Functional connectivity of resting-state, working memory and inhibition networks in perceived stress

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ABSTRACT

Experimental imaging studies on the effects of acute stress have revealed functional changes in the amygdalae, hippocampi and medial frontal cortices. However, much less is known about the association between perceived stress and neurological function which may have implications for the development of stress related disorders. Participants completed a working-memory task and an inhibition task whilst undergoing a functional magnetic resonance imaging (fMRI) scan. Task related and resting-state fMRI data from 22 women and 24 men were analysed to investigate changes in task activations and functional connectivity associated with perceived stress over the past month. Analyses were stratified by gender due to gender differences in the stress response. Stress was associated with faster working memory response time in women, but not men. Stress was not associated with any differences in task activations in either gender. There were many significant associations between stress and connectivity: findings in women were consistent with increased emotional regulation; men exhibited decreases in connectivity between affective processing areas during the tasks and showed no relation between perceived stress and resting-state connectivity; very few of the within gender differences were significantly different between gender. Dysregulated connectivity between areas involved in the neural stress response and self-referential thoughts (e.g. the default mode network) suggests that perceived stress may have a subtle impact on cognitive processing and neural correlates.

1. Introduction

1.1. Stress & cognitive function

Stress can be interpreted as appraising a situation as beyond the organism's resources (Lazarus and Folkman, 1984). The acute stress response involves the release of adrenocorticotrophin hormone (ACTH) and culminates in the release of glucocorticoids (cortisol in humans) from the adrenal cortex. The release of cortisol results in temporary changes to the metabolic, immune and nervous systems. In the short-term this stress response could be adaptive, but prolonged exposure to high levels of stress has been associated with poorer health and cognitive impairment (Lucassen et al., 2014; S. J. Lupien et al., 2009; Steptoe and Kivimaki, 2013).

Stress has been shown to impact on the function of many regions of the limbic system, including the medial prefrontal cortex (mePFC), anterior cingulate (ACC), hippocampus, amygdalae and insulae (Dedovic et al., 2009; Heimer and Van Hoesen, 2006). The most consistent areas associated with stress appear to be in the hippocampus and amygdala, followed by the cingulate (Dedovic et al., 2009) in keeping with the neuroendocrinological process of the stress response (Pruessner et al., 2010). However, more recently the role of the frontal lobes in stress regulation (Dedovic et al., 2009) and the impact of stress on frontal lobe function has become apparent (Lataster et al., 2011; Lucassen et al., 2014; S. J. Lupien et al., 2009). Whilst chronic stress (over many years) has long been associated with smaller hippocampal volume,

dysregulated function and poorer hippocampal dependent memory (Gianaros et al., 2007; S. J. Lupien et al., 1998) the indication that stress impacts on frontal lobe function has implications for many executive functions (Sonia J Lupien et al., 2007; Mika et al., 2012). For instance, studies have shown that frontal dependent working memory is more sensitive to the effects of cortisol administration than hippocampal dependent declarative memory (Sonia J Lupien et al., 2007).

1.2. Stress, WM and inhibition

The relationship between stress and cognitive function depends not only on the task, but also the type, stage and intensity of stress (Sandi and Pinelo-Nava, 2007). Interestingly, a meta-analysis showed that for the first 75 min after cortisol administration, working memory is impaired, but after 75 min the administration results in improved working memory (Shields et al., 2015). The reverse was true for inhibition; whereby for the first 135 min after cortisol administration inhibition performance was improved, but after 135 min cortisol administration was associated with impaired inhibition performance (Shields et al., 2015). There was no association between cortisol administration and set-shifting performance (Shields et al., 2015).

Longer term perceived stress (last two weeks) has been associated with improved working memory performance (Lewis et al., 2008; Vedhara et al., 2000), but poorer attention (Vedhara et al., 2000), consistent with the delayed effects of acute stress (Shields et al., 2015). Similarly, chronic stress was associated with slower interference inhibition in a sample of caregivers (Oken et al., 2011). However, long term work-stress related sick leave has also been associated with poorer performance in verbal working memory and digit span tasks (Jovanovic et al., 2011), suggesting that whilst acute stress improves working memory, prolonger stress may result in impaired working memory. Nonetheless, this finding may be related to the effects of stress related illness, rather than the impact of stress.

1.3. Stress and imaging

A meta-analysis reported acute intrinsic stress resulted in greater activation of the right superior temporal gyrus, inferior frontal gyrus and insula (Kogler et al., 2015), but fewer studies have investigated the neural correlates of extrinsic stress on cognitive function. Two studies have investigated the effect of stress on inhibition (Liston et al., 2009; Rahdar and Galvan, 2014) in mixed gender groups. Self-reported high state stress (on the day of testing) was associated with poorer inhibition performance and greater mePFC activation during the task, compared to participants who reported average state stress (Rahdar and Galvan, 2014). Similarly, one month of higher perceived stress was associated with impaired attention shifting and decreased neural coupling between

the dorsolateral prefrontal cortex and many areas of the fronto-parietal attention network employed in the task, but greater coupling between the left dorsolateral prefrontal cortex and middle temporal gyrus (Liston et al., 2009).

1.4. Gender differences in the stress response

Many studies have shown a gender difference in the cognitive, endocrine and neural stress response (Kogler et al., 2016; Lighthall et al., 2012; McLaughlin et al., 2009; Wang et al., 2007) and further gender differences depending on the type of stress (Wang et al., 2007). There are gender differences in the stress hormone response to psychosocial stress (Kirschbaum et al., 1999). In addition, women are thought to be more sensitive to social stress, whereas men are considered to be more sensitive to achievement related stressors (Kudielka et al., 2009; Wang et al., 2007). Wang and colleagues (2007) reported acute stress was associated with greater activations in the right prefrontal cortex in men and deactivated the left orbitofrontal cortex and inferior frontal cortex. However, in women, stress ratings were associated with more limbic activations; in the insulae and ventral striatum during the task and the anterior cingulate (ACC) and posterior cingulate cortex (PCC) one hour after the task. The authors speculated that men respond to stress with a fight or flight, whereas women employ emotional coping strategies. Others have supported the notion that women cope with stress using more emotionally focused strategies, but propose men to be more reward oriented when stressed (Kogler et al., 2016; Lighthall et al., 2012). There is some support for this hypothesis from a study indicating basal cortisol level is positively associated with greater connectivity between the amygdala and emotion related areas (e.g. PCC and inferior frontal gyrus, IFG) in women and between the amygdala and reward areas (e.g. striatum) in men (Kogler et al., 2016). Given these gender differences, it is not surprising that many studies investigating the neural effects of stress focus on single gender samples.

1.4.1. Women
Relatively few studies investigating stress and cognition have focused on women; one study demonstrated no association between basal cortisol and inhibition task performance in a small sample of women (Tops et al., 2006); two studies have demonstrated associations between stress and altered resting-state neural connectivity from the amygdala. The first study demonstrated an enhanced coupling between the amygdala and ACC and insula after acutely induced stress (van Marle et al., 2009), conversely, chronic stress was associated with decreased coupling between the amygdala and ACC (Jovanovic et al., 2011).

1.4.2. Men
High perceived stress did not impair inhibition performance in a sample of 60 men (Wu et al., 2014). However, one study reported decreased working memory accuracy after induced stress (Qin et al., 2012), but another study reported improved recall the next day (Henckens et al., 2009). Other studies reported no association between acute stress and working memory (Weerda et al., 2010) or cognitive flexibility (Ohira et al., 2011). Imaging studies have reported decreased memory task related activity (Qin et al., 2012; Weerda et al., 2010). However, acute stress was associated with less middle temporal lobe deactivation in one study – where there was also poorer performance in the stressed group (Qin et al., 2012), but the other study reported decreased middle temporal lobe activity, but only during the maintenance phase – with no relationship between stress and performance (Weerda et al., 2010), suggesting that the phases of memory and performance may contribute to differences in imaging findings.

Imaging studies focusing on stress and connectivity have reaped more consistent findings than studies investigating task activations. Vaisvaser and colleagues (2013) analysed connectivity in resting-state data after acute stress and employed the hippocampus and PCC as seeds. They reported greater coupling between the hippocampus and amygdala and middle temporal gyrus, but no PCC associations. Studies with a similar stress-then-scan protocol but using the amygdala as a seed have reported increased coupling between the amygdala and PCC, and mePFC (Veer et al., 2011), as well as decreased coupling between the amygdala and hypothalamus, middle frontal gyrus and middle temporal gyrus (Henckens et al., 2009). Studies investigating associations between cortisol measures and coupling between the amygdala and limbic areas, reported similar results but with differences in directions: basal cortisol variation was associated with greater coupling between the amygdalae and the ACC and mePFC (Veer et al., 2012). Whereas, Kiem et al. (2013) reported that whilst increased coupling between the amygdala and hippocampus was predictive of greater ACTH reactivity, most of the findings showed that decreased coupling within the limbic lobe was predictive of greater reactivity (Kiem et al., 2013).

