International Journal of Neuropsychopharmacology, Page 1 of 10. © CINP 2014
doi:10.1017/S1461145714000339

ARTICLE

The effects of catecholamine depletion on the neural response to fearful faces in remitted depression

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Abstract

Recent evidence suggests that increased psychophysiological response to negatively valenced emotional stimuli found in major depressive disorder (MDD) may be associated with reduced catecholaminergic neurotransmission. Fourteen unmedicated, remitted subjects with MDD (RMDD) and 13 healthy control subjects underwent catecholamine depletion with oral α-methyl-*para*-tyrosine (AMPT) in a randomized, placebo-controlled, double-blind crossover trial. Subjects were exposed to fearful (FF) and neutral faces (NF) during a scan with [^{15}O]H_2O positron emission tomography to assess the brain–catecholamine interaction in brain regions previously associated with emotional face processing. Treatment with AMPT resulted in significantly increased, normalized cerebral blood flow (CBF) in the left inferior temporal gyrus (ITG) and significantly decreased CBF in the right cerebellum across conditions and groups. In RMDD, flow in the left posterior cingulate cortex (PCC) increased significantly in the FF compared to the NF condition after AMPT, but remained unchanged after placebo, whereas healthy controls showed a significant increase under placebo and a significant decrease under AMPT in this brain region. In the left dorsolateral prefrontal cortex (DLPFC), flow decreased significantly in the FF compared to the NF condition under AMPT, and increased significantly under placebo in RMDD, whereas healthy controls showed no significant differences. Differences between AMPT and placebo of within-session changes in worry-symptoms were positively correlated with the corresponding changes in CBF in the right subgenual prefrontal cortex in RMDD. In conclusion, this study provided evidence for a catecholamine-related modulation of the neural responses to FF expressions in the left PCC and the left DLPFC in subjects with RMDD that might constitute a persistent, trait-like abnormality in MDD.

Received 20 November 2013; Reviewed 7 January 2014; Revised 15 January 2014; Accepted 18 February 2014

Key words: Catecholamines, depression, DLPFC, fearful faces, PCC, PET.

Introduction

Altered processing of negatively valenced emotional stimuli has been consistently found in MDD. However, the neurobiological underpinnings and the pathogenetic relevance of abnormal reactions to such stimuli in MDD have not yet been fully elucidated. One established method to examine the processing of negative stimuli involves the exposure to pictures of human faces portraying fearful expressions. Previous research found that the response to fearful faces (FFs) in MDD is associated with increased activity in brain regions associated with emotional evaluation (i.e. amygdala, orbitofrontal cortex (OFC), striatum) (Cavassila et al., 1999; Drevets, 2000; Fales et al., 2008) and decreased activity in regions associated with emotional regulation (i.e. dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC)) (Johnstone et al., 2007; Siegle et al., 2007; Fales et al., 2008). These findings appear partly compatible with a model of emotional processing that involves top-down regulation of limbic and subcortical structures associated with rapid processing of emotionally salient stimuli (Mayberg, 1997; Phillips et al., 2008) by the DLPFC and OFC (Ochsner et al., 2002; Phillips et al., 2008). Interestingly, it has been found that a single dose of reboxetine, a norepinephrine reuptake inhibitor, reversed the altered response to happy faces in depressed subjects, suggesting that catecholamines are involved in the modulation of emotional processing at a very early stage in antidepressant treatment, before symptom changes are evident (Harmer et al., 2009). These data, taken together with evidence that catecholamines affect functions of the limbic–cortical–striatal–pallidal–thalamic circuitry implicated in the pathophysiology of MDD

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(Hasler et al., 2008; Price and Drevets, 2010), suggest that examination of brain–catecholamine interactions in cerebral structures associated with the differential neural response to FF stimuli in MDD subjects *vs.* healthy controls may illuminate the neural bases of the negative emotional processing biases associated with MDD (Victor et al., 2010; Harmer et al., 2011). The current study aimed to address this goal without the potential confound of a medication bias (Stuhrmann et al., 2011) by assessing unmedicated, fully remitted patients with MDD (RMDD) during exposure to neutral and fearful facial expressions.

To study the brain–catecholamine interaction, subjects received either placebo or alpha-methyl-para-tyrosine (AMPT) (Berman et al., 1999) before the [^{15}O]H_2O-positron emission tomography (PET) scan. AMPT competitively inhibits tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, and is, therefore, able to decrease catecholaminergic neurotransmission by depleting central dopamine and norepinephrine stores (Stine et al., 1997; Verhoeff et al., 2003). Given that previous research found an increased activity in subcortical brain regions and a decreased top-down control by the DLPFC in response to FFs, we hypothesized that catecholamine depletion with AMPT but not placebo would result in an increased behavioral response to FFs in RMDD but not in healthy controls, and in an increased hemodynamic response in the limbic–striatal–pallidal–thalamic circuit along with a decreased response in the DLPFC.

