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ORIGINAL ARTICLE

Brain mechanisms of anxiety's effects on cognitive control in major depressive disorder

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Background. Adults with major depressive disorder (MDD) demonstrate increased susceptibility to interfering effects of anxiety on cognitive control; although under certain conditions adults with MDD are able to compensate for these effects. The brain mechanisms that may facilitate the ability to compensate for anxiety either via the recruitment of additional cognitive resources or via the regulation of interference from anxiety remain largely unknown. To clarify these mechanisms, we examined the effects of anxiety on brain activity and amygdala–prefrontal functional connectivity in adults diagnosed with MDD.

Method. A total of 22 unmedicated adults with MDD and 18 healthy controls (HCs) performed the Tower of London task under conditions designed to induce anxiety, while undergoing a functional magnetic resonance imaging assessment.

Results. During the easy condition, the MDD group demonstrated equivalent planning accuracy, longer planning times, elevated amygdala activity and left rostrolateral prefrontal cortex (RLPFC) hyperactivity relative to HCs. Anxiety mediated observed group differences in planning times, as well as differences in amygdala activation, which subsequently mediated observed differences in RLPFC activation. During the easy condition, the MDD group also demonstrated increased negative amygdala–dorsolateral prefrontal cortex (DLPFC) connectivity which correlated with improved planning accuracy. During the hard condition, HCs demonstrated greater DLPFC activation and stronger negative amygdala–DLPFC connectivity, which was unrelated to planning accuracy.

Conclusions. Our results suggest that persons with MDD compensate for anxiety-related limbic activation during low-load cognitive tasks by recruiting additional RLPFC activation and through increased inhibitory amygdala–DLPFC communication. Targeting these neural mechanisms directly may improve cognitive functioning in MDD.

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Key words: Anxiety, cognitive control, depression, norepinephrine, stress.

Introduction

Anxiety occurring during attempts to engage in goal-directed activity can inhibit prefrontal control (Fales *et al.* 2008) and impair performance (Vytal *et al.* 2012; Clarke & Johnstone, 2013). This appears to be particularly true for adults diagnosed with major depressive disorder (MDD; Jones *et al.* 2015). Although, under certain conditions, adults with MDD appear to compensate for the interfering effects of anxiety (Jones *et al.* 2015), the brain mechanisms underlying the effect of anxiety on prefrontal control in MDD remain largely unknown, as do the mechanisms that facilitate compensating for the effects of anxiety during goal-directed tasks. Clarifying these mechanisms may identify neural circuits that could be targeted by alternative treatments (e.g. brain stimulation) to improve cognitive functioning (Kalu *et al.* 2012). This is important given that poor cognitive functioning in MDD is impairing (Godard *et al.* 2011), inadequately responds to medication (Trivedi & Greer, 2014), and costs society up to $31 billion a year in lost productivity (Stewart *et al.* 2003).

Anxiety may be characterized as an emotional response to vague, potential threats (Tovote *et al.* 2015) generated, in part, by activation of the amygdala (Etkin & Wager, 2007; Adhikari *et al.* 2015; McCall *et al.* 2015). During difficult cognitive tasks, individuals with MDD appear more sensitive to the effects of anxiety, such that elevated levels of anxiety are more strongly associated with decreased behavioral accuracy and decreased task-evoked pupillary responses (TEPRs) relative to healthy controls (HCs) (Jones *et al.* 2015). TEPRs are thought to index phasic locus coeruleus–norepinephrine (LC-NE) firing in the cortex, which is positively associated with task-related brain activity (Siegle *et al.* 2003; Minzenberg *et al.* 2008). As such, these results are consistent with the inhibitory effect of amygdala activation on the recruitment of

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prefrontal cognitive control. However, in Jones *et al.* (2015) brain activation was not directly examined, hence the effect of anxiety on brain activation in MDD remains unclear. Interestingly, a subset of individuals with MDD, who were highly engaged with the task, demonstrated greater TEPRs relative to HCs, indicative of increased phasic LC-NE firing and increased recruitment of task-related brain activity in order to compensate for the presence of anxiety (Jones *et al.* 2015). Indeed, increased left rostrolateral prefrontal cortex (RLPFC) activation has been shown to facilitate the ability to compensate for the presence of emotional interference in MDD (Etkin & Schatzberg, 2011). However, in Etkin & Schatzberg (2011), state anxiety occurring during the task was not assessed, thus it remains unclear whether the left RLPFC helps individuals with MDD compensate for anxiety interference in the context of a cognitive task. Compensation may also involve the inhibition of the emotion's interfering effects. In healthy populations increased amygdala–lateral PFC and amygdala–ventrolateral PFC connectivity is associated with reduced interference from anxiety during goal-directed tasks and with improved cognitive performance (Dolcos *et al.* 2006; Clarke & Johnstone, 2013; Gold *et al.* 2015). MDD is associated with decreased resting amygdala–ventrolateral PFC connectivity (Tang *et al.* 2013; Ramasubbu *et al.* 2014), as well as decreased amygdala–dorsolateral prefrontal cortex (DLPFC) connectivity during emotional processing (Siegle *et al.* 2007), and emotion regulation (Erk *et al.* 2010). As such, individuals diagnosed with MDD may demonstrate decreased amygdala–PFC connectivity, representing difficulty regulating interference from anxiety during goal-directed tasks. However, compensation via increased inhibition of the amygdala by the PFC could potentially occur.

In the current study, we extend our previous findings (Jones *et al.* 2015) by examining the brain mechanisms underlying the effect of anxiety on prefrontal control in MDD; and the brain mechanisms that facilitate compensating for the effects of anxiety during goal-directed tasks. Participants completed a modified version of the Tower of London (TOL) task (Shallice, 1982; Unterrainer *et al.* 2004) while undergoing a functional magnetic resonance imaging (fMRI) assessment with concurrent pupillometry. The TOL task engages multiple cognitive processes, including working memory supported by the DLPFC and planning supported by the left RLPFC (Wagner *et al.* 2006). We modified the TOL task to induce anxiety (see online Supplementary material) which allowed us to examine the effects of anxiety on limbic and prefrontal activity and the role of amygdala–prefrontal connectivity in MDD.