1.5. Summary

The majority of neuroimaging studies have investigated the effects of acute laboratory induced stress (Henckens et al., 2009; Pruessner et al., 2008; Qin et al., 2012; Rahdar and Galvan, 2014; Vaisvaser et al., 2013; van Marle et al., 2009; Veer et al., 2011; Weerda et al., 2010), cortisol administration (Henckens et al., 2012), hormonal measures of stress (Kiem et al., 2013; Veer et al., 2012) or investigated differences in samples with stress-related disorders compared to healthy controls (Jovanovic et al., 2011; Ohira et al., 2011). Whilst these studies have made valuable contributions to the field, they may not be generalisable to the normal experiences of the general population. Some studies have employed the perceived stress scale (Bergdahl et al., 2005; Liston et al., 2009), a retrospective scale assessing perceived stress over the past month (Cohen et al., 1983). This questionnaire is inherently subjective, and may be biased by the current state of the respondent at the time of completion. However, given the subjective nature of the questionnaire it provides an important naturalistic angle to stress assessment, likely incorporating a combination of recent stress exposure, vulnerability and coping and offering potential for generalisable results. The perceived stress scale is reported to correlate with life events and cortisol levels and has prospectively predicted health outcome measures even after adjusting for psychological symptoms (Monroe, 2008; Pruessner et al., 1999).

The current literature suggests that different forms of stress may have differential effects on working memory and inhibition (Shields et al., 2015). However, to date, no studies have comprehensively investigated the relationship between a naturalistic measure of stress, such as perceived stress, and working memory and inhibition and the corresponding neural correlates in both women and men. Furthermore, given most studies employ single gender samples, few studies have investigated whether the within gender effects differ significantly. This study characterises the neural correlates of perceived stress by investigating (1) associations between perceived stress and task performance and activations in women and men and (2) associations between perceived stress and functional connectivity of limbic areas in task and resting-state data in women and men. Our study employs an inhibition task and a working memory task, on a sample of healthy adults, stratified by gender. It is hypothesised that:

1. higher perceived stress will be associated with improved working memory performance, but no effect on inhibition (e.g. Shields et al., 2015)
2. higher perceived stress will be associated with decreased task-related frontal lobe activations (e.g. Qin et al., 2009; Weerda et al., 2010)
3. higher perceived stress will be associated with greater connectivity between limbic areas (the hippocampi, amygdalae and ACC) during resting-state (e.g Kiem et al., 2013; Veer et al., 2011).
4. higher perceived stress will be associated with greater connectivity between limbic areas during the task (similar to resting-state).
5. higher perceived stress will be associated with decreased connectivity in ACC in flanker task (e.g Liston et al., 2009)
6. higher perceived stress will be associated with decreased coupling from the amygdala and ACC during resting-state in women (e.g. Jovanovic et al., 2011),
7. higher perceived stress will be associated with greater coupling between the amygdalae and the ACC and mePFC in resting-state in men (e.g. Veer et al., 2012).

2. Methods

2.1. Participants

Data for this study were extracted from a large lifespan dataset of 210 participants recruited from the general population by advertisements aged 21–79 years old with no self-reported major physical illness, neurological or psychiatric disorder and no history of head injury resulting in loss of consciousness. Of the 210 participants 87 completed the perceived stress scale. Only a subset of participants completed the scale because it was not included in the study protocol at the beginning of data collection. As there is evidence for different responses to stress depending on age and gender (Kudielka et al., 2004; Lighthall et al., 2012; Wang et al., 2007), participants over the age of 65 were excluded and analyses were stratified by gender, resulting in a sample of 31 women and 30 men. All participants provided informed written consent and the study was granted ethical approval according to the Declaration of Helsinki by the National University of Singapore Institutional Review Board.

2.2. Questionnaires

The Patient Health Questionnaire (PHQ) is a 9 item self-report measure of depressive symptoms. The questions are based on the Diagnostic Statistical Manual IV criteria for a depressive disorder and it has been validated for use as a screening tool for depression in the general population (Martin et al., 2006). The PHQ was used to identify participants who showed high levels of depressive symptoms indicative of a moderate depressive episode. Any participants who scored greater than 15 were excluded from the stress analyses.

The 10-item Perceived Stress Scale (PSS) (Cohen et al., 1983) includes two factors; six negatively phrased questions assess experienced levels of stress and four positively phrased questions assess coping over the past month. Items are rated on a five point Likert scale. Responses to the perceived coping items are reverse coded and all items are summed with higher scores (out of 40) indicating higher perceived stress.

The 24-item Stressful Life Events Questionnaire (LEQ) was adapted from the National Population Health Survey Stress Questionnaire that is routinely administered to the general Canadian population by Statistics Canada. The questionnaire focused on three types of stressors: six questions on childhood adversity, which measured the number of traumatic events that participants were

exposed to during their childhood or adolescence, e.g. "Did you spend 2 weeks or more in a hospital?"; six questions relating to life events in the past year that are considered detrimental to well-being and would require a significant degree of adjustment within a short period of time, e.g. "In the last 12 months did you or someone in your family have a major financial crisis?"; and 11 questions about ongoing stress and feeling able to cope, e.g. "You don't have enough money to buy the things you need". Participants responded yes or no to each event; higher number of events indicated greater number of stressors.

2.3. Functional MRI tasks

The Spatial Addition Task (SAT) is a visuospatial working memory task that allows evaluation of processes involved in maintenance only and maintenance plus processing. There were four conditions in this task: Low Load (LL); High Load (HL); Low Maintenance (LM); High Maintenance (HM) (Fig. 1a). In each condition, subjects saw the word "ready" for 500 ms to cue them to the start of a trial, followed by the first 'target' (T1) which was shown for 1500 ms. Then there was a fixation cross for 500 ms, followed by the second 'target' (T2) for 1500 ms. After T2 an equals sign was presented for 1000 ms, representing the maintenance period, finishing with the 'Probe' which was presented for 3000 ms. The targets and Probe were 5×5 square matrices formed of white lines on a black background where a number of squares were shaded in white, depending on the condition; for the LL condition, one square was shaded for both targets, whereas two squares were shaded in both targets for the HL condition. For LM trials only one square was shaded for T1 and no squares were shaded for T2, for the HM condition, two squares were shaded for T1, but no squares were shaded for T2 (Fig. 1a). For half of the trials in each condition the probe correctly represented the addition of T1 and T2 (correct) and the other half the probe incorrectly represented the addition of T1 and T2 (incorrect). Subjects responded with a button press during the Probe period using left thumb if the Probe was correct and their right thumb if the Probe was incorrect. The presentation of correct and incorrect trials was randomized. Each trial lasted for eight seconds, and each block included two trials. Each run included three cycles lasting a total of 192s (3 min 12s). The contrast HM > LM represents maintenance and the contrast HL > LL represents manipulation load. Participants completed a practice task which included feedback before entering the scanner and completed three runs in the scanner without feedback.

The Flanker Task is an event-related fMRI inhibition task that relies heavily on limiting interference, based on the Eriksen Flanker task (Eriksen and Eriksen, 1974). In this task a fixation cross is presented for 300 ms, followed by a horizontal array of five arrows for 1300 ms and then a blank screen for 400s. The total time for one event was two seconds, so the presentation of events was out of sync with the image acquisition time. Participants were required to press a button on the right if the central arrow pointed to the right and on the left if the central arrow pointed to the left (Fig. 1b). In each run, twenty-four congruent trials (in which all the arrows pointed in the same direction), 24 incongruent trials (in which the central arrow pointed in the opposite direction to that of the surrounding arrows) and 24 neutral trials (in which the flanking arrows were replaced by dashes of the same size) were presented. Half of the trials in each condition were presented at the top of the screen and the other half at the bottom of the screen, this was to help participants fixate to the centre, before the saccade to the stimuli in order to allow for distraction by the flanking arrows. An equal number of trials with central arrows pointing to the left or right were presented for each condition. The sequence of the conditions was pseudo-randomized with no trials of the same

condition/position occurring consecutively. Participants were free to respond during the presentation of the arrows and the blank screen, responding during the presentation of the fixation cross was considered a null response and coded as incorrect in the analysis. Each trial took two seconds, each run took 2 min 24s. Each participant completed a practice task with feedback outside of the scanner, followed by three runs in the scanner with no feedback.

During the resting-state scan participants were instructed to rest with their eyes closed for eight minutes. Subjects were instructed not to fall asleep or to think of anything in particular. Participants were required to communicate with the researchers just before and after the resting-state scan to verify that they were awake.

2.4. Functional imaging acquisition

Images were acquired with a Siemens 3T Trio MRI scanner using a 32-channel quadrature headcoil at the Clinical Imaging Research Centre, National University of Singapore. Whole brain structural scans were obtained prior to functional Magnetic Resonance Imaging (fMRI), consisting of an MP-RAGE anatomical sequence (192 axial slices of 1 mm thickness, repetition time (TR) = 2300 ms, echo time (TE) = 1.9 ms, flip angle (FA) = 9°, field of view (FOV) = 256 mm, matrix = 256 × 256, interleaved acquisition). Whole brain fMRI data were obtained using an Echo Planar Imaging (EPI) sequence (48 axial slices of 3 mm thickness with no gap, TR = 2400 ms, TE = 25 ms, FA = 90°, FOV = 192 mm, matrix = 64 × 64, interleaved acquisition) for the task data and a functional EPI whole brain sequence (48 axial slices of 3 mm thickness with no gap, TR = 2300 ms, TE = 25 ms, FA = 90°, FOV = 192 mm, matrix = 64 × 64, interleaved acquisition) for the resting-state data. All images were acquired co-planar with the anterior commissure − posterior commissure line and the first three images of any run were discarded. A total of 206 vol were obtained during the resting-state scan, 80 images were acquired for each SAT run and 60 images for each flanker run.

2.5. Procedure

Participants completed the stress questionnaires first, along with some neuropsychological tests. This was followed by MRI scanning, starting with structural scans, followed by the resting-state scan and then the task scans. All participants completed three runs of each task and the order of the tasks was counterbalanced by alternating allocation within each decade of the life-span and gender on the day of testing. Stimuli were presented using Eprime v2.0 (Psychology Software Tools, Pittsburgh, PA, USA) and accuracy and response times (RTs) were recorded.