Methods

The current study was part of a larger project that investigated the role of catecholamines in the pathogenesis of MDD. To this end, we assessed the behavioral, neuropsychological and neurobiological response to catecholamine depletion in subjects with low and high risk of depression. The neuropsychological assessment included the behavioral and hemodynamic responses to FFs. FFs were used to activate the stress system that is thought to be overactive in depression. Specifically, using a double-blind, placebo-controlled, crossover design, fully remitted, unmedicated participants with MDD were studied under catecholamine depletion as well as under placebo administration. During the depletion procedures, cerebral blood flow (CBF) was assessed using [^{15}O]H_2O PET to measure the neural response to emotionally valenced stimuli. Afterwards, the study participants also underwent [^{18}F]-fluordeoxyglucose PET scanning to compare metabolic correlates of catecholamine depletion between RMDD subjects and healthy controls, and neuropsychological testing, from which the results have been reported previously (Hasler et al., 2008, 2009).

Participants

The experimental group comprised individuals aged 18–56 yr who met DSM-IV criteria for MDD in full remission. The healthy controls had no personal history of any psychiatric disorder and no family history of a major psychiatric disorder in first-degree relatives. Diagnosis was established using the Structured Clinical Interview for DSM-IV (First, 2001) and confirmed by an unstructured interview with a psychiatrist. The subjects were recruited through the outpatient clinical services of the National Institute of Mental Health and by advertisements in local newspapers and posters on the National Institutes of Health (NIH) campus. Exclusion criteria included major medical illnesses, pregnancy, psychotropic drug exposure (including nicotine) within 3 months, substance abuse within 1 yr, lifetime history of substance dependence, psychiatric disorders other than MDD, and structural brain abnormalities on MRI. Inclusion criteria required that RMDD subjects had remained in remission without medications for at least 3 months and had manifested depression onset before 40 yr of age. Written informed consent was obtained as approved by the Combined Neuroscience Institutional Review Board of the NIH.

Experimental design

Using a randomized, double-blind, placebo-controlled crossover design, subjects underwent two identical sessions separated by at least 1 wk, in which they received either a body-weight adjusted AMPT dose or placebo (see (Hasler et al., 2008) for details). To reduce risk of adverse reactions, a body-weight adjusted AMPT dose of 40 mg/kg of body weight orally, to a maximum of 4 g, over 22 h was used. Each session took 3 d and was performed on an inpatient basis at the NIH Clinical Center. To reduce the risk of crystalluria during AMPT administration, subjects received sodium bicarbonate, drank at least 2 l of water daily, and underwent urinalysis twice daily. During the PET scan, blocks of FF stimuli were alternated with control scans in which the faces showed a neutral expression (NF). Behavioral ratings included a visual analog scale (VAS) of three different emotional dimensions (anxiety, sadness, worry). Ratings were conducted inside the camera by short interviews with the research subjects after a series of faces with the same emotional expression were presented, i.e. at the beginning of the interval between blood flow scans. In addition, as a pharmacodynamic assessment to verify target engagement within the central nervous system, the effect of AMPT on serum prolactin levels was measured.

Statistical analysis of behavioral data

To account for the repeated measurements in the same subjects, full factorial linear mixed models with restricted maximum likelihood estimations were used to examine the effects of the primary and secondary outcome measures. Schwarz's Bayesian criteria were used to determine

the best fitting covariance structure for each set of measures in cases where the typical compound symmetry approach used by ANOVA did not provide the optimal structure for the extant data. The effects of treatment, diagnosis, and stimulus on the VAS scores were assessed with linear mixed models with a first-order autoregressive covariance structure. The effects of treatment, diagnosis, and time on prolactin levels were assessed with linear mixed models with a first-order autoregressive covariance structure. Subject number and treatment sequence were included as random effects in all models. *Post-hoc t*-tests involved a Tukey-correction for multiple comparisons. The significance thresholds for these contrasts were set at $\alpha = 0.05$, two-tailed. SAS 9.3 (SAS Institute Inc., USA) was used for all analyses. The means of the data are reported with their associated standard deviation (S.D.).