We hypothesized that the MDD group would demonstrate longer planning times and either intact or poorer planning accuracy than HCs and that anxiety would mediate observed differences in performance. Consistent with the aforementioned pattern, we predicted that the MDD group would demonstrate greater amygdala activity, and altered PFC activation relative to HCs. We expected the direction of PFC activation to depend on whether or not preserved behavioral accuracy was observed in the MDD group. In addition, we predicted that anxiety would mediate observed differences in amygdala activation, which would subsequently mediate observed differences in PFC activation. We further hypothesized that amygdala–DLPFC functional connectivity would be altered in the MDD group relative to HCs and that connectivity would be associated with planning accuracy.

Method

Participants

A total of 22 unmedicated adults diagnosed as having a current major depressive episode (mean age = 25.6 years, S.D. = 7.8 years; 12 females; 82% Caucasian, 9% African-American, 4.5% Asian, 4.5% Hispanic/Latino) via a structured clinical interview (SCID-I; First *et al.* 1996) were compared with an age-matched group of 18 never-depressed HCs with no current or past psychiatric diagnoses based on the SCID-I (First *et al.* 1996), and with no known first-degree relatives with psychiatric diagnoses (mean age = 24.5 years, S.D. = 6.24 years; 12 females, 89% Caucasian, 6% African-American, 5% Asian). Of the depressed participants, 14 (64%) had a co-morbid anxiety diagnosis. All participants were required to have a full-scale intelligence quotient equivalent estimate >80 based on the North American Adult Reading Test (NAART; Nelson & Willison, 1991). All participants reported no significant eye problems, health problems, psychoactive drug or alcohol abuse within the past 6 months, history of psychosis, or manic episodes and were not on psychotropic medications during the past month.

Procedure

Participants were recruited from the community. During an initial assessment, after providing written informed consent, all participants were screened using a SCID-I interview (First *et al.* 1996), a color-recognition test, and the NAART. Participants were then trained on the TOL task and completed a questionnaire battery assessing mood symptoms via computer. During a subsequent fMRI assessment, participants completed symptom questionnaires and practised the task. This study was approved by the

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University of Pittsburgh Institutional Review Board. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Experimental paradigm

Participants completed 21 TOL problems, whose optimal solution required 4, 5 or 6 moves over the course of three blocks (Unterrainer *et al.* 2004). Each level of difficulty was interspersed within each block. The five 5-move problems and five 6-move problems were combined into one level of difficulty to improve estimation of the blood oxygen level-dependent signal during planning difficult problems. Participants were presented with a 1.67 s fixation cue, followed by a TOL problem that they were instructed to solve according to standard rules. Participants had 35.07 s to plan their solution and select their answer using a mouse. Next, participants were given 20.04 s to show their plan. Subsequently, participants were given performance feedback for 5.01 s, followed by an 11.69 s fixation (see Jones *et al.* 2015 – Fig. 1). Planning time (time needed to plan their response) and accuracy (percentage of problems solved correctly) were recorded. At the start of the task and after each block, participants were asked to rate their mood. One HC had a mouse malfunction during the first block of the TOL task; performance data for this subject were excluded from relevant analyses.

Anxiety measure

Immediately prior to beginning the task (i.e. baseline), participants rated how anxious they were feeling in the current moment on a scale from (1) 'not at all' to (6) 'a great deal'. After each block, they completed analogous ratings in reference to the last block of problems. Single-item measures of anxiety are ideal in situations where rapid unobtrusive measurement of anxiety is warranted; these types of measures demonstrate good reliability, validity and sensitivity (Davey *et al.* 2007; Abend *et al.* 2014).

fMRI data acquisition/preprocessing

fMRI data were collected using a 3.0-T Siemens Tim Trio scanner. Each functional volume contained 32 oblique axial slices (repetition time = 1.67, echo time = 29; flip angle = 75°, field of view = 205, 3.2 mm thickness, 64 × 64 matrix, 3.2 × 3.2 mm in-plane resolution) parallel to the anterior commissure/posterior commissure plane. Axial anatomical images were acquired using a standard T1-weighted spin-echo pulse sequence using a finer in-plane resolution (1 mm thickness, field of view = 25 mm, 256 × 256 matrix, 1 × 1 mm in-plane resolution). Preprocessing of the functional imaging data was completed using a variety of packages primarily including AFNI (Cox, 1996) and NIS (Fissell *et al.* 2003). Preprocessing included iterated least-square motion correction (AFNI 3dVolReg), realigning of volume slices to the same temporal origin (AFNI 3dTshift), detrending and outlier correction (NIS Correct), temporal smoothing with a five-point middle-peaked filter (NISfilter), and voxelwise conversion to percentage change from the median of the dataset. Functional images were transformed into a common space using the parameters from co-registering the anatomical images to the Montreal Neurological Institute (MNI) template (32 parameter non-linear warp to MNI brain) (Woods *et al.* 1992), and then spatially smoothed (6 mm full width at half maximum; FWHM) to accommodate individual anatomical differences (NIS gsmooth).

Pupil dilation acquisition/preprocessing

As participants lay in the moderately lit fMRI scanner, stimuli were presented on a back-projection screen approximately 127 cm from a mirror that was placed approximately 12 cm above their eye. Pupil dilation was acquired using an ASL Long Range Optics unit (Applied Science Laboratories, Inc.). Pupil data were cleaned and preprocessed as described in Jones *et al.* (2015). Removal of trials with over 50% blinks resulted in mean loss of 1 trial (S.D. = 2). Due to acquisition errors, pupillary data from eight participants (two HC, eight MDD) could not be analysed.