2.6. Statistical analysis

2.6.1. Behavioural data

Regression analyses were conducted between the PSS score and age and accuracy and RTs for the two tasks using Stata v.11 (StataCorp). Two-way mixed ANalyses Of Variances (ANOVAs) were conducted with task performance measures as the dependent variable and gender as a between subject independent variable and task condition as a within subject independent variable. A linear regression was employed to investigate an association between age and PSS score; age was included as the independent variable and PSS the dependent variable. Further linear regression analyses were conducted with PSS as the dependent variable and the task performance measures as the independent variables. Accuracy and RTs were computed to reflect the task contrasts (HL-LL, HM-LM, I-C). Analyses were conducted for women and men separately and, for
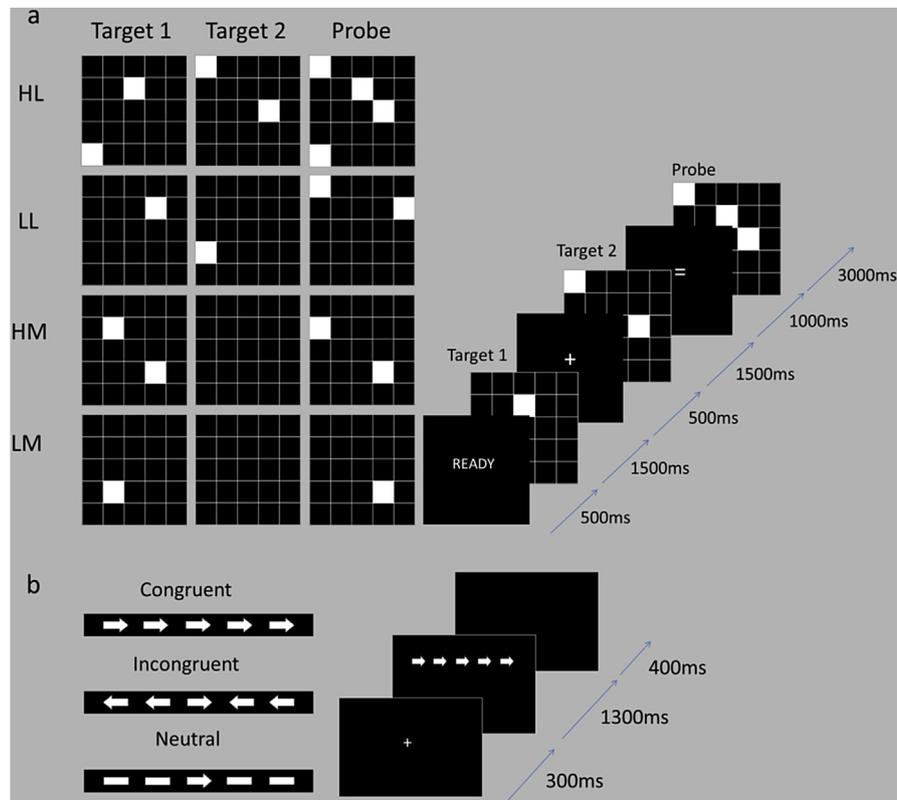
Fig. 1. Task descriptions. a) The spatial addition task: HL = high load; LL = low load; HM = high maintenance; LM = low maintenance. b) The flanker task.

the between gender analyses, as a whole group with gender included as an interaction term.

2.6.2. fMRI data

All fMRI Images were preprocessed in Statistical Parametric Mapping 8 (SPM8, Wellcome Department of Imaging Neuroscience, London, UK http://www.fil.ion.ucl.ac.uk/spm) in Matlab 7.9.0 (The Mathworks, Inc.,Natick, USA) using the steps (1) slice timing correction (to the middle slice using Fourier phase shift interpolation), (2) realignment for motion correction (to first image), (3) coregistration (using entropy correlation coefficient) and (4) the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) pipeline (Ashburner, 2007) was applied to obtain a group specific structural template for segmentation and normalisation to the standard (Montreal Neurological Institute 152, MNI) space and smoothing using an $8 \times 8 \times 8mm$ full width half maximum Gaussian kernel.

Fixed effect general linear models at single subject level were conducted in SPM8 to obtain the task activation contrasts of interest. **SAT.** The SAT task design was a block design with a time course regressor for each condition. All correct and incorrect trials were included within the same regressor. The task activations of interest were HL > LL and HM > LM for the SAT, with LL > HL and LM > HM representing the respective task deactivations. **Flanker.** The flanker task design included a time course regressor for each correct congruent, incongruent and neutral trial and an additional regressor for incorrect trials. Stimuli onset was entered as the event time with a duration of 0. The task contrast of interest for the flanker task was Incongruent > Congruent (I > C) representing inhibition. Task deactivations were assessed with the contrast C > I.

For both tasks, the task design function was convolved with a canonical haemodynamic response function as the main effect of

interest and motion parameters were included as covariates. Low frequency variation was eliminated using a 128 s high pass filter and a one-lag autoregression model was applied globally. Runs with lower than 60% accuracy on any condition were excluded from the analyses, as were runs which included greater than 1.5 mm translational movement or more than 2° rotation.

The effect of perceived stress on task activations was investigated in Robust Biological Parametric Mapping (BPM) (Yang et al., 2011) using robust (Huber) random effect analyses. Within group analyses were conducted with PSS entered as the variable of interest and age and grey matter probability included as covariates. Between group analyses were conducted as interactions between PSS score and gender including age as a covariate of no interest, thus indicating a difference in the relationship between PSS score and activation. HL-LL RT was included as a covariate for the SAT analyses as there was a significant association between perceived stress and RT in women (see behavioural results).

Based on the stress literature, the amydalae, hippocampi and ACC were considered key Regions of Interest (ROIs) with which to conduct a seed-to-voxel connectivity analysis (Blix et al., 2013; Dedovic et al., 2009; Henckens et al., 2009; Jovanovic et al., 2011; Kiem et al., 2013; Qin et al., 2009; Thomason et al., 2011; van Marle et al., 2009; Weerda et al., 2010). Both tasks and resting-state followed a very similar connectivity analysis procedure, the following steps apply to all the connectivity data unless otherwise stated. The seed ROIs were constructed in MNI space: the anatomy toolbox (Eickhoff et al., 2005) was used to create the hippocampal and amygdalae ROIs; the MAsks for Region of INterest Analysis (MARINA) (Walter et al., 2003) toolbox was used to create the ACC ROIs (Fig. 2). These ROIs were masked at subject level using the subject specific grey mater probability map. Connectivity analyses were conducted in the CONN functional connectivity toolbox v14

(Whitfield-Gabrieli and Nieto-Castanon, 2012). For each participant, data were band pass filtered (0.008−0.09 Hz) and the effects of six motion parameters and their temporal derivatives and the blood oxygen level dependent (BOLD) time series obtained from within white matter and cerebrospinal fluid (CSF) masks were regressed out using a component based noise reduction method for each functional imaging run. For the SAT and flanker all the aforementioned covariates were regressed out for each run before effectively concatenating the sessions to conduct first level connectivity analyses, this was not necessary for resting-state as there was only one run. For all tasks and resting-state first level connectivity analyses, the BOLD time series from each ROI was regressed against all voxels for each participant to form a first level map of Fisher-transformed correlation coefficients, which were submitted for second level analyses. The processing of data and first level analyses were conducted as a whole group, but for the gender stratified analyses only the male or female participants were selected for second level. A one-sample t-test of the first level connectivity maps was conducted to identify areas with significant functional connectivity with the seed region. Perceived stress score was included as the second level regressor of interest to investigate if perceived stress score was associated with the level of functional connectivity between the seed and the rest of the brain in a random effects general linear model. For the SAT the contrast HL > LL was selected; for the flanker task the contrast I > C was selected and for resting-state "rest" was selected as the contrast variable. For resting-state and both tasks PSS score was entered as a between subject variable for the gender stratified analyses and a gender and PSS score interaction was entered for the between gender results. Restricted Maximum Likelihood analyses provided F-statistical parameter maps for thresholding. Age was adjusted for in all second level analyses. Response time was included as a covariate for the SAT analysis because perceived stress was associated with SAT RT in the female sample (see results).

As in the introduction, past studies indicate that the stress response differs between genders (S. J. Lupien et al., 2005; Otte et al., 2005). Sex differences in resting-state amygdala connectivity have also been reported (Kilpatrick et al., 2006). Thus, most studies focus on single gender samples (e.g. Gianaros et al., 2007; Henckens et al., 2009; Kiem et al., 2013; Qin et al., 2009; van Marle et al., 2009; Weerda et al., 2010). Some of the hypotheses in this study may only apply to one gender because they have been informed by past studies focusing on single gender samples. There were also significant gender differences in the behavioural measures and a significant gender interaction (see behavioural results). Thus, so as not to mask within gender effects by combining both genders the analyses were stratified by gender. Given there were qualitative differences between the two genders a further analysis was conducted to investigate whether any of the stress associations

within each gender were significantly different between genders (a PSS score by gender interaction effect). Whole group results adjusted for gender are presented in Supplement 1.

All imaging results are reported to voxel level $p < 0.001$ and a cluster threshold of $p < 0.05$ family wise error (FWE) corrected. Anatomical labels were identified by converting MNI coordinates to Talairach using a non-linear transform (Lancaster et al., 2007) and referencing the coordinates in the Talairach Atlas (Talairach and Tournoux, 1988). Cerebellum activations were located using MNI coordinates and the Schmahmann and colleagues (Schmahmann et al., 1999) cerebellar atlas. All coordinates are presented in MNI space.