[^{15}O]H_2O-PET imaging

The PET scanning was performed during the expected nadir of plasma catecholamine levels on the second day of AMPT administration. Subject preparation consisted of intravenous catheterization and immobilization of the head using a thermoplastic mask. PET scans were acquired using a GE Advance (35 contiguous slices with 4.25 mm plane separation; 3D resolution = 6–7 mm FWHM, 3D acquisition mode). (Townsend et al., 1998) First, a transmission scan (about 8 min) using rotating rods of 68Ge/68Ga with electronic windowing around the rods to minimize scatter was obtained for attenuation correction of the emission scans during the tracer uptake period. (Huang et al., 1979) Following transmission scan, five CBF scans were acquired at an 8 min inter-scan interval using bolus injection of 10 mCi of [^{15}O]H_2O. To provide an anatomical framework for analysis of the PET images, structural MRI scans were acquired with a 3.0-T scanner (Signa; GE Medical Systems) and a T1-weighted pulse sequence (magnetization prepared rapidly acquired gradient echo (MP-RAGE); voxel size, 0.9 × 0.9 × 1.2 mm).

Behavioral task

Gray-scale, static face picture stimuli (Ekman, 1976) modified as previously described by Morris et al. (1996) were presented during each scan. These stimuli depicted the faces of actors and actresses displaying multiple distinct emotional expressions. The photographs were digitized and modified by cropping out hair, neck, and ears, achieving isoluminance across stimuli, and centering each face's eyes so they appeared in a uniform location on the computer monitor, as described and illustrated in Morris et al. (1996, 1998). The photographed subject's eyes provide a natural fixation point for the viewers' gaze. Since we were only interested in determining the responses to a block of emotionally expressive faces that are of a single emotion, within an individual scan all face stimuli shown were of the same emotional valence, and face pictures depicting different grades of a particular emotion (e.g. moderately *vs.* markedly fearful) were displayed in random order within a particular scan. Scans containing blocks of FF stimuli were alternated with control scans containing blocks of NF stimuli. The FFs were presented in two different blood flow scans. To reduce order effects, the sequence in which either NF or FF types were presented was counterbalanced across subjects. The task instructions were the same for all scans, as subjects were asked to indicate the gender of each face by pressing the left button on a two-button fiberoptic key pad for male and the right for female (Morris et al., 1996). Stimulus presentation was initiated approximately 7 s before the start of the emission scan to minimize the amount of habituation that may occur prior to the arrival of the ^{15}O bolus to the head (the fixation point CBF scan that precedes the experimental scans permitted estimation of the lag time between [^{15}O]H_2O injection and the rise in radioactive counts from the head). The face stimuli were displayed at one Hz on a Macintosh computer monitor using Super Lab™ software. This frequency was selected because in pilot studies it permitted adequate time to assign gender, and the short stimulus duration minimized the likelihood of scanning eye movements. Eye movement was not evident during random checks during stimulus presentation, as at the 1 Hz rate of stimulus presentation, the experimental subject's eyes usually remain fixed on the pictured subject's eyes and do not scan the faces. Starting with a fixation point control (Fx Pt), the subjects were randomized to one of two possible scan sequences within each group: 1. Fx Pt–FF–NF–FF–NF, 2. Fx Pt–NF–FF–NF–FF.

Analysis of PET image data

Matlab (Matlab v.7, release 2010b; The MathWorks, Inc., USA), SPM8 (Wellcome Trust Centre for Imaging, England; www.fil.ion.ucl.ac.uk/spm8), and the toolbox aslm (Homan et al., 2012) were used for image analysis. The PET images were co-registered to each subject's structural image, spatially normalized to the Montreal Neurological Institute brain template and smoothed using a 6 mm gaussian smoothing kernel. The statistical models that were applied to compare the CBF across stimuli, treatments and diagnostic groups included the main effects of FF *vs.* NF stimuli, placebo *vs.* drug, patients *vs.* healthy controls, and subject. In a first step, the flexible factorial model in SPM8 was used to model each subject's response to FF and NF stimuli for AMPT and placebo, respectively. The contrast images then were used in a second level analysis, in which the three-way interaction (stimulus × treatment × diagnosis) was assessed in a two-sample *t*-test of both diagnostic groups (i.e. RMDD and

Table 1. Demographic and clinical characteristics of unmedicated subjects with remitted major depressive disorder (RMDD) and healthy controls

Characteristic	RMDD (n=14)	Controls (n=13)
Sex, No. females/males	13/1	12/1
Age, mean (S.D.), yr	38.1 (11)	39 (10)
Age at onset, mean (S.D.), yr	23 (8.2)	NA
Past Major Depressive Episodes, mean (S.D.), No.	2.5 (1.3)	0
Time in remission, months		
Mean (S.D.)	33 (29)	NA
Range	7–118	NA
First-degree relative(s) with a mood disorder, No.	14	0
Remote (>1 yr ago) history of alcohol abuse, No.	3	1
History of drug abuse, No.	0	0
MADRS score at study entry, mean (S.D.)	1.4 (2.2)	0.4 (0.9)