Behavioral analyses

Mixed-effects models, conducted in SAS/STAT® version 9.4 (SAS Institute Inc., USA), were used to examine group differences in anxiety and behavioral performance. To test whether baseline levels of anxiety mediated observed group differences in cognitive performance, mediation analyses were conducted using the MEDCURVE macro (Hayes & Preacher, 2010), which allows constituent paths in the mediation to be non-linear. This method was chosen given the expected Yerkes–Dodson association between arousal and performance (Yerkes & Dodson, 1908). Given that traditional methods used to examine mediation are typically underpowered (MacKinnon *et al.* 2002) and can be biased when used with small samples, we employed an indirect effects approach (Preacher & Hayes, 2008). We used a bootstrapping method with 95% bias-corrected confidence intervals (CIs) to test the significance of these mediation effects.

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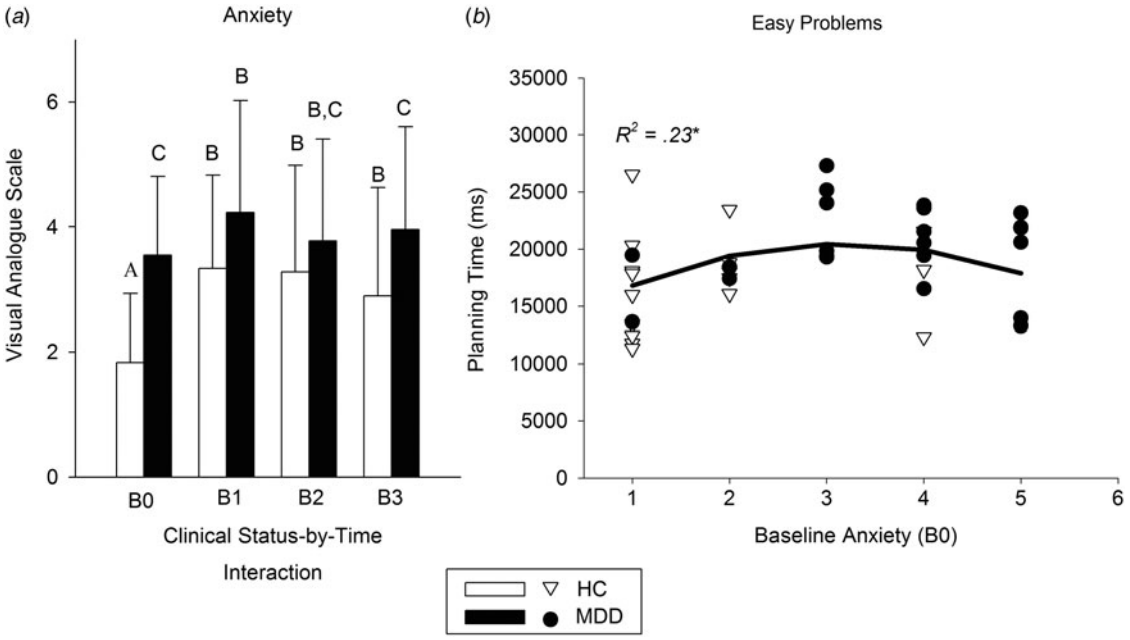

Fig. 1. (*a*) Group differences and changes in anxiety across task blocks. Values are means, with standard deviations represented by vertical bars. ^{A,B,C} Mean values with unlike letters were significantly different ($p < 0.05$). (*b*) Non-linear mediation of group differences in easy planning time by baseline anxiety. HC, Healthy control; MDD, major depressive disorder.

Neuroimaging analyses

Region of interest (ROI) analysis

Our *a priori* analyses focused on left hemispheric activation in the RLPFC, DLPFC and amygdala. We were particularly interested in activation within the left RLPFC which has been shown to specifically be involved in planning (e.g. Wagner *et al.* 2006; Trujillo *et al.* 2015); in addition, there is evidence to suggest that MDD is associated with greater left hemispheric dysfunction (Thibodeau *et al.* 2006). However, the current study was not powered to detect hemispheric differences. By focusing our *a priori* analyses on the left hemisphere we also controlled the number of independent comparisons we examined (see online Supplementary Fig. S1 for right-hemispheric activation).

To obtain an ROI for the DLPFC, we used a mask of the left DLPFC ($x = -40$, $y = 33$, $z = 37$) derived from a meta-analysis of published TOL studies (see online Supplementary data for details of the meta-analysis). We used a mask of the left RLPFC (Brodmann area 10) by placing a 10 mm sphere centered on MNI coordinates ($x = -37$, $y = 49$, $z = 12$) reported in Wagner *et al.* (2006)[1]†. We used an anatomically derived left amygdala mask ($x = -23$, $y = -4$, $z = -17$) as described in Siegle *et al.* (2002) to examine limbic activity during planning[2].

Percentage signal change time-series data during the fixation and planning phases of the task for both levels of difficulty were extracted for the left amygdala, left RLPFC and left DLPFC ROIs. Hierarchical mixed-effects analyses of each respective ROI were conducted using SAS/STAT proc glimmix. The hemodynamic response function (HRF) was modeled as a linear combination of seven cubic B-spline functions. This approach has been shown to overcome a significant issue in standard multi-subject fMRI data analyses, in that it allows both the shape and timing of the HRF to vary across regions and subjects (Degras & Lindquist, 2014). When utilizing multiple basis functions to analyse time-series data with independent variables (IVs), the statistical term of interest represents the interaction between the IVs and the combined-basis-functions. Probing the interaction entails testing the significance of the association between the IVs and dependent variable at each time point within the original time-scale by combining and fixing the Basis functions to the appropriate values corresponding to the original time-scale. Type I error rates were controlled within each analysis using the Guthrie and Buchwald method (Guthrie & Buchwald, 1991). See online Supplementary data for the detailed analytic plan.

Brain mediation analyses

We conducted a serial mediation analyses in MPLUS (Muthén & Muthén, 1998–2006) with 'robust' estimation of S.E. to determine if baseline anxiety mediated observed group differences in amygdala activation,

† The notes appear after the main text.

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and whether elevated levels of amygdala activation mediate observed differences in prefrontal cortex activation. Because true mediation analyses require precise knowledge of the temporal ordering of effects (Baron & Kenny, 1986; Kraemer *et al.* 2002), we refer to models in which temporal sequencing is unknown as statistical mediation, which represents the degree to which one set of relationships can statistically account for (or explain) another.