3. Results

3.1. Participants

Only participants who had data for both tasks and resting-state data were included. Thus, after excluding participants with incomplete data due to technical errors, poor compliance or below threshold accuracy the sample consisted of 27 women and 25 men. A further five women and one man were excluded due to extensive movement in the resting-state scan. Thus, the final sample consisted of 22 women and 24 men. This sample size was considered sufficient based on previous imaging studies on single gender groups typically including 16−27 participants (Bergdahl et al., 2005; Henckens et al., 2009; Jovanovic et al., 2011; Kiem et al., 2013; Liston et al., 2009; Qin et al., 2009; Thomason et al., 2011; van Marle et al., 2009; Weerda et al., 2010). Thirty-three (72%) participants were right handed, four (9%) were left handed and the remaining participants were ambidextrous based on the Edinburgh Handedness Inventory (Oldfield, 1971) with no significant difference between genders ($\chi^2 = 4.06$, $p = 0.131$). Twelve participants were educated up to high school or less, 22 participants had a higher education qualification other than a degree and 12 participants were educated to university degree level or higher, with no significant difference between genders ($\chi^2 = 2.57$, $p = 0.766$). The neuropsychological test scores showed men tended to outperform women in spatial cognitive tests and an associate learning task, whereas women outperformed men in a memory task (see Supplement 2 for more details).

No participants scored greater than 12 on the PHQ, with the majority scoring below eight. In addition, no participants endorsed more than two items on the childhood stress or stress in the last year components of the LEQ, with the majority of participants endorsing no childhood stress items and no stressful life events in the last year. Given the low level of endorsement, childhood stress and stressful life events over the last month were not included in the analyses. Participants endorsed up to five items on the current
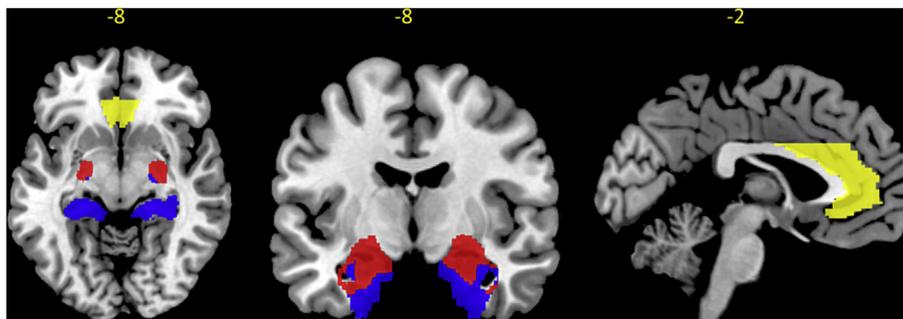

Fig. 2. Region of Interest seeds used in connectivity analyses. Amydalae in red, hippocampi in blue, anterior cingulate in yellow. Yellow numbers indicate slice number in MNI space. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stress component of the LEQ, but as these items overlapped with the PSS and the PSS is a more sensitive measure due to the larger range of possible responses, analyses were conducted using only the PSS. Participant demographics and PSS descriptives are presented in Table 1, the responses for the PHQ and LEQ are presented in Table 2.

3.2. Behavioural results

3.2.1. Task performance and gender differences

Task accuracy and RTs are presented in Table 3. **SAT.** Two-way mixed ANOVAs (condition x gender) revealed a significant main effect of condition and gender for both accuracy and RT for the SAT [accuracy $F(44,3) = 9.32$, $p < 0.001$, $F(44,1) = 19.66$, $p < 0.001$; RT $F(44,3) = 22.93$, $p < 0.001$, $F(44,1) = 16.16$, $p < 0.001$ for condition and gender respectively] and no interaction between condition and gender for either accuracy or RT [$F(44,3) = 0.95$, $p = 0.420$; $F(44,3) = 0.43$, $p = 0.730$ respectively]. Mean scores indicated that men were significantly faster and more accurate than women. Posthoc analyses indicated there was no difference in accuracy between HL and LL ($p = 0.144$), but participants were significantly slower in the HL condition compared to the LL condition ($p < 0.001$). Participants were slower and less accurate in the LM condition compared to the HM condition, although this was not significantly different ($p = 0.364$ for accuracy, $p = 0.145$ for RT). However, to check the lack of significance was not a power issue this comparison was analysed using the whole sample, whereby the differences were significant [$F(185,3) = 17.54$, $p < 0.001$, Bonferroni posthoc HM vs LM $p < 0.001$; $F(185,3) = 117.91$, $p < 0.001$, Bonferroni posthoc HM vs LM $p < 0.001$ for accuracy and RT respectively]. Given the LM condition was intended to be easier than the HM condition this may indicate different strategies were being employed for the LM condition, thus, as a precautionary measure the HM and LM contrast was not investigated further. **Flanker.** There was a significant effect of condition for both accuracy and RT [$F(44,2) = 11.24$, $p < 0.001$; $F(44,2) = 121.52$, $p < 0.001$ respectively]. Posthoc analyses and mean scores indicated participants were significantly slower and less accurate in the incongruent condition compared to the congruent and neutral condition (all $p < 0.001$), but there was no significant difference between congruent and neutral for either accuracy or RT ($p = 0.878$ and $p = 0.466$ respectively). There was no main effect of gender for accuracy, but there was a main effect of gender for RT in the flanker task [$F(44,1) = 0.39$, $p = 0.534$; $F(44,1) = 34.84$, $p < 0.001$ respectively] indicating men were faster than women. There was no interaction between gender and condition in either accuracy or RT [$F(44,2) = 2.36$, $p = 0.100$; $F(44,2) = 0.04$, $p = 0.959$ respectively].

3.2.2. Associations between task performance, age and PSS score

There was a significant decrease in perceived stress score with increasing age for the entire sample [$N = 87$, β(95% confidence interval, CI) = -0.12(-0.21, 0.04), $p = 0.006$]. Although the association was not significant in the smaller age restricted sample [$N = 46$, β(CI) = -0.05(-0.18, 0.07), $p = 0.403$] as a precautionary

measure age was still included as a covariate for subsequent imaging analyses. **SAT.** Perceived stress was associated with significantly faster RTs in women for HL > LL, with and without adjusting for age (with adjustment for age $N = 22$, β(CI) = -13.12(-21.21, −5.02), $p = 0.003$). There was a trend towards a significant association between perceived stress and slower RTs in men after adjusting for age ($N = 24$, β(CI) = 9.03(-1.22,19.29), $p = 0.081$) and a significant gender interaction ($N = 46$, β(CI) = 23.96(11.17,36.75), $p < 0.001$). As a result, RT was included as a covariate in the SAT analyses. There were no other significant associations. **Flanker.** There were no significant associations between perceived stress and accuracy or RT.

3.3. Task activations

3.3.1. Women

SAT. The HL > LL contrast activated a right precuneus/parietal activation cluster in women, accompanied by deactivations in the right and left ACC and left angular gyrus. **Flanker.** There were no significant activations for the I > C contrast.

3.3.2. Men

SAT. There was a right precuneus/superior parietal lobule activation cluster for the HL > LL contrast, as well as right and left fusiform, left middle occipital gyrus, precentral gyrus, right inferior frontal gyrus and middle frontal gyrus. Men showed deactivations in the left inferior frontal gyrus and middle temporal gyrus/precuneus. **Flanker.** There was a significant activation in the lingual gyrus for the I > C flanker contrast.

No other activations or deactivations were significant for either men or women and there were no significant differences between genders (Table 4, Fig. 3).

3.4. Effect of PSS on task activations

There were no significant associations between PSS scores and task activations at the voxel threshold of $p < 0.001$ and cluster threshold of $p < 0.05_{FWE}$ in women or men (with or without adjustment for RT in women) and there were no significant differences between gender.

3.5. Connectivity results

3.5.1. Women

SAT. There were no significant task related alterations in connectivity for the HL > LL contrast. **Flanker.** There was decreased connectivity from the left ACC to the left middle frontal gyrus, and from the right ACC to the left orbital gyrus in the incongruent compared to the congruent condition, thus reflecting decreased coupling between these areas for the I > C contrast. **Rest.** The seed to voxel analysis showed high levels of connectivity within seed regions (the hippocampi, amygdalae and ACC) and between the seed regions and surrounding structures and bilateral anatomical regions.

3.5.2. Men

SAT. There was a decrease in connectivity between the left hippocampus and left inferior temporal gyrus for the HL > LL contrast. **Flanker.** There was an increase in connectivity between the left amygdala and the left and right medial frontal gyrus/right ACC; from the right hippocampus to the right superior temporal gyrus and within the right ACC in the incongruent condition compared to the congruent condition, reflecting increased connectivity between these areas for the I > C task contrast. **Rest.** The seed to voxel analysis showed high levels of connectivity within the

Table 1
Participant demographics and perceived stress score (PSS) descriptives.

	N	Age		PSS	
		M (sd)		M (sd)	range
women	22	48.81 (15.21)		12.59 (6.04)	0–24
men	24	45.04 (13.25)		11.37 (5.68)	0–23

Note. No significant difference between men and women [age $t(44) = 0.90$, $p = 0.373$; PSS $t(44) = 0.71$, $p = 0.482$]. M = mean, sd = standard deviation.

Table 2
Life event stress questionnaire (LEQ) and patient health questionnaire (PHQ).