Abbreviations: f/m, female/male; MADRS, Montgomery–Åsberg Depression Rating Scale; NA, not applicable; S.D., standard deviation.

healthy controls). *A priori* hypotheses were tested by assessing changes in the predefined ROI including the amygdala, DLPFC, medial and lateral OFC, hippocampus, anteroventral striatum (AVS), thalamus, posterior cingulate cortex (PCC) and the subgenual and pregenual ACC. These ROI were selected according to the findings and model of Drevets and Price (Drevets et al., 1992; Price and Drevets, 2010) using anatomic boundaries and methods as described by Drevets et al. (2001) and Neumeister et al. (2004). To assess changes in the normalized blood flow the regional tissue radioactivity was scaled to the global activity, and then compared across conditions using the Small Volume Analysis option within SPM8 software to evaluate the significance of group differences in the contrasts obtained in the second level analysis.

In order to assess AMPT-induced CBF changes across the whole brain, an exploratory voxelwise analysis of the PET data also was performed *post-hoc*. Additional correlational analyses were performed using the behavioral VAS scores for anxiety, sadness, and worry as covariates of interest in the second level analysis. Therefore, the difference between the within-session behavioral score change between the FF and the NF condition for each subject in the AMPT session *vs.* the placebo session was calculated to reflect the magnitude of the AMPT-induced effect on symptom ratings. In ROI for which we had *a priori* hypotheses, results were small-volume corrected for familywise error, and reported at a voxel-level threshold of $p<0.05$. Additional clusters derived from the whole-brain analysis are reported with a voxel-level threshold of $p<0.05$, whole-brain corrected for familywise error. For significant regions, β estimates of regional CBF were extracted in order to calculate *post-hoc* tests and to depict the direction of the three-way-interaction.

Results

The clinical and demographic characteristics of the subject samples are detailed in Table 1.

From 28 subjects, 14 subjects with RMDD and 13 healthy controls were included in the study. One female subject with RMDD did not participate in the [^{15}O]H_2O-PET imaging. The 13 healthy volunteers (12 females; mean±[S.D.] age=39±10 yr) were not significantly different from the 14 RMDD subjects (13 females; mean±S.D. age=38.1±11 yr) with respect to gender ratio and mean age.

Behavioral results

Behavioral results of catecholamine depletion compared to placebo in RMDD subjects and healthy controls as measured with the Montgomery–Åsberg Depression Rating Scale (MADRS), Hamilton Rating Scale for Depression (HAMD), and Beck Anxiety Inventory (BAI) have been published previously (Hasler et al., 2008). Briefly, depressive symptoms were increased after the administration of AMPT to a greater extent in subjects with RMDD than in healthy controls, and anxiety ratings also increased in RMDD under catecholamine depletion, whereas the corresponding changes in healthy subjects were statistically not significant.

Figure 1 shows VAS scores of anxiety, sadness, and worry categorized by stimulus, treatment, and diagnosis. Anxiety scores as measured after each condition using the VAS were higher in the FF compared to the NF conditions in the whole group ($F_{1,203}$=6.09, p=0.01), and were significantly higher in the RMDD subjects compared to the controls ($F_{1,203}$=12.58, p=0.0005) as well as under AMPT administration compared to placebo ($F_{1,203}$=12.84, p=0.0004). The stimulus×diagnosis interaction also was significant ($F_{1,203}$=7.81, p=0.006); this interaction