Psychophysiological interaction (PPI) analysis

We used PPI to measure changes in functional connectivity modulated by task difficulty. We conducted whole-brain PPI tests, reflecting greater correlation with the left amygdala seed time series for easy *v.* hard conditions. This contrast was selected given that the interfering effects of anxiety on the PFC are more readily observed during low relative to high levels of cognitive load (Bishop, 2009; Vytal *et al.* 2012; Clarke & Johnstone, 2013). We used AFNI 3dClustSim to perform a Monte Carlo simulation within the volume (using the group smoothness from level 2 analyses estimated using 3dFWHMx) and found that a voxel-wise $p < 0.005$ combined with an extent threshold of 54 voxels corresponded to a family-wise error rate of $p < 0.050$. Greater connectivity in one condition *v.* another can result from either more positive or less negative connectivity in one condition *v.* the other. To clarify the nature of the PPI interaction we decomposed the interaction by examining β weights for the contrast of amygdala–PFC connectivity *v.* baseline for each condition for each group. To determine if the strength of the observed limbic–prefrontal connectivity was correlated with planning accuracy we conducted robust regression analyses in SAS/STAT using an MM estimation method (Yohai, 1987).

TEPRs data analyses

Mean Pearson correlations were used to evaluate the association between TEPRs and activity in extracted ROIs for each condition. Hierarchical mixed-effects analyses were used to analyse group differences in TEPRs (see online Supplementary material for details).

Results

Did the groups differ in their experience of anxiety across the task?

The effects of clinical status ($F_{1,38} = 6.50$, $p = 0.015$) and time ($F_{3,114} = 12.49$, $p < 0.001$) on anxiety were qualified by a clinical status × time interaction ($F_{3,114} = 2.88$, $p = 0.039$). The MDD group reported higher levels of anxiety than the HCs at baseline ($t_{114} = 3.52$, $p < 0.001$, $d = 1.12$), and during block 3 ($t_{114} = 2.19$, $p = 0.031$, $d = 0.70$), but not block 1 ($t_{114} = 1.84$, $p = 0.069$, $d = 0.58$) or block 2 ($t_{114} = 1.02$, $p = 0.311$, $d = 0.32$). The MDD group demonstrated an increase in anxiety from baseline to block 1, which decreased by block 2 and remained stable at indistinguishable levels from baseline (see Fig. 1*a*). Levels of anxiety in the HCs increased from baseline and remained stable across task blocks (online Supplementary Table S5). Given that, unlike in Jones *et al.* (2015), the task did not cause a sustained increase in anxiety in the MDD group we used baseline anxiety as our potential mediator of performance and brain activity. Mediation analyses of observed group differences in performance and brain activity conducted with task anxiety were not statistically significant (see online Supplementary data analyses).

Did the groups differ in their cognitive performance?

Planning time

There were main effects of clinical status ($F_{1,37} = 6.53$, $p = 0.015$, $d = 0.81$) and task difficulty ($F_{1,37} = 98.1$, $p < 0.001$, $d = 3.14$), but no clinical status × difficulty interaction ($F_{1,37} = 0.39$, $p = 0.535$) on planning time. These results indicate that the MDD group demonstrated longer planning times (mean = 23 367, S.E. = 642 ms) relative to the control group (mean = 20 881, S.E. = 731 ms).

Planning accuracy

Main effects of clinical status ($F_{1,37} = 2.33$, $p = 0.136$, $d = 0.49$) and task difficulty ($F_{1,37} = 52.58$, $p < 0.001$, $d = 2.34$) were not qualified by a clinical status x difficulty interaction ($F_{1,37} = 0.09$, $p = 0.763$) on planning accuracy. These results indicate that the MDD group demonstrated intact planning accuracy (mean = 65, S.E. = 4.6%) relative to HCs (mean = 75, S.E. = 5.5%) during the easy condition, and that both groups demonstrated reduced planning accuracy (MDD: mean = 38, S.E. = 4.8%, HC: mean = 46, S.E. = 5.5%) during the difficult task condition.

Did baseline anxiety explain why the MDD group demonstrated longer planning times relative to HCs?

The quadratic effect of baseline anxiety mediated group differences in easy planning times (instantaneous indirect effect: $\theta = 3323$, S.E. = 2469, 95% CI 69–10185 ms; see Table 1). As shown in Fig. 1*b*, baseline anxiety explained the presence of longer planning times in the MDD group relative to HCs in an inverted U-shaped fashion. Baseline anxiety was positively correlated with longer planning times in individuals experiencing low to moderate levels of anxiety (anxiety < 3.5), whereas individuals high in anxiety (anxiety >

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Table 1. *Results of behavioral and brain mediation analyses*

Dependent variable	Predictor	β	b	S.E.	t	p	R^2
Behavioral data							
$Anxiety_{Baseline}$	Clinical status	0.58	1.66	0.39	4.29	<0.001	0.33
Easy: planning time	Clinical status	0.24	2009	1512	1.33	0.193	0.23
	$Anxiety_{Baseline}$	1.71	4909	2289	2.14	0.039	
	$Anxiety^2_{Baseline}$	−1.59	−773	380	−2.04	0.049	
Hard: planning time	Clinical status	0.30	2133	1387	1.54	0.133	0.09
	$Anxiety_{Baseline}$	0.43	1041	2100	0.50	0.623	
	$Anxiety^2_{Baseline}$	−0.47	−193	348	−0.55	0.584	
fMRI data							
$Anxiety_{Baseline}$	Clinical status	0.59	1.71	0.37	4.61	<0.001	0.35
Amygdala	Clinical status	0.67	16.00	2.81	5.67	<0.001	0.45
Amygdala	$Anxiety_{Baseline}$	0.28	2.29	0.89	2.58	0.010	0.50
	Clinical status	0.50	12.03	3.02	3.98	<0.001	
RLPFC	Amygdala	0.54	0.87	0.28	3.11	0.002	0.42
	Clinical status	0.15	0.58	6.35	0.92	0.357	
RLPFC	Amygdala	0.51	0.83	0.28	3.03	0.002	0.42
	$Anxiety_{Baseline}$	0.07	0.98	2.41	0.41	0.683	
	Clinical status	0.12	4.78	7.41	0.65	0.518	

S.E., Standard error; fMRI, functional magnetic resonance imaging; RLPFC, rostrolateral prefrontal cortex.