Number of endorsements	Women				Men			
		LEQ				LEQ		
	PHQ	child	last year	current	PHQ	child	last year	current
0	7	17	16	9	9	17	18	11
1–2	5	5	6	8	9	7	6	7
3–5	5	0	0	5	3	0	0	6
6–8	4	–	0	0	3	–	0	0
9–12	1	–	0	0	0	–	0	0

Note. No significant difference between men and women.

Table 3
Accuracy and response times (RTs) for the two tasks.

	Mean accuracy (sd)		Mean RT (sd)	
	women	men	women	men
SAT*				
HL	0.89 (0.09)	0.93 (0.06)	1087.28 (267.35)	940.32 (211.63)
LL	0.91 (0.10)	0.95 (0.05)	899.53 (182.81)	790.47 (175.33)
HM	0.96 (0.06)	0.98 (0.03)	852.34 (156.83)	766.02 (135.60)
LM	0.94 (0.07)	0.95 (0.06)	909.63 (263.22)	790.1 (123.24)
Flanker^				
Con	0.99 (0.01)	0.98 (0.02)	529.07 (97.35)	520.42 (57.38)
Incon	0.95 (0.06)	0.97 (0.04)	586.69 (110.47)	576.76 (51.31)
Neutral	0.99 (0.01)	0.99 (0.02)	533.4 (100.11)	525.22 (62.36)

Note. *Significant gender difference in accuracy and RT ($p < 0.001$). ^Significant gender different in RT ($p < 0.001$). HL = high load, LL = low load, HM = high maintenance, LM = low maintenance, Con = congruent, Incon = incongruent, sd = standard deviation.

seed regions and between the seed regions and surrounding structures and bilateral anatomical regions.

3.5.3. Women vs men

There were no other significant differences between women and men at a voxel threshold of $p < 0.001$, cluster threshold of $p < 0.05_{FWE}$, results are presented in Table 5 and Fig. 4.

3.6. Association between perceived stress and connectivity

3.6.1. Women

SAT. Greater stress was associated with increased connectivity from the right hippocampus to the middle frontal gyrus/ACC, and from the right ACC to the medial frontal gyrus/middle frontal gyrus. Increased stress was also associated with decreased connectivity from the left amygdala to the middle occipital gyrus/cuneus and from the right amygdala to the precentral gyrus. Unadjusted results are presented in Supplement 3. **Flanker.** Greater stress was associated with increased connectivity from the left ACC to the left cerebellum crus II and from the right hippocampus to the right ACC, but decreased connectivity from the right amygdala to the right lingual gyrus. **Rest.** Greater stress was associated with increased connectivity from both the left and right ACC to the middle cingulate, and decreased connectivity from the left hippocampus to the precuneus; and from the right hippocampus to the left middle frontal gyrus (Table 6, Fig. 5a).

3.6.2. Men

SAT. Greater stress was associated with increased connectivity from the left amygdala to the right fusiform gyrus and from the right hippocampus to the right superior temporal lobe. Greater stress was also associated with decreased connectivity from the left ACC to the medial superior frontal gyri. Unadjusted results are presented in Supplement 3. **Flanker.** Greater stress was associated

with increased connectivity from the right amygdala to midbrain/cerebellum. Increased stress was also associated with decreased connectivity from the left amygdala to the right medial frontal gyrus and left middle cingulate; and from the left hippocampus to the ACC/medial frontal gyrus in the flanker task (Table 6, Fig. 5b).

3.6.3. Women vs men

SAT. The negative correlation between PSS score and connectivity from the right amygdala to the left precentral gyrus in women was significantly more negative compared to the relationship between PSS score and connectivity from the right amygdala in men (Table 6, Fig. 6).

There were no other significant findings at the voxel threshold of $p < 0.001$ and cluster threshold of $p < 0.05_{FWE}$ for the within gender analyses and no other areas overlapped between the within and between gender analyses.

4. Discussion

This study characterised the relationship between perceived stress and neural cognitive processing in both women and men. Due to a paucity of data on perceived stress and cognitive function the hypotheses were based on previous research using psychosocially induced or hormonally simulated stress or psychological or hormonal measures of chronic stress as well as perceived stress studies. Some, but not all of these hypotheses have come to light.

4.1. Women

4.1.1. Stress, task performance and activations

The first aim was to investigate associations between perceived stress and task performance and activations. There were no significant associations between perceived stress and task accuracy. However, in support of our hypothesis, higher stress was associated with faster reaction times in the working memory task, but not the inhibition task, in accordance with mixed gender studies showing improved working memory performance in acute (Lewis et al., 2008; Shields et al., 2015) and perceived stress (Vedhara et al., 2000). However, mixed gender studies have also shown poorer verbal working memory in chronic stress (Jovanovic et al., 2011), suggesting the effects of perceived stress are closer to the effects of acute than chronic stress. No association between stress and flanker performance was apparent and nor was it expected based on previous reports of stress and inhibition in mixed gender and women only samples (Liston et al., 2009; Oken et al., 2011; Rahdar and Galvan, 2014; Schlosser et al., 2013; Tops et al., 2006).

There were no significant associations between perceived stress and task activations in women with or without adjusting for RT. This is in contrast to the hypotheses and to findings from previous studies investigating the effects of acute stress in mixed gender samples (Rahdar and Galvan, 2014) and studies on women

Table 4
Task activations.

	BA	x	y	z	T	Z	k	Cluster P(FWE)
Women								
SAT - activations								
R SPL	7	24	−66	57	5.52	4.29	282	<0.001
SAT - deactivations								
R/L ACC	32	6	33	−18	6.78	4.88	274	<0.001
L AG/MTG/SOG	39	−51	−72	36	5.00	4.01	73	0.037
Men								
SAT - activations								
R Precuneus/SPL	7	18	−69	63	9.32	5.94	1343	<0.001
R ITG/Cerebellum Lobule VI	37	51	−60	−9	7.97	5.47	118	0.008
L MOG/IPL/Precuneus	19	−33	−87	15	6.55	4.87	975	<0.001
L Fusiform Gyrus	19	−45	−69	−9	6.36	4.78	125	0.006
R Precentral Gyrus/MFG	9	48	9	27	5.81	4.51	141	0.003
L Precentral Gyrus/IFG	6	−45	6	27	5.31	4.25	90	0.025
R SFG	6	33	6	60	4.47	3.75	75	0.048
SAT - deactivations								
L IFG/Caudate	47	−24	36	−18	5.26	4.22	186	0.001
L MTG/Precuneus	39	−48	−72	33	4.63	3.86	85	0.031
Flanker - activations								
L Lingual Gyrus	19	−27	−66	0	4.74	3.92	57	0.029

Note. Results are presented in MNI space at $p < 0.001$ (unc), cluster $p < 0.05_{FWE}$. ACC = anterior cingulate gyrus, AG = angular gyrus, CG = cingulate gyrus, MCG = middle cingulate gyrus, IFG = inferior frontal gyrus, IPL = inferior parietal lobule, ITG = inferior temporal gyrus, MFG = middle frontal gyrus, MOG = middle occipital gyrus, MTG = middle temporal gyrus, SOG = superior occipital gyrus, SPL = superior parietal lobule, BA = Brodmann area, R = right, L = left.
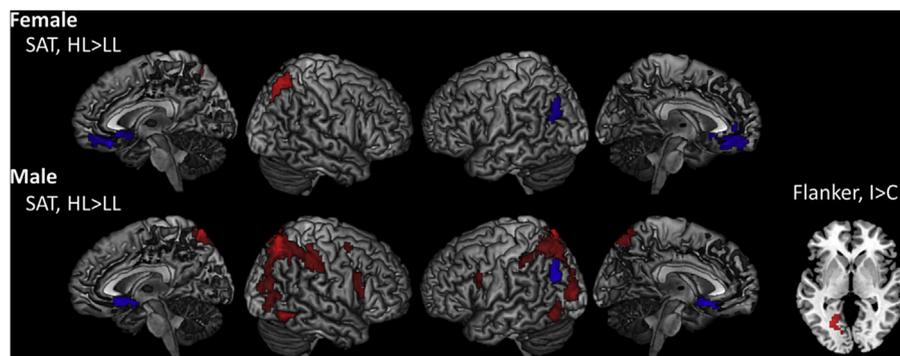
Fig. 3. Task activations. Red = activation, blue = deactivation. HL > LL = high load > low load. I > C = Incongruent > congruent. Voxel: $p < 0.001$, cluster: $p < 0.05_{FWE}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Effect of task on connectivity from seed regions.

Seed Region	Voxel cluster result	BA	x	y	z	T	Z	k	Cluster p(FWE)
Women									
Flanker - I > C									
Anticorrelations									
L ACC	L MFG/MeFG	10	−18	57	−15	4.85	3.93	31	0.040
R ACC	L Orbital Gyrus	11	−12	54	−24	5.81	4.44	32	0.030
Men									
SAT - HL > LL									
Anticorrelations									
L Hippocampus	L ITG	20	−57	−57	−12	6.41	4.81	38	0.030
Flanker - I > C									
Correlations									
L Amygdala	L MeFG/ACC	25	−12	27	−24	5.24	4.21	54	0.004
R Hippocampus	R STG	39	54	−57	30	5.72	4.47	32	0.047
R ACC	R ACC	32	6	45	−15	4.55	3.80	33	0.041

Note. Results are presented in MNI space at $p < 0.001$ (unc), cluster $p < 0.05_{FWE}$. ACC = anterior cingulate cortex, ITG = inferior temporal gyrus, MeFG = medial frontal gyrus, SFG = superior frontal gyrus, STG = superior temporal gyrus. BA = Brodmann area, L = left, R = right.

subjected to intrinsic (task-related) stress (Wang et al., 2007). This may be due to the more complex and naturalistic measure of perceived stress compared to induced acute stress under laboratory conditions.