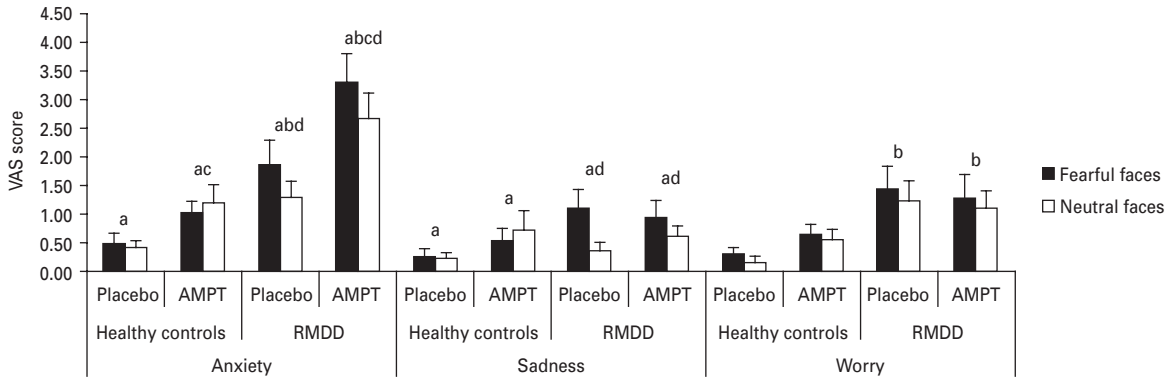

Fig. 1. Mean anxiety, sadness and worry symptoms with S.E. as measured with a visual analogue scale (VAS) after each condition (fearful faces, neutral faces) categorized by stimulus, treatment and diagnosis. a = significant stimulus-effect; b = significant diagnosis-effect; c = significant treatment-effect; d = significant stimulus × diagnosis interaction; all effects significant at $p<0.05$.

was attributable to higher anxiety scores in the FF condition in RMDD subjects compared to controls ($t_{1,203}=4.19$, $p=0.0002$) and in the FF compared to the NF conditions in RMDD ($t_{1,95}=3.76$, $p=0.001$). The ratings of sadness were higher in the FF compared to the NF conditions in the whole group ($F_{1,203}=8.6$, $p=0.004$), and there was a stimulus-by-diagnosis interaction evident ($F_{1,203}=12.98$, $p=0.0004$) that was attributable to higher sadness in the FF compared to the NF condition in RMDD ($t_{1,203}=4.67$, $p<0.0001$). The VAS scores of worry were higher in RMDD subjects compared to controls ($F_{1,203}=4.83$, $p=0.03$).

The central effect of AMPT was evidenced by an increase of serum prolactin levels following catecholamine depletion ($F_{1,25}=361.87$, $p<0.0001$), and no effect of diagnosis ($p=0.69$) or treatment × diagnosis interaction ($p=0.97$) were evident.

[^{15}O]H_2O-PET imaging results: ROI analysis

Figure 2 shows the ROI analysis of the PCC, where a significant stimulus × treatment × diagnosis interaction was evident in the PCC cortex corresponding to Brodmann area 30, situated on the posterior cingulate gyrus and the anterior-most aspect of the cuneate gyrus (peak: *x*, *y*, *z* = −8, −60, 8; cluster-size: 38 voxels; $t_{25}=5.65$, $p<0.05$, small-volume corrected for familywise error). This interaction was attributable to an increased regional CBF in the PCC as response to FFs under AMPT compared to placebo for RMDD and a respective decrease in healthy controls. The ROI analysis of the DLPFC revealed a significant stimulus × treatment × diagnosis interaction in the left dorsolateral prefrontal cortex (Brodmann area 46; locus for peak voxel *t*-value: *x*, *y*, *z* = −54, 26, 28; cluster-size: 25 voxels; $t_{25}=6.37$, $p<0.05$, small-volume corrected for familywise error; Figure 3). This interaction was attributable to a decreased regional flow with the FFs under AMPT compared to an increased regional flow with the FFs under placebo for RMDD that was not evident in healthy controls. No interaction was found in the other ROI (amygdala, medial and lateral OFC, hippocampus, AVS, thalamus, and ACC).

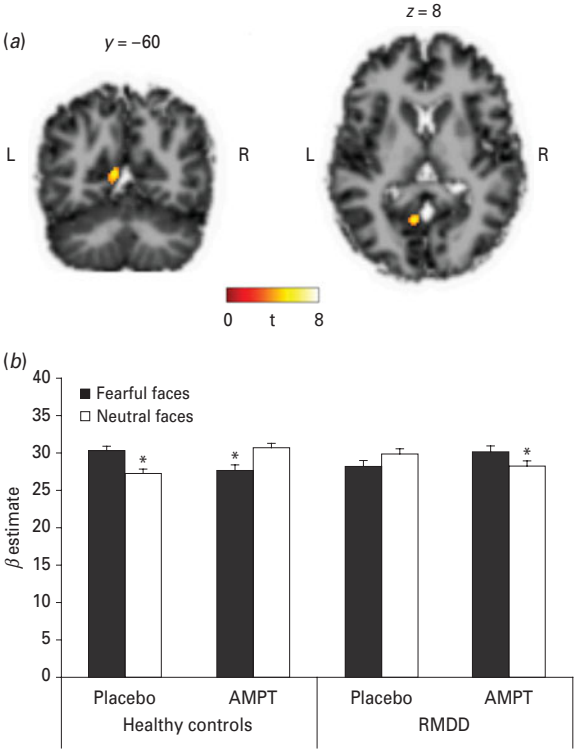

Fig. 2. (*a*) Stimulus × treatment × diagnosis interaction in the region of interest selected according to the *a prioiri* hypotheses. A cluster of 38 voxels was found in the posterior cingulate cortex (PCC), corresponding to Brodmann area 30 (peak: *x*, *y*, *z* = −8, −60, 8; $t_{25}=5.65$, $p<0.05$, small-volume corrected for familywise error). (*b*) Mean beta estimates with S.E. at the region of interest depicted in (*a*) categorized by stimulus, treatment and diagnosis. *Indicates a significant difference in the *post-hoc* tests at $p<0.05$.