3.5) demonstrated faster planning times. The quadratic effect of baseline anxiety did not mediate group differences in hard planning times (instantaneous indirect effect: $\theta = 525$, S.E. = 1983, 95% CI −2715 to 5765 ms). Given the observed interference from anxiety on planning times and intact planning accuracy in the easy condition, we expect neural compensation via increased PFC activation and/or increased amygdala–PFC connectivity to be observed in the following analyses.

Did MDD and HC groups differ in the degree of activation in the amygdala and prefrontal regions?

Left amygdala

There was a significant clinical status × difficulty × combined-basis-function interaction ($F_{7,385.7} = 2.92$, $p = 0.006$) on amygdala activity. Probing the interaction indicated that during easy problems the MDD group demonstrated less of a decrease in amygdala activity from 10.02 to 23.38 s relative to HCs (p's < 0.050, corrected, $d = 0.69$). No differences were observed during hard problems (p's > 0.050, corrected, $d = 0.19$, see Fig. 2*a*). These results indicate that the MDD group demonstrated increased limbic activation associated with anxiety during the task.

Left RLPFC

There was a significant clinical status × difficulty × combined-basis-function interaction ($F_{7,487.1} = 2.14$, $p = 0.038$) on RLPFC activity. Probing the interaction indicated that during easy problems the MDD group demonstrated greater RLPFC activity from 15.03 to 23.38 s relative to the HCs (p's < 0.050, corrected, $d = 0.78$). Group differences were not observed during hard problems (p's > 0.050, corrected, $d = 0.28$, see Fig. 2*b*). These results, combined with the positive association between RLPFC activation and planning accuracy, suggest that the MDD group needed to compensate in order to maintain their planning accuracy.

Left DLPFC

There was a significant clinical status × difficulty × combined-basis-function interaction ($F_{7,266.4} = 2.21$, $p = 0.034$) on DLPFC activity. Probing the interaction indicated that during hard problems the MDD group demonstrated decreased DLPFC activity from 15.03 to 35.07 s relative to HCs (p's < 0.050, corrected, $d = 0.74$). Group differences were not observed during easy problems (p's > 0.050, corrected, $d = 0.24$, Fig. 2*c*). Consistent with strong correlations between TEPRs and left DLPFC activation (easy/hard: mean = 0.64/0.73, S.D. = 0.41/0.43), group differences in TEPRs followed an identical pattern of activation as the left DLPFC (see online Supplementary Fig. S5). These DLPFC and pupil findings suggest that during difficult problems, HCs recruited additional working memory resources relative to the MDD group; however, this did not translate into improved accuracy. See online

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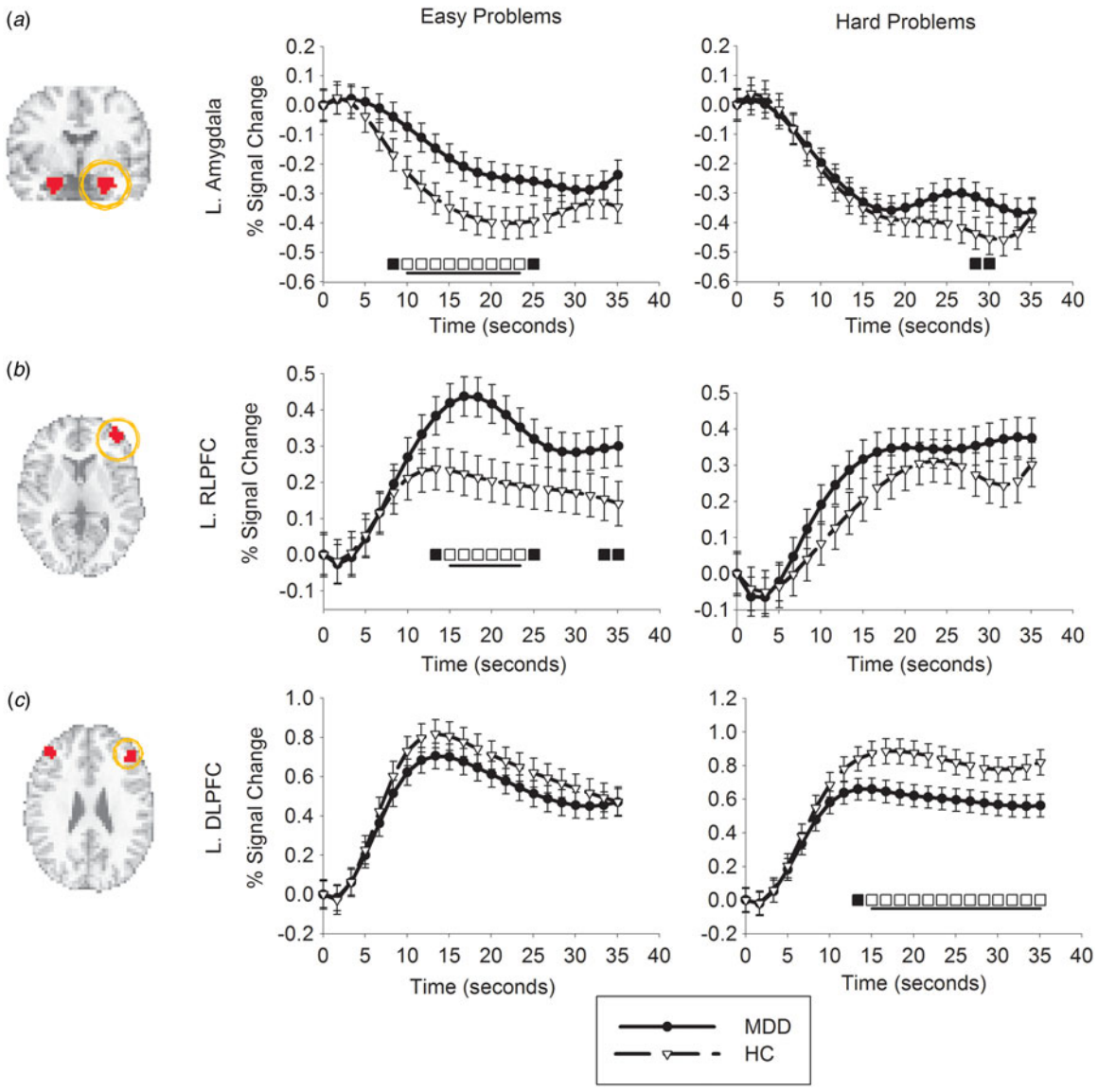