4.1.2. Stress and connectivity

The second aim was to investigate associations between perceived stress and functional connectivity from the limbic lobe. **SAT.** We hypothesised that higher perceived stress would be
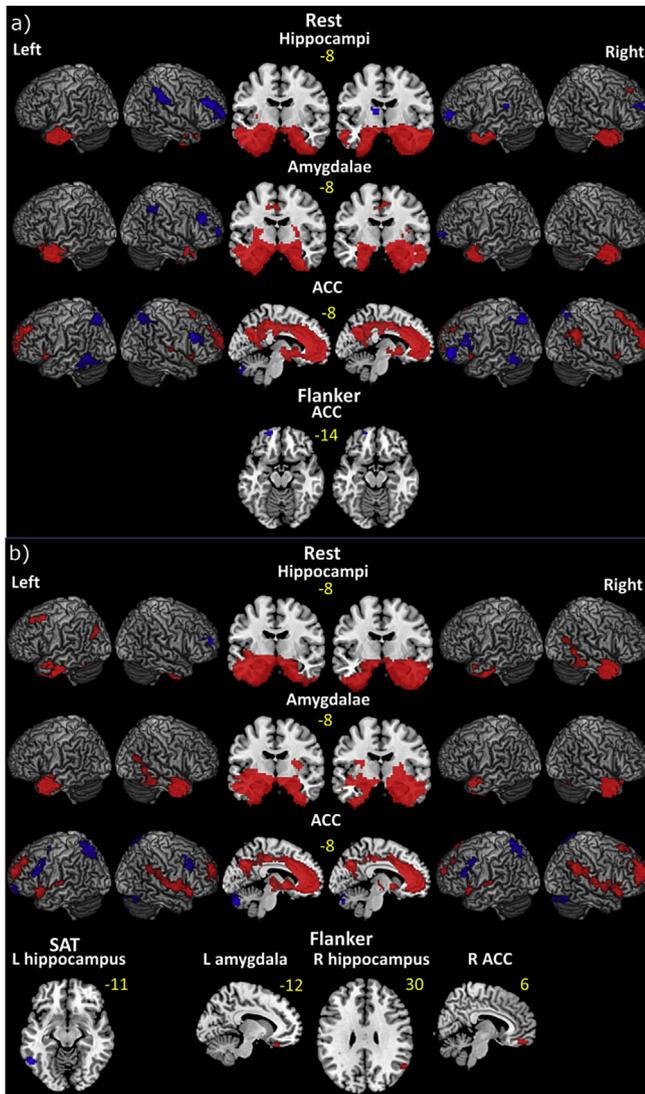
Fig. 4. Connectivity maps for a) women, b) men. Resting-state and task dependent functional connectivity. Red = positive correlation. Blue = negative correlation. Yellow numbers indicate slice in MNI space. Voxel: $p < 0.001$, cluster: $p < 0.05_{FWE}$. ACC = anterior cingulate cortex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated with greater connectivity between limbic areas during the task. Consistent with this hypothesis, increased stress was associated with an increase in hippocampal to ACC connectivity during higher task loads. However, the effect was not prominent enough to result in differences in task activation and was unrelated to task performance as this finding was apparent both with and without adjustment for RT. There was also a decrease in connectivity from the left amygdala to the middle occipital gyrus/cuneus, and from the right amygdala to the left precentral gyrus in females during the SAT. The middle occipital gyrus has been implicated in visual processing aspects of working memory (Cohen et al., 1997) and the precentral gyrus has also been implicated in load and temporal aspects of working memory processing (Cohen et al., 1997). The middle occipital gyrus/cuneus and precentral gyrus have also previously been shown to be anti-correlated with the amygdalae (Roy et al., 2009). Thus, perceived stress is associated with greater anti-correlations between limbic regions and higher memory task load regions during working memory. Conversely, there was increased connectivity from the ACC to medial and

middle frontal gyri. Previous reports have shown associations between cortisol and increased connectivity between the amygdala and the middle frontal gyrus during resting-state in men (Henckens et al., 2012) and the involvement of the ACC and medial prefrontal regions in the default mode network (DMN) and working memory deactivation (Mayer et al., 2010). On the one hand, this suggests increased communication between non-task directed areas (Mayer et al., 2010), but on the other hand, much of the cluster is in the middle frontal gyrus (BA6) which has also been implicated in temporal and load aspects of working memory (Cohen et al., 1997).

In general, there was increased connectivity within limbic and DMN areas, and decreased connectivity from limbic to task related areas. Only the association between the ACC to the hippocampus was significant both with and without adjustment for RT. As stress was associated with faster RT in women this suggests that the associations between limbic areas and task performance areas are indirectly related to their improved performance, which requires further investigation.

Flanker. The hypothesis that higher perceived stress will be associated with decreased connectivity in the ACC was not supported. Although, many other associations between stress and connectivity were observed: stress was associated with increased connectivity between the left ACC to Crus II of the cerebellum, previously found to be functionally connected to the DMN (Buckner et al., 2011); stress was also associated with decreased coupling between amygdala to lingual gyrus, an area involved in visual processing in tasks (Fink et al., 1996; Goldin and Gross, 2010; Menon et al., 2001). Finally, similar to the SAT stress was associated with increased hippocampal to ACC coupling. Overall, these results indicate increased coupling from limbic areas to other limbic and DMN related areas, and decreased coupling between limbic areas and task related areas.

Rest. The hypothesis that higher perceived stress will be associated with decreased coupling from the amygdala and ACC during resting-state was not supported. However, that hypothesis was formed from a past study investigating the effects of chronic stress (Jovanovic et al., 2011), whereas acute stress increased coupling between the amygdala and ACC (van Marle et al., 2009). Thus, this discrepancy may relate to the nature of perceived stress; the one month duration of assessment likely represents a longer duration than previous studies on acute stress, but a shorter and less severe form of stress than that described in the study on chronic stress (Jovanovic et al., 2011).

Nevertheless, perceived stress was associated with a decrease in connectivity between the left hippocampus and precuneus and from the right hippocampus to left superior/inferior temporal gyrus. The precuneus has been reported to be heavily involved in self-referential processing (Fransson and Marrelec, 2008) so this may reflect a decrease in hippocampal to precuneus communication during self-relational thoughts in resting-state in those feeling stressed. However, connectivity between the ACC and the middle cingulate was positively associated with stress. This aspect of the middle cingulate is associated with emotion and social interaction and appraisal of negative emotions (Etkin et al., 2011). Lesions to this area in macaques led to less value in social interaction (Ptacek et al., 1994). Thus, this may relate to rumination of stressful social events, or social coping mechanisms frequently used by women (Ptacek et al., 1994). Taken together these findings may indicate a decrease in self-related free thoughts, but more socially directed thoughts in those feeling more stressed during resting-state.

4.1.3. Summary for women

To date, few, if any, studies have investigated perceived stress in a women only sample. The results are variable, but generally consistent with the broader interpretation of previous reports

Table 6
Perceived stress and functional connectivity in women and men.

Seed region	Voxel cluster result	BA	x	y	z	T	Z	k	Cluster P(FWE)
Women									
SAT									
Perceived stress associated with increased connectivity									
R hippocampus	L MFG/ACC	47	−18	33	−21	6.15	4.46	29	0.044
R ACC	R MeFG/MFG	6	9	12	63	6.04	4.41	151	<0.001
Perceived stress associated with decreased connectivity									
L amygdala	R MOG/Cuneus	18	30	−93	9	5.40	4.11	45	0.005
R amygdala	L Precentral Gyrus	6	−42	9	33	5.38	4.10	31	0.035
Flanker									
Perceived stress associated with increased connectivity									
R hippocampus	R ACC	32	3	45	12	4.68	3.77	32	0.030
L ACC	L Cerebellum Crus II		−15	−87	−45	5.96	4.42	41	0.009
Perceived stress associated with decreased connectivity									
R amygdala	R Lingual Gyrus	17	15	−99	−3	6.14	4.50	29	0.049
Rest									
Perceived stress associated with increased connectivity									
L ACC	L/R MCG	24	−9	−3	39	6.23	4.55	74	0.005
R ACC	L/R MCG	24	−3	3	33	5.86	4.37	72	0.005
Perceived stress associated with decreased connectivity									
L hippocampus	L/R Precuneus	7	6	−72	45	4.95	3.92	116	0.000
R hippocampus	L ITG/MTG/FG	37	−48	−75	3	4.86	3.87	50	0.042
Men									
SAT									
Perceived stress associated with increased connectivity									
L amygdala	R Fusiform Gyrus	19	33	−90	−12	5.18	4.08	40	0.020
R hippocampus	R STG/ITG	38	36	6	−48	5.46	4.22	40	0.019
Perceived stress associated with decreased connectivity									
L ACC	R/L SFG	8	9	48	39	6.13	4.55	90	<0.001
Flanker									
Perceived stress associated with increased connectivity									
R amygdala	R Mid brain/Cerebellum Lobule III	18	−27	−21		5.41	4.23	37	0.022
Perceived stress associated with decreased connectivity									
L amygdala	R MeFG/MCG	10	18	69	−6	6.02	4.54	46	0.008
	L MCG	31	0	−27	33	5.66	4.36	32	0.047
L hippocampus	R ACC/MeFG	32	3	48	−9	4.77	3.88	45	0.010
More positive association between perceived stress and connectivity in Women compared to Men									
SAT									
L ACC	L MeFG/SFG	6	−3	9	54	5.39	4.63	59	0.004
R ACC	L MeFG	6	−3	12	51	5.44	4.66	80	0.001
	R MFG	6	36	9	60	4.91	4.30	66	0.002
Flanker									
L amygdala	L MTG/AG	39	−42	−57	30	5.27	4.58	72	0.001
Rest									
L amygdala	R Paracentral Lobule	5	12	−36	57	4.83	4.27	135	0.001
L hippocampus	L MFG	10	−42	48	12	5.51	4.74	117	0.002
More positive association between perceived stress and connectivity in Men compared to Women									
SAT									
L amygdala	L MFG/SFG/MeFG	9	−39	54	21	5.79	4.89	69	0.001
L hippocampus	R Cerebellum Crus II		51	−66	−42	5.65	4.80	45	0.017
R amygdala	L IFG/Precentral Gyrus	9	−45	9	30	4.94	4.33	39	0.029
Rest									
L ACC	L Cerebellum Lobule VIIIA		−21	−63	−42	5.40	4.67	80	0.009
R ACC	L Cerebellum Lobule VIIIA		−21	−63	−42	4.89	4.32	62	0.030

