 (4.8)	7.15 (9.1)	0.83	0.42
Smoking (pack-years)	13.15 (14.5)	11.54 (17.1)	0.26	0.80
BMI (kg/m^2)	29.75 (3.3)	29.8 (3.15)	0.04	0.97
E2 (pg/mL) mean (S.D.)	0.11 (0.08)	0.09 (0.04)	0.25	0.81
Education (years) mean (S.D.)	12.35 (3.3)	15.1 (4.75)	1.70	0.10

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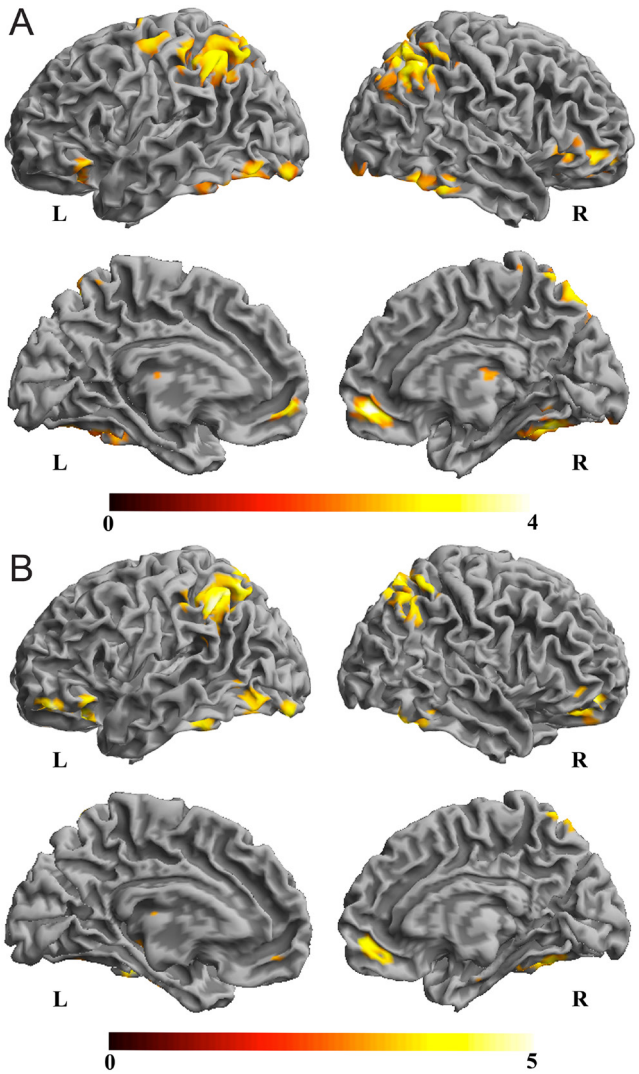


Figure 1

Statistical parametric map showing the main effect of the rehearsal (A) and recall (B) conditions of the verbal memory task (VMT) across both groups. Color bars represent standardized *Z*-scores. Right hemispheres are shown on the right. Results are corrected for multiple comparisons ($P < 0.05$; see 'Methods' for more details).