Fig. 2. Region of interest time series analyses. (*a*) Group differences in the time series of left amygdala (L. Amygdala) activity for easy and hard problems. (*b*) Group differences in the time series of left rostrolateral prefrontal cortex (L. RLPFC) activity for easy and hard problems. (*c*) Group differences in the time series of left dorsolateral prefrontal cortex (L. DLPFC) activation for easy and hard problems. Values are means, with standard errors represented by vertical bars. The squares under each time series indicate where the groups differed (□ = $p < 0.050$, ■ = $p < 0.100$). Black underlined segments indicate regions that were statistically significant after contiguity thresholding to control for multiple comparisons ($p < 0.050$). MDD, Major depressive disorder; HC, healthy control.

Supplementary Table S7 for full models predicting amygdala, RLPFC and DLPFC activation.

Did baseline anxiety explain the presence of elevated amygdala activation and did amygdala activation account for elevated levels of RLPFC activation observed in the MDD group?

As shown in Fig. 3*a*, baseline anxiety mediated differences between MDD and HCs in left amygdala activation (indirect effect = 0.16, S.E. = 0.08, $p = 0.037$, 95% CI 0.01–0.32), supporting the conclusion that elevated amygdala activity during the task in the MDD group was explained by having increased levels of baseline anxiety. In addition, increased amygdala activation statistically accounted for observed differences between MDD and HCs in RLPFC activation (indirect effect = 0.36, S.E. = 0.13, $p = 0.007$, 95% CI 0.10–0.62; see Fig. 3*b*). Serial mediation analyses indicated a marginal indirect effect of clinical status on RLPFC activation acting through baseline anxiety and amygdala activation (indirect effect = 0.08, S.E. = 0.05, $p = 0.095$, 95% CI

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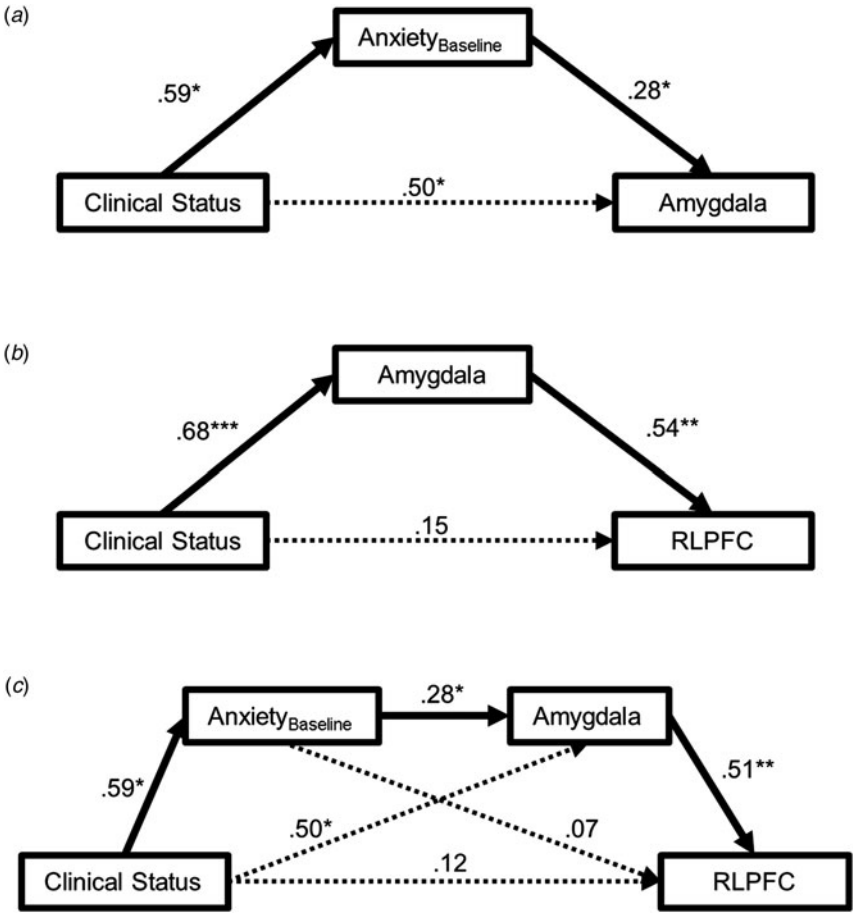

Fig. 3. Path analyses demonstrating the mediation of group differences in brain activity. (*a*) Mediation of group differences in amygdala activation by baseline anxiety. (*b*) Statistical mediation of group differences in rostrolateral prefrontal cortex (RLPFC) activation by amygdala activity. (*c*) Serial mediation of group differences in RLPFC activation via anxiety and amygdala activation. Solid lines reflect mediation paths. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

−0.02 to 0.18; see Fig. 3*c*). These results provide additional support for the conclusion that increased RLPFC activation in the MDD group reflects compensatory recruitment of prefrontal control due to elevated anxiety-related amygdala activation during the task.

Did MDD and HC groups differ in the magnitude of amygdala–prefrontal functional connectivity?