Note. Results are presented in MNI coordinates at $p < 0.001$ (unc), cluster $p < 0.05_{FWE}$. ACC = anterior cingulate cortex, AG = angular gyrus, IFG = inferior frontal gyrus, ITG = inferior temporal gyrus, MCG = middle cingulate gyrus, MFG = middle frontal gyrus, MeFG = medial frontal gyrus, MOG = middle occipital gyrus, MTG = middle temporal gyrus, FG = fusiform gyrus, SFG = superior frontal gyrus. BA = Brodmann area, L = left, R = right.

indicating altered connectivity within the limbic lobe during stress in mixed and single gender samples (e.g. (Jovanovic et al., 2011; Kiem et al., 2013; Kogler et al., 2016; van Marle et al., 2009)). and tending towards increased connectivity within the limbic lobe and decreased connectivity between limbic areas and task areas. Interestingly, in both the SAT and the flanker task the hippocampus had greater connectivity to more ventral ACC areas which have greater baseline connectivity with affective areas such as the hippocampus and amygdalae and areas associated with non-task directed thoughts (Margulies et al., 2007). This may reflect an increased communication between the ACC and the hippocampus to self-regulate whilst strained by task demands. However, it was not associated with task performance and there was no related association between stress and task activations.

This study did not replicate the dysregulated amygdala connectivity findings previously reported in resting-state in women (Jovanovic et al., 2011; Kogler et al., 2016; van Marle et al., 2009). However, there was altered connectivity between emotion and self-referential processing areas in resting state. Taken together, these findings are consistent with previous reports suggesting women employ social and emotion focused coping mechanisms when feeling stressed (Kogler et al., 2016; Ptacek et al., 1994).
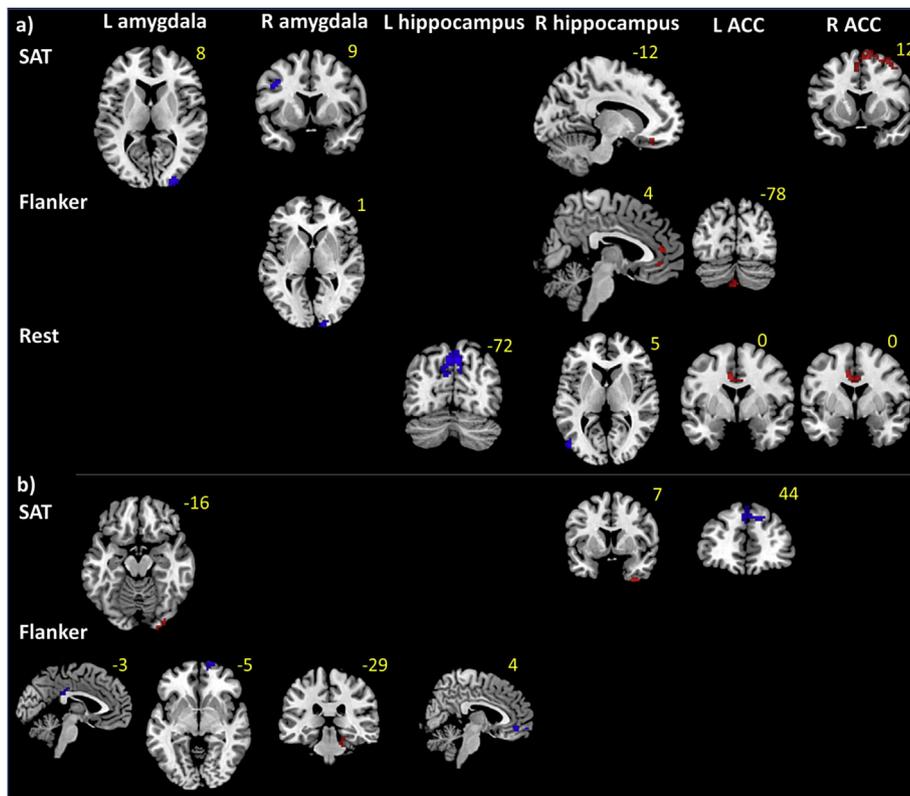
Fig. 5. Clusters where seed-to-voxel connectivity is associated with PSS score in a) women, b) men. Red = increased connectivity with stress, blue = decreased connectivity with stress. Top line indicates seed region. Left column indicates task or resting-state. Yellow numbers indicate slice in MNI space. Voxel: $p < 0.001$, cluster: $p < 0.05_{FWE}$. ACC = anterior cingulate cortex, L = left, R = right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
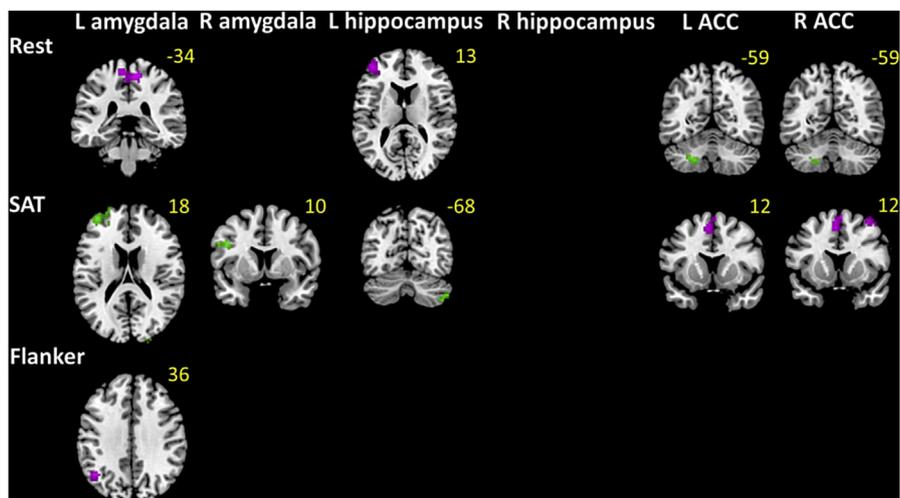
Fig. 6. Clusters where there is a gender difference in the association between seed-to-voxel connectivity and PSS score. Purple = more positive association between PSS score and connectivity in women compared to men. Green = more positive association between PSS score and connectivity in men compared to women. Top line indicates seed region. Left column indicates task or resting-state. Yellow numbers indicate slice in MNI space. Voxel: $p < 0.001$, cluster: $p < 0.05_{FWE}$. ACC = anterior cingulate cortex, L = left, R = right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Men

4.2.1. Stress, task performance and activations

In men there were no significant relationships between stress and task performance. This is supportive of the hypothesis for inhibition, but not the hypothesis for working memory and is in contrast to mixed gender studies and a meta-analysis suggesting acute stress is associated with improved working memory performance (Lewis et al., 2008; Shields et al., 2015; Vedhara et al., 2000). However, studies on men only samples have also reported no effect or decreased working memory accuracy after acute stress (Qin et al., 2012; Weerda et al., 2010). In accordance with this, this sample demonstrated a trend towards slower RTs in men experiencing higher perceived stress. There were no associations between

PSS and task activations, despite past studies employing male only samples indicating effects (Qin et al., 2012; Weerda et al., 2010). However, those studies conducted the task-related imaging immediately after an acute stress induction. Whilst the studies also included stress ratings, the results were related to completion of the stress induction paradigm which was chosen to reliably induce stress. Thus these results show that the more naturalistic level of stress does not provide significant effects on task performance or task activations in men.

4.2.2. Stress and connectivity

SAT. Perceived stress was associated with increased connectivity between the left amygdala and the fusiform gyrus, and the right hippocampus and more lateral temporal gyri. There was also decreased connectivity between the left ACC and medial superior frontal areas. Of these connections, the medial superior frontal areas (Brodmann area 8) and the superior (Brodmann area 38), but not inferior, temporal gyri (Ochsner et al., 2004) and the fusiform gyri (Phillips et al., 2008) have previously been implicated in emotional regulation. Thus, stress was associated with increased coupling from the amygdala and hippocampus to posterior emotional regulation areas, but decreased ACC to anterior emotional regulation areas. Notably, these findings were only apparent after adjusting for RT, suggesting they are indirectly related to performance and this warrants further investigation in future studies.