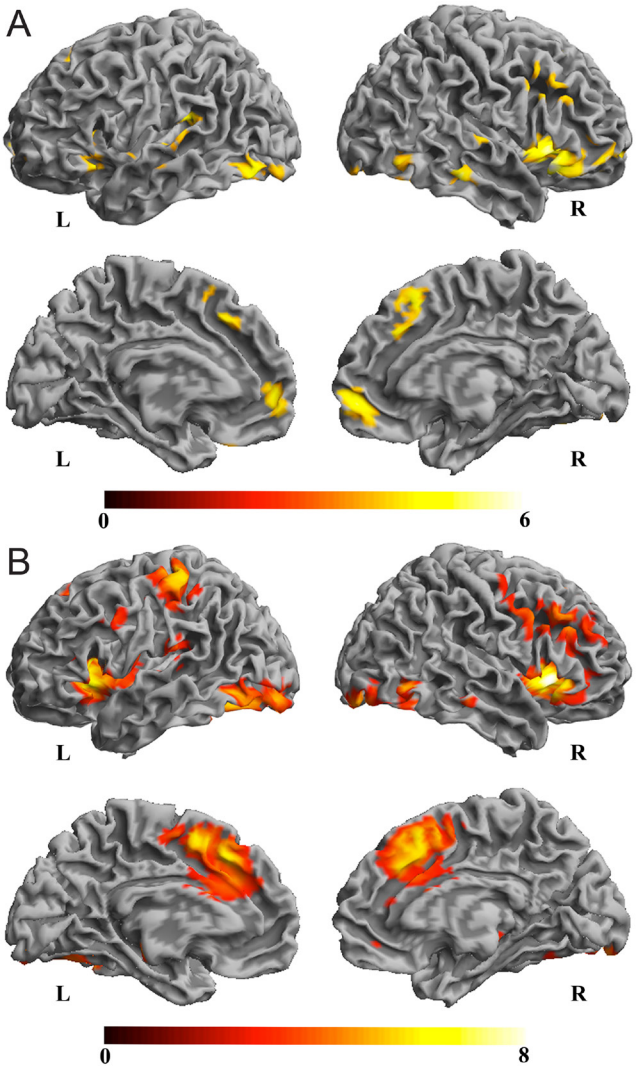


Figure 2

Statistical parametric map showing the main effect of sad affect (A) and fearful affect (B) across both groups in the emotion recognition task (ERT). Color bars represent standardized *Z*-scores. Right hemispheres are shown on the right. Results are corrected for multiple comparisons ($P < 0.05$; see 'Methods' for more details).

Behavioral analysis

The VMT

There was no longitudinal difference in reaction time or accuracy in the 'Recall' blocks (interaction term $P > 0.05$) or point difference at baseline or at 6-month follow-up between the two groups (Table 2).

The ERT

There was no difference in reaction time or accuracy in the sad or fearful affect or shapes blocks longitudinally (interaction term $P > 0.05$). Neither were there significant differences at baseline or at 6-month follow-up between the two groups (Table 3).

The VBM analysis

There was no significant longitudinal difference in gray matter, white matter, or hippocampal volume between the two groups (interaction term $P > 0.05$).

Discussion

We studied the effect of EAT on brain activation related to cognitive function and affect, as well as putative

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Table 2 Comparison between the placebo and the EAT group in reaction time and accuracy in the Recall condition of the VMT at baseline and after 6 months.

	Placebo	EAT	*t* stats	*P*
Baseline trial				
Reaction time (s) mean (SD)	10.2 (1.4)	9.5 (0.5)	1.40	0.16
Accuracy % mean (S.D.)	46 (4)	45 (4)	2.04	0.84
6-month trial				
Reaction time (s) mean (S.D.)	9.8 (0.5)	9.7 (2.5)	0.01	0.93
Accuracy % mean (S.D.)	45 (2)	45 (4)	0.02	0.99

changes in hippocampal gray and white matter volume in testosterone depleted subjects with prostate cancer. We did not find any significant change in brain activation associated with verbal memory or emotion recognition, or in hippocampal volume following 6 months of treatment with transdermal E2 (0.9 mg per day).

Our findings may seem incongruent with earlier imaging studies that found reduced gray matter volume, brain activation and functional connectivity in cognitive brain networks in prostate cancer patients on ADT treatment (18, 19, 20, 21). One study reported a positive correlation between cerebral metabolism in cognitive brain areas including the cingulate cortex, middle temporal area, and parietal lobe and performance on a working memory task (20). Another study reported reduced gray matter volume in the dorsolateral prefrontal cortex in ADT patients that was not evident in an age matched nonexposed control group. The reduced gray matter volume was associated with longer reaction time on a working memory task (21). In contrast to our study, these studies utilized healthy control subjects or prostate cancer patients that did not receive ADT as control subjects (18, 19, 21). In addition, the latter study (20) was a prospective cohort study and had no control group.

It is possible that the duration of ADT in our inclusion criteria was not sufficient for our participants to have developed changes in cognition. Cherrier and colleagues detected altered brain metabolism and reduced brain activation in cognitive brain networks during spatial memory and mental rotation tasks in ADT treated patients after 9 months when compared to healthy control subjects (19, 43). However, these studies had a relatively smaller number of subjects, with five subjects participating in the fMRI study and nine subjects in the positron emission tomography study (19, 43). In addition, these studies utilized healthy control subjects or ADT naive prostate cancer patients as control subjects. Nonetheless, other studies reported evident changes at the neurobiological (18, 21) and behavioral (24) levels with similar or shorter duration of treatment to the one used in this study.

Table 3 Comparison between the placebo and the EAT group in reaction time and accuracy in the three active conditions of the ERT at baseline and after 6 months.