A test of group differences in the hard *v*. easy PPI contrast revealed that HCs demonstrated greater left amygdala–middle frontal gyrus connectivity (Brodmann area 9/46) ($t_{max} = 5.10$, 64 voxels), hereafter referred to as amygdala–DLPFC connectivity for ease of comparison with extant literature, and –frontal operculum ($t_{max} = 5.21$, 107 voxels) connectivity than the MDD group (see Fig. 4*a*, *b*). Decomposing the PPI interaction for the amygdala–DLPFC region (see Fig. 4*c*) indicated that this was due to HCs having less negative connectivity in the easy relative to hard condition (Cohen's $d = 1.26$), and a lack of a differentiation between the easy and the hard condition in the MDD group (Cohen's $d = 0.03$). The amygdala and DLPFC demonstrate mutually inhibitory influences on one another, such that stronger negative connectivity may reflect the use of prefrontal resources to inhibit amygdala activation or *vice versa* (Anticevic *et al.* 2010; Yun *et al.* 2010; Clarke & Johnstone, 2013). The MDD group demonstrated stronger negative connectivity in the easy condition relative to controls (Cohen's $d = 0.56$), whereas the reverse was true for the difficult condition (Cohen's $d = -0.79$). These results suggest that the MDD group needed to regulate limbic activity across both levels of difficulty, whereas the controls only needed to regulate during the hard condition.

Was amygdala–DLPFC functional connectivity associated with greater planning accuracy?

There was a significant easy planning accuracy x clinical status interaction ($b = -0.03$, S.E. = 0.01, $p = 0.002$), indicating that the strength of the association between

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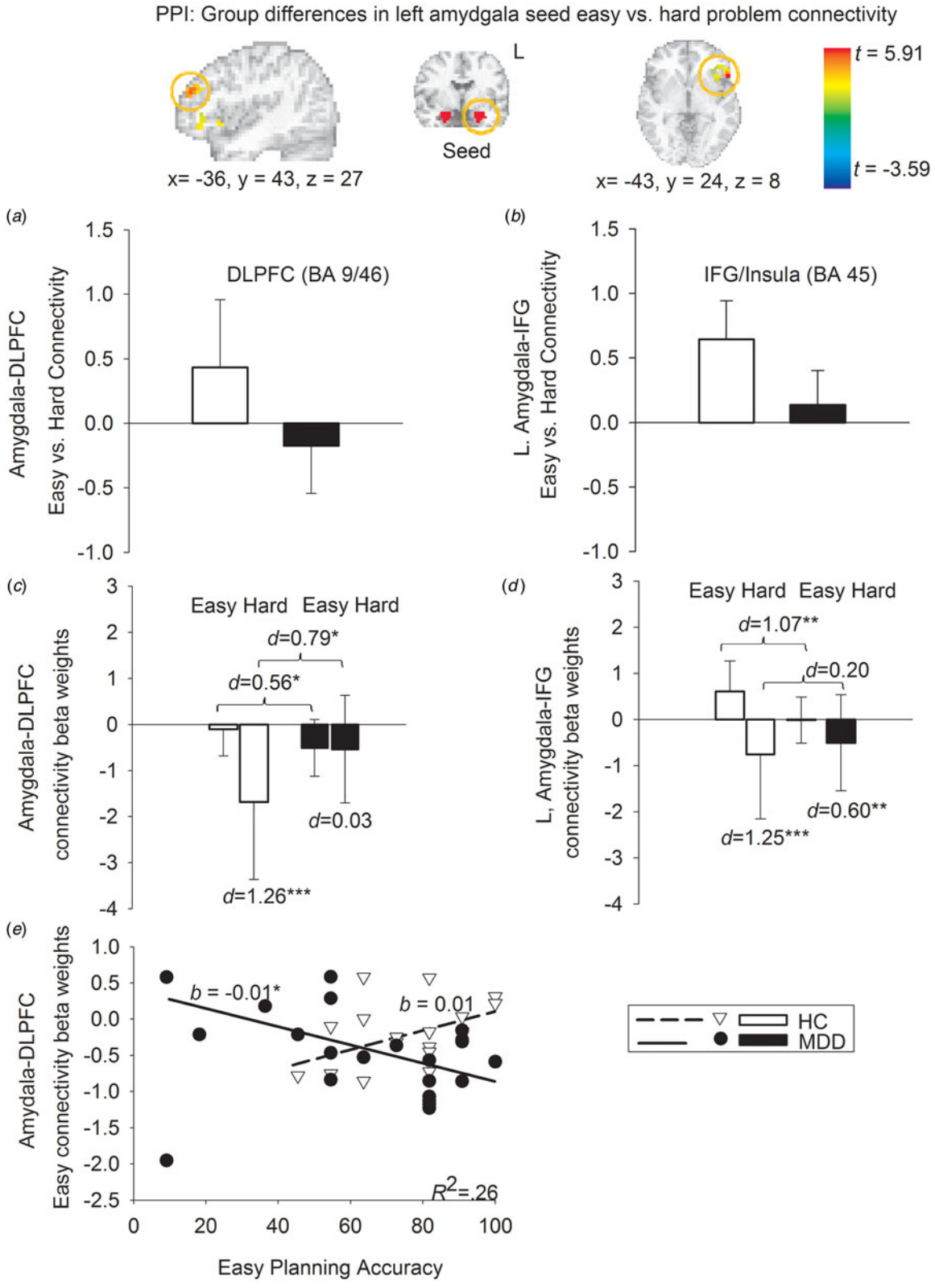

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planning accuracy and the amygdala–DLPFC functional connectivity in the easy condition differed as a function of group. As shown in Fig. 4*e*, probing the interaction indicated that planning accuracy was positively correlated with negative amygdala–DLPFC connectivity in the MDD group ($b = -0.01$, S.E. = 0.004, $p = 0.003$) and was not significantly correlated with planning accuracy in the HCs ($b = 0.01$, S.E. = 0.01, $p = 0.069$) (model $R^2 = 0.26$) in the easy condition. The MDD group demonstrated greater variance in easy planning accuracy relative to HCs ($F_{21,17} = 2.58$, $p = 0.052$) indicative of a restriction of range in HCs. These results suggest that within the MDD group, greater prefrontal–limbic regulation was associated with greater planning accuracy. There was not a significant hard planning accuracy × clinical status interaction ($b = -0.03$, S.E. = 0.01, $p = 0.002$) and planning accuracy was not correlated with amygdala–DLPFC connectivity in the difficult condition. Amygdala–frontal operculum connectivity was not associated with performance (see online Supplementary material).