[^{15}O]H_2O PET imaging results: voxelwise analysis

After AMPT administration, blood flow was higher in the left inferior temporal gyrus (ITG), Brodmann area

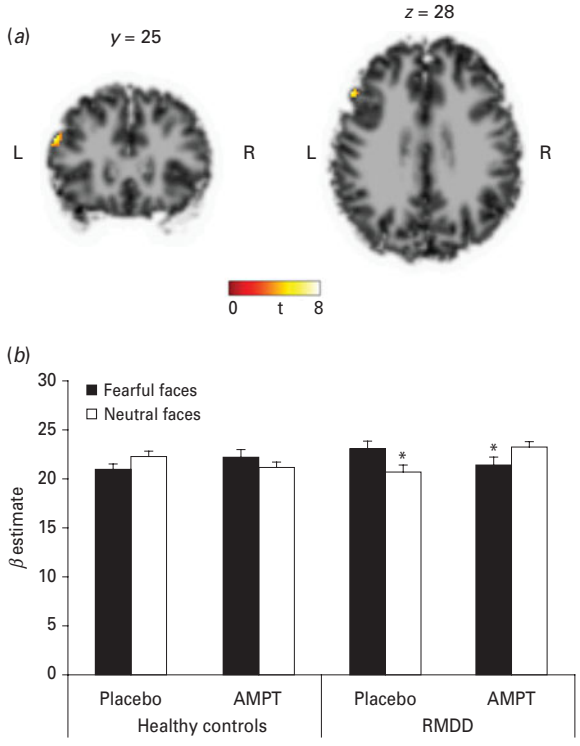

Fig. 3. (*a*) Stimulus × treatment × diagnosis Interaction in the left dorsolateral prefrontal cortex, a region of interest selected according to the *a priori* hypotheses. A cluster of 25 voxels was found in Brodmann area 46 (peak: *x, y, z*=−54, 26, 28; t_{25}=6.37, p<0.05, small-volume corrected for familywise error). (*b*) Mean beta estimates with S.E. at the region of interest depicted in (*a*) categorized by stimuli, treatment and diagnosis. *Indicates a significant difference in the *post-hoc* tests at p<0.05.

20 (peak: *x, y, z*=−54, −11, −28; t_{26}=7.47; p=0.004, corrected for familywise error) and lower in the anterior lobe of the right cerebellum (peak: *x, y, z*=28, −42, −38; t_{26}=7.77, p=0.002, corrected for familywise error) compared to placebo across conditions and groups. Testing for a stimulus-effect, a treatment-by-diagnosis interaction, a stimulus × diagnosis interaction, a stimulus × treatment interaction, and a stimulus × treatment × diagnosis interaction did not reveal any significant results.

[^{15}O]H_2O PET imaging results: correlational analysis

Additional correlational voxelwise analyses were performed including the difference (AMPT *vs.* placebo) of within-session (FF *vs.* NF) changes of anxiety, sadness and worry VAS scores as covariates. There was a positive association of the AMPT-induced, within-session change of worry VAS score and the corresponding CBF change in the right subgenual prefrontal cortex (corresponding to Brodmann area 10/32 (Ongur et al., 2003; Price and Drevets, 2010); peak voxel *t*-value at: *x, y, z*=6, 34, −14; t_{24}=6.68; p<0.05, corrected for familywise error; Fig. 4). Re-computing the correlational analysis for each group separately revealed that RMDD showed a significant positive correlation of the AMPT-induced, within-session change of worry VAS score and the corresponding CBF change (r=0.89, p<0.0001) that was only present at a trend-level in controls (r=0.48, p=0.09).