	Placebo	EAT	*t* stats	*P*
Baseline trial				
Fearful				
Reaction time (s) mean (S.D.)	1.15 (0.48)	1.15 (0.47)	0.49	0.63
Accuracy % mean (S.D.)	93 (5)	91 (7)	0.63	0.53
Sad				
Reaction time (s) mean (S.D.)	1.15 (0.48)	1.05 (0.33)	1.03	0.31
Accuracy % mean (S.D.)	91 (6)	85 (3)	0.74	0.46
Shapes				
Reaction time (s) mean (S.D.)	1.12 (0.04)	1.10 (0.03)	0.93	0.36
Accuracy % mean (S.D.)	93 (4)	92 (5)	0.60	0.60
6-month trial				
Fearful				
Reaction time (s) mean (S.D.)	1.15 (0.37)	1.15 (0.46)	0.59	0.56
Accuracy % mean (S.D.)	91 (8)	90 (7)	0.40	0.69
Sad				
Reaction time (s) mean (S.D.)	1.20 (0.36)	1.15 (0.50)	0.55	0.59
Accuracy % mean (S.D.)	91 (5)	94 (5)	1.22	0.24
Shapes				
Reaction time (s) mean (S.D.)	1.20 (0.25)	1.10 (0.37)	1.14	0.27
Accuracy % mean (S.D.)	91 (4)	93 (3)	1.24	0.23

Similarly, the duration of the EAT may not have been sufficient to restore E2 levels to normal range or to effect a detectable change in cognitive function. We previously showed that 28 days of treatment with transdermal E2 patches of the same concentration used in this study in testosterone deplete men with prostate cancer was sufficient to restore serum E2 concentrations into the reference range reported for healthy men (33). Serum levels of E2 were maintained throughout the 6 months of the trial (33). Furthermore, our past reports of indicators of peripheral actions of E2 suggest adequate serum levels and physiologic response. The most compelling of these was bone remodeling markers showing a strong antiresorptive effect of E2 administration over 6 months (31). Additionally, E2 treatment reduced average daily hot flush frequency by about 50% and gynecomastia and nipple tenderness were twice as common in the E2 group (25).

Worth noting is that changes in brain activation and metabolism as demonstrated by neuroimaging studies

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did not translate to improvement in cognitive function in ADT exposed patients (18, 19, 20, 21). In our study, the results from the in-scanner behavioral analysis suggest no longitudinal difference in reaction time or accuracy on the fMRI tasks between the two groups. These results are in agreement with our findings from full cognitive battery analysis in the same group (44) and the extant but limited literature. In a randomized controlled trial, Matousek and Sherwin demonstrated no differences between subjects receiving ADT for 24 weeks who received 12 weeks of EAT compared to subjects that were not exposed to EAT, on measures of working and verbal memory or visuospatial ability (45). Similarly, Taxel and colleagues showed no significant improvement in 15 out of the 17 cognitive and affective battery tests including tests of memory and visuospatial ability (45). Only a small improvement in reaction time on the trail-making test was observed in the EAT group which was difficult to interpret in light of nonsignificant MANOVA. Beer and colleagues on the other hand, reported a significant decline in verbal memory in ADT exposed subjects compared to age-matched healthy control subjects on individual repeated measures analysis (24). The decline in verbal memory improved in the EAT group only compared to patients continuing ADT therapy and healthy control subjects. In addition, both patient groups showed slower processing speed when compared to healthy control subjects but performed equally to healthy control subjects on a working memory task (24). The results of the Beer *et al.* study suggest that ADT may affect specific cognitive domains related to verbal memory that are reversible with EAT, however, the overall interaction term of group × visit was not statistically significant in their study. Our study did not have a nonexposed control group and hence, it was not possible to address the effect of ADT on cognitive function and affect.

Our study has several limitations, the most salient of which was the small sample size that may have underpowered the study to detect subtle effects. This is due primarily to the effects of the coronavirus disease 2019 pandemic curtailing our study, as we had planned for a significantly larger participant cohort but were forced to terminate recruitment earlier than planned. The need to have patients with castrate level of testosterone who underwent 6 months or more of ADT restricted the number of included patients in the study. Unfortunately, we were unable to assess changes in brain activation with the MRT and visuospatial memory is a domain that the above studies suggested was impacted by ADT. Another limitation is the absence of a control group who were not exposed to ADT, which may have shed light on the effect of ADT on cognitive function and affect. Similarly, the lack of a healthy control group with normal androgen profile; however, our aim was to specifically assess the effect of estradiol on brain activation and volume; hence, we chose the double blind randomized controlled clinic trial to address this question. Nonetheless, the study was well designed to capture putative changes in neurobiology related to cognitive function and affect in response to EAT.

In conclusion, this study supports earlier findings that EAT has no detectable effect on verbal memory or affect in testosterone-depleted men with prostate cancer as assessed with fMRI. Our study further demonstrates no detectable effect on brain morphology in brain regions previously believed to experience changes in response to EAT

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-23-0409.

Declaration of interest

The authors have nothing to declare.

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Author contribution statement

OD: study design, image acquisition, data analysis, and drafting of manuscript; JA: recruitment of participants, logistic support, data collection, data analysis, and editing of manuscript; AM: data analysis and drafting of manuscript; NR, MG, and RK: study design and methodology, and editing of manuscript. AG provided templates for MRI paradigm and edited the manuscript.

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