Discussion

We aimed to clarify the mechanisms underlying the impact of anxiety on goal-directed activity in MDD and to identify compensatory neural mechanisms that may facilitate behavioral performance. Results indicated that during the easy condition persons diagnosed with MDD recruited additional RLPFC-mediated cognitive control and demonstrated increased negative amygdala–DLPFC connectivity in order to maintain planning accuracy due to interference from anxiety. In the hard condition the MDD group demonstrated decreased DLPFC activation and decreased inhibitory amygdala–DLPFC connectivity relative to HCs.

Unexpectedly, increased anxiety in the MDD group was not sustained during the task. This was probably due to the MDD group feeling an increased sense of competence as the task progressed (see online Supplementary data). Despite this unexpected finding, there was evidence indicating that patients diagnosed with MDD took longer to plan TOL problems and demonstrated decreased amygdala inhibition during the easy condition relative to controls due to elevated baseline anxiety. Consistent with the need to compensate for increased anxiety, persons diagnosed with MDD demonstrated intact planning accuracy, and increased RLPFC recruitment whose magnitude was accounted for by the degree of amygdala activation. Past research indicates that the RLPFC is critical for planning (Wagner *et al.* 2006) and may facilitate planning by integrating information held in working memory (Kim *et al.* 2015) and resolving uncertainty regarding the action sequence required to solve the presented problem (Desrochers *et al.* 2015). As such, these results are consistent with the hypothesis that persons diagnosed with MDD recruit additional cognitive control to maintain cognitive performance, due to interference from anxiety-related limbic activation (Harvey *et al.* 2005; Etkin & Schatzberg, 2011). Of note, task anxiety did not mediate differences in planning times or amygdala activation. This may have resulted from the fact that increased task anxiety more strongly reflected worry as opposed to baseline anxiety (see online Supplementary material). Evidence indicates that worry does not make an impact on reaction times or cognitive control, whereas trait anxiety makes an impact on both (Forster *et al.* 2015).

The pattern of results from the connectivity analyses also provided evidence that patients with MDD compensated for anxiety during the easy condition by using prefrontal control mechanisms to regulate limbic activation. Individuals with MDD who demonstrated stronger negative amygdala–DLPFC connectivity during the easy condition also demonstrated greater planning accuracy. The association between connectivity and accuracy was not observed in the HCs, but this was probably due to a restriction of range in accuracy in the HCs during the easy condition. Given that there are minimal direct connections between the amygdala and the DLPFC (Ray & Zald, 2012), abnormal amygdala–DLPFC connectivity may reflect a failure of the DLPFC to decrease amygdala activation through more proximal regulatory structures such as the ventrolateral prefrontal cortex (Dolcos *et al.* 2006; Dolcos & McCarthy, 2006; Clarke & Johnstone, 2013). Although the MDD group did demonstrate decreased

Fig. 4. Voxel-wise results. Left (L) amygdala psychophysiological interaction (PPI) analysis. Increased connectivity, ($p < 0.050$ cluster corrected) with the left amygdala seed during the easy *v.* hard trials was observed in: (*a*) the dorsolateral prefrontal cortex (DLPFC) extending into the rostrolateral prefrontal cortex and (*b*) the inferior frontal gyrus (IFG) extending into the insula. No other regions survived cluster thresholding. (*c*) β Weights for the contrast of amygdala–DLPFC connectivity *v.* baseline for each condition for each group. (*d*) β Weights for the contrast of amygdala–IFG connectivity *v.* baseline for each condition for each group. (*e*) Amygdala–DLPFC functional connectivity is more strongly associated with increased planning accuracy in the easy condition in patients with major depressive disorder (MDD) relative to healthy controls (HCs). BA, Brodmann area. Values are means, with standard deviations represented by vertical bars. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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amygdala–frontal operculum connectivity relative to HCs during the easy condition, amygdala–frontal operculum connectivity was not associated with anxiety or planning accuracy. It is likely that altered amygdala–DLPFC connectivity may stem from alterations in the LC-NE system, which modulates both amygdala and DLPFC activation (Ray & Zald, 2012). Our finding that HCs demonstrated greater DLPFC activation relative to persons with MDD during the hard condition also fits within a regulatory account; however, increased DLPFC activity and stronger negative connectivity did not result in greater planning accuracy relative to the MDD group during the hard condition. This was probably due to overloading working memory resulting in poor performance for all participants (Yun *et al.* 2010).

Limitations

Study limitations include a modest sample size and reliance on retrospective self-report measures of anxiety. In addition, the task did not induce sustained anxiety within the MDD group. As such, our results highlight the impact of baseline anxiety probably stemming from co-morbid anxiety. Given our current sample size and distribution of co-morbid anxiety diagnoses, we are not able to examine differences in cognitive control between MDD with *v.* without a formal anxiety disorder.

Conclusion

In summary, this study demonstrated that individuals diagnosed with MDD compensate for anxiety-related limbic activation during cognitive tasks by recruiting additional prefrontal cognitive control and by increased inhibitory amygdala–DLPFC connectivity when resources are available. Targeting these mechanisms in MDD may lead to improve cognitive functioning.

Supplementary material

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0033291716001185

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Declaration of Interest

None.

Notes

[1] Activation within the RLPFC was not detected in the meta-analysis of the TOL task. This was probably due to including activations resulting from planning *v.* control contrasts due to their dominance in the literature and not activations generated by parametrically varying planning load.

[2] As shown in Supplementary analyses, the left DLPFC and left RLPFC were associated with planning time and planning accuracy. In addition, left amygdala activity was associated with anxiety. These results support the proposed functions of these regions during the TOL task.

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