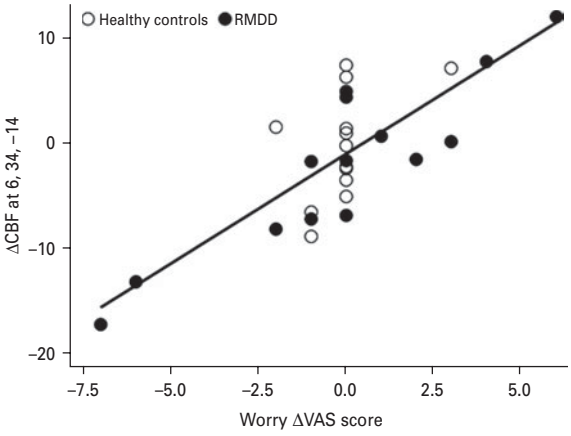

Fig. 4. Worry VAS scores as calculated by the difference (AMPT *vs.* placebo) of within-session (fearful faces *vs.* neutral faces) changes plotted against the corresponding normalized cerebral blood flow (CBF) values. The CBF values were extracted from the region where significant correlations were evident in the voxelwise correlational analysis including worry VAS score changes as covariates. This analysis revealed a positive association of the AMPT-induced within-session change of worry VAS score and the corresponding CBF changes in the right subgenual prefrontal cortex, Brodmann area 10 m/32 (peak: *x, y, z*=6, 34, −14; t_{24}=6.68; p<0.05, corrected for familywise error).

Discussion

To our knowledge, this is the first study to compare the effect of catecholamine depletion on the neural response to FFs *vs.* NFs in unmedicated RMDD and healthy controls. Catecholamine depletion was associated with behavioral and neurophysiological effects that in some cases were similar and in others were distinct in the RMDD sample relative to the control sample. The administration of AMPT increased anxiety in the entire sample, and to a greater extent in RMDD. AMPT-administration compared to placebo resulted in a higher CBF in the left inferior temporal gyrus and a lower CBF in the anterior lobe of the right cerebellum across conditions and groups. In the left PCC flow increased in the FF compared to the NF condition after AMPT but remained unchanged after placebo in RMDD, whereas healthy controls showed an increase under placebo and a decrease under AMPT. In the left DLPFC, flow decreased in the FF compared to the NF condition under AMPT and increased under placebo in RMDD, whereas healthy controls showed no such differences. Differences between AMPT and placebo of within-session changes in worry symptoms were positively correlated with the corresponding changes in CBF in the right subgenual prefrontal cortex in RMDD.

The current study applied catecholamine depletion with AMPT to assess the brain–catecholamine interaction of cortical structures responding to fearful emotional stimuli. Consistent with our *a priori* hypothesis, the behavioral responses were higher levels of anxiety in RMDD compared to controls in response to FFs, supporting previous findings that found that the risk for MDD is associated with an increased sensitivity to fearful stimuli (Bhagwagar et al., 2004; Neumeister et al., 2006; Norbury et al., 2010). However, since both RMDD subjects and controls showed more anxiety under AMPT compared to placebo, the change in anxiety under catecholamine depletion did not have a differential effect on RMDD in this study sample.

The behavioral response to FFs was associated with regional hemodymanic responses. In two regions of the limbic–cortical–striatal–pallidal–thalamic circuitry where we had *a priori* hypotheses, AMPT-induced changes in blood flow between the FF and NF conditions differed significantly between groups. With respect to the PCC, monosynaptic anatomical connections have been described previously between this region and the rostral ACC (Saleem et al., 2008; Price and Drevets, 2010), which extends into the subgenual prefrontal cortex region implicated herein, and forms part of the medial prefrontal (visceromotor) network (Price and Drevets, 2010) implicated in the pathophysiology of depression (Drevets et al., 2002; Neumeister et al., 2004; Hasler et al., 2008), as well as in the processing of self-consciousness (Kjaer et al., 2002; Lou et al., 2004) involved in the 'default mode network' that has been shown to be aberrant in depression (Sheline et al., 2009). Furthermore, studies examining neural face processing found less functional connectivity of the precuneus and the OFC in patients with MDD (Frodl et al., 2010) and aberrant neural responses in this extrastriatal area (Lawrence et al., 2004; Fu et al., 2007) that might be mediated by interconnections with the amygdala (Haxby et al., 2002; Phillips et al., 2003). Inconsistent with our *a priori* hypothesis, no difference in the amygdala response to FFs was found in the current study. Notably, the functional connectivity of the amygdala response to negatively valenced faces, which previously was reported to be abnormal in depression by Fu et al. (2007), could not be assessed using PET data due to the limited numbers of images obtained per condition. Fu et al., also found that the PCC showed decreased activation in response to happy faces in patients with MDD which improved significantly upon treatment with antidepressants. However, in contrast to our study, the authors assessed the response to happy faces, and the patients were treated with fluoxetine, a selective serotonin reuptake inhibitor, which limits the comparability to our study. The study populations in the studies by Lawrence et al., and Frodl et al., comprised subjects with MDD who were receiving medications at the time of scanning (Lawrence et al., 2004; Frodl et al., 2010), which also limits the comparability to our study. Due to our different study design involving subjects with RMDD, our study adds to the aforementioned findings by showing that the distinct CBF-response compared to healthy controls may reflect a risk factor for MDD that is associated with catecholaminergic neurotransmission.