Flanker. The hypothesis that higher perceived stress would be associated with decreased connectivity in ACC in flanker task was not supported. Perceived stress was associated with a decrease in the connectivity between the left amygdala and medial frontal and middle cingulate and between the hippocampus to the ACC and medial frontal cortices. Activity in these areas has previously been reported to be positively correlated with amygdalae activity (Roy et al., 2009) and overlap with areas of non-task directed activity (Margulies et al., 2007; Mayer et al., 2010), suggesting that in those with higher levels of perceived stress compared to lower levels of perceived stress, connectivity between affective and anterior self-referential processing areas diminishes during the flanker task. Furthermore, in this case it may have implications for the role of the ACC in the flanker task, as indicated from the increased connectivity within the ACC in the flanker task contrast.

Rest. Again, the hypothesis that higher perceived stress would be associated with greater coupling between the amygdalae and the ACC and mePFC in resting-state in men was not supported, despite many studies reporting stress related dysregulation in resting-state connectivity in mixed and male only samples (Henckens et al., 2012; Kogler et al., 2016; Vaisvaser et al., 2013; Veer et al., 2012). However, none of the previous resting-state studies employed a measure of perceived stress, so whilst naturalistic, this measure may not result in the same effect sizes as acute induced stress or hormone related measures or the results may be confounded by the perceptive aspects of the measure.

4.2.3. Summary for men

Despite findings that are inconsistent with the hypotheses, there are common decreases in connectivity between seed regions and anterior DMN areas involved in self-referential processing with increasing task load in the flanker and SAT. Although, there is also an increase in seed to posterior DMN areas in SAT. Whilst this pattern has not been reported before, the variations seen here may be due to the subjective, perceptual nature of the stress measure used in this study.

These findings do not support the suggestion that men are reward focused during stress (Kogler et al., 2016; Wang et al., 2007). Ryan and colleagues (Ryan et al., 2011) showed that levels of

connectivity from the posterior cingulate to the ACC were associated with agreeableness. Thus, when already feeling stressed further task demands may affect pro-social states.

4.3. Summary and gender comparisons

On the behavioural level, there was a significant gender interaction on the association between stress and working memory RT; stress was associated with improved performance in women, compared to a non-significant decrease in performance in men. Notably, previous findings of improved working memory are from mixed gender studies (Shields et al., 2015), whereas the male only samples appear to present more mixed and inconclusive results (Henckens et al., 2009; Qin et al., 2012; Weerda et al., 2010; Wu et al., 2014). This indicates a need for future studies investigating the relationship between stress and task performance to stratify by gender.

Qualitatively, the results challenge past suggestions that men are more reward seeking than women during stress. Although the results are different within each gender, they indicate a similar pattern of dysregulated limbic connectivity, but differences in the form of dysregulation in our neuroimaging results. For instance, women exhibited a more consistent increase in coupling between emotion and self-regulation areas (Banks et al., 2007; Heimer and Van Hoesen, 2006), but decreased limbic to task related areas during tasks. This is contrasted with decreased coupling between emotion and self-referential processing areas (Banks et al., 2007) in the resting-state. These findings are consistent with increased emotional regulation demands and findings that women tend to use self-referenced speech to cope with stressful situations (Kogler et al., 2016). However, this self-regulating speech may also be of a ruminative nature (Kogler et al., 2016) so, given the correlational nature of these analyses, increasing the perception of being stressed. Conversely, men exhibited decreases in connectivity between affective processing areas and self-referential processing areas such as medial frontal and cingulate cortices, but only during the tasks and showed no relation of perceived stress and resting-state connectivity. This may be indicative of reduced ability to regulate emotions when feeling stressed and undergoing tasks (Ryan et al., 2011).

The gender and stress interaction analyses indicated that the majority of the findings were not significantly different between genders. This could suggest that whilst men and women process stress differently (Kilpatrick et al., 2006; S. J. Lupien et al., 2009; Otte et al., 2005), the differences are not big enough for a sample of this size to detect a gender interaction. There was a significant interaction in the SAT, which showed that the increased connectivity from the ACC to medial frontal gyrus in women, is not increased in men. Taken together, the results partially support suggestions that women are more likely than men to engage in emotionally focused stress management strategies (Kogler et al., 2016), but men also exhibit changes in connectivity in emotion regulation circuits when feeling stressed.

4.4. Limitations

This study contributes to the understanding of the effects of stress on task processing and neural activity. However, there are some limitations. First, given the moderate sample size it was important that we did not run too many tests, thus three ROIs which covered the entire anatomical region were chosen. Even so, it is acknowledged that sub-regions within the ROIs may be more relevant than others (Kiem et al., 2013; Margulies et al., 2007) and other areas previously implicated in the stress network were not included. Second, the accuracy for the LM condition in the SAT task

was lower than that of the HM task. This may raise questions as to the validity of the HL vs LL contrast. However, there were some notable differences between the LM condition compared to the other conditions such as 1) it would be easier to verbalise the LM condition than the other conditions due to only having to remember one square; 2) all other conditions have at least two boxes shaded in the Probe, thus could be considered pattern recall, rather than location recall for LM (Jiang et al., 2009) and 3) relating to point 2, for all other conditions the shaded squares can be 'anchored' to each other (Lew and Vul, 2015) and 4) unlike the LM condition the accuracy for the HL And LL conditions were congruent with our expectations. Third, there were very few associations between PSS and task performance. This could be explained by the comparatively small sample size and naturalistic stress measure. Nonetheless, the SAT RT results are consistent with previous reports, indicating some external validity to laboratory, hormonal and perceived measures of stress. The lack of task activation findings may be because - based on near ceiling accuracy levels and the smaller frontal task activations compared to previous working memory and inhibition imaging studies (Nee et al., 2013; Nee et al., 2007) - participants did not find the tasks very challenging; more differences may have been apparent in tasks with a more challenging high load condition as have been employed in previous studies (Bergdahl et al., 2005; Liston et al., 2009). Fourth, the analyses were stratified based on previous research indicating differences between genders (Kirschbaum et al., 1999; Kogler et al., 2016; Kudielka et al., 2009; Lighthall et al., 2012; McLaughlin et al., 2009; Wang et al., 2007), but very few of the gender stratified findings were significantly different between men and women. However, this could reflect the different nature of the stratified analyses, which indicates simple effects within gender, compared to the nature of an interaction analysis alongside a moderate sample size for investigating interaction effects. Finally, the perceived stress scale does not tease apart the many factors involved in an individual's perception of stress. These include, for instance past stress during key developmental periods, the duration of past and present stressors, vulnerabilities or availability of support. This sample reported low levels of depressive symptoms, childhood adversity and life events over the past year, but the scale may be unduly influenced by the participants' mood at the time of completing the questionnaire or social desirability effects (Bowling, 2005), rather than an accurate report of average perceived stress over the last month, therefore resulting in a more acute measure of perceived stress. However, more 'objective' measures of stress such as life event checklists are also subject to biases (Bowling, 2005; Monroe, 2008). The PSS has been well validated and documented to prospectively predict health outcomes independent of psychological symptoms or life events and correlates with biological measures of stress (Monroe, 2008). Nonetheless, by screening out participants with high levels of depressive symptoms and the fact that few participants in this sample had experienced many life events either in childhood or the last year may have resulted in decreased sensitivity. Using the PSS may have different implications from that of more objective or comprehensive measures, and highlights the correlational nature of the results. For instance the results may reflect differences in the neural processing of stress perception and, as mentioned before, higher self-referential processing may be related to rumination which could increase stress perception. Finally, these results did not replicate past findings from other studies, though they were generally consistent with themes from previous studies. However, many studies investigating the neurobiology of stress have failed to exactly replicate past results, but show complementary results despite different protocols (e.g. Henckens et al., 2009; Henckens et al., 2012; Kiem et al., 2013; Vaisvaser et al., 2013; Veer et al., 2011, 2012).

4.5. Conclusions

In summary, this study characterises the relationship between perceived stress and (1) task performance and processing and (2) task and resting-state connectivity in men and women. We demonstrated a significant gender difference in the relationship between stress and response time in working memory, which is consistent with past studies (e.g. Lewis et al., 2008; Qin et al., 2012; Shields et al., 2015; Vedhara et al., 2000). There were no associations between stress and task activations in either gender, which may relate to the naturalistic measure of stress, or the less challenging nature of the tasks compared to other studies (Bergdahl et al., 2005; Liston et al., 2009). Nevertheless, there were many significant associations between stress and connectivity. Findings in women were consistent with increased emotional regulation demands and support the notion that women tend to use self-referenced speech to cope with stressful situations (Kogler et al., 2016). However, this self-regulating speech may also be of a ruminative nature (Kogler et al., 2016) therefore increasing the perception of being stressed. Men exhibited some increases and some decreases in connectivity between affective processing areas during the tasks and showed no relation of perceived stress and resting-state connectivity. Perhaps indicative of less ability to regulate emotions when feeling stressed and engaging in a task (Ryan et al., 2011). This challenges previous reports of men being reward focused during stress (Kogler et al., 2016), but again, perhaps this is influenced by the perceptive nature of the stress assessment. However, very few of the within gender findings were significantly different between genders. This study supports previous reports of gender differences in stress processing and has shown perceived stress is associated with altered functional connectivity in task and resting-state, which demonstrates the contribution of a naturalistic measure of stress to the understanding of stress processing and cognitive function.

Conflict of interest

No authors have no conflict of interest to declare. The funding source had no involvement in the design; the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ynstr.2017.01.002.

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