The reduction in CBF in the left DLPFC in the FF compared to the NF condition under AMPT in RMDD implicates a region within the prefrontal cortex that has been associated with voluntary and automatic emotional processing (reviewed in Price and Drevets (2010)). Previous PET studies found decreased resting-activity in the left DLPFC in individuals with MDD (Baxter et al., 1989; Bench et al., 1993), and more recent functional magnetic resonance imaging (fMRI) studies of emotional face processing in MDD have shown reduced DLPFC activity in response to negative facial expressions (Fu et al., 2004; Surguladze et al., 2005; Siegle et al., 2007). Notably, most of these findings have been localized to the left hemisphere, in line with a proposed hemispheric localization of emotion (Davidson, 1992) and lesion-based studies in MDD (Shimoda and Robinson, 1999). In addition, previous neuroimaging studies in RMDD have confirmed abnormalities in the left DLPFC and, therefore, suggest that alterations in this region might be a trait marker in MDD (Liotti et al., 2002; Neumeister et al., 2006; Norbury et al., 2010; Victor et al., 2010; Kerestes et al., 2012), although those studies included medicated subjects (Liotti et al., 2002) or subjects with co-morbid illnesses (Neumeister et al., 2006; Norbury et al., 2010), which limits its conclusions that can be drawn about trait markers of MDD. A recent study in unmedicated MDD subjects reported reduced hemodynamic activity in response to FFs in the left DLPFC (Kerestes et al., 2012), which is supported by our finding and extended by the observation that the increase in regional CBF in the left DLPFC in response to FFs under placebo that was inverted by AMPT might reflect a top-down control of anxiogenic stimuli depending on catecholaminergic neurotransmission.

The fact that healthy controls showed a decreased flow under catecholamine depletion when presented FFs compared to NFs, whereas subjects with RMDD showed an increased flow when presented FFs compared to NFs, might indicate that brain activity in the PCC can be interpreted as compensatory brain activity that would usually maintain remission in RMDD but is vulnerable to a depletion of catecholamines. Compensatory brain activity has been suggested as a counter-measure used by the brain to cope with depression (Lewis, 2000; Norbury et al., 2010; Kessler et al., 2011), i.e. 'an augmentation of existing circuits to (unsuccessfully) cope with the disease' (Kessler et al., 2011). The fact that AMPT led to a 'normalization' of flow in the DLPFC suggests that this brain region shows catecholamine-dependent activity associated with remission in RMDD.

Finally, there was an association of the behavioral and the neural response to FFs that was evidenced by the

positive correlation of within-session changes in worry-symptoms and the corresponding CBF changes in a small locus within the right medial frontal gyrus (MFG). The greater the difference in CBF in the right MFG between the FF and the NF tasks and between the AMPT and placebo conditions, the more worry-symptoms were expressed by the RMDD subjects. The right MFG may be viewed as an interface mediating prefrontal and limbic neurotransmission within the limbic–cortical–striatal–pallidal–thalamic circuit implicated in the pathophysiology of MDD (Hasler et al., 2008; Price and Drevets, 2010). Specifically, our finding suggests that the risk for MDD reflected by an increase in worry-symptoms is associated with an interaction of limbic and cortical regions mediated by increased activity in the right MFG.

The current study had several limitations. The study sample was relatively small and predominantly consisted of female subjects. Since we did not include an additional condition with emotional stimuli except FFs, we cannot rule out that the observed emotion effects represent general effects for any emotional stimuli. Next, our method of PET imaging did not allow us to assess central catecholamine concentrations, and we did not measure plasma or urinary catecholamine levels to monitor the magnitude of catecholamine depletion achieved. Since there is no consistent association of central and peripheral catecholamine levels, we assessed the central AMPT-effect indirectly by measuring serum prolactin levels, which is the standard method to assess the effect of central catecholamine depletion (Hasler et al., 2008, 2009). An additional limitation is the relatively large range of remission-time in the RMDD sample, a possible confounder that may have had an impact on catecholaminergic neurotransmission in the brain regions found in the current study, for instance for reasons of age-related declines in CBF. However, we tried to take this possible confounder into account by repeating the SPM analysis including remission-time in a correlational analysis in RMDD only without any significant results, using a significance threshold of $p<0.05$ and a familywise error correction for multiple comparisons. We also repeated the SMP analysis with age as a covariate of no interest in the whole sample, which did not alter any of the results. In addition, no correlations were found between age and CBF in a voxelwise analysis using a significance threshold of $p<0.05$ and a familywise error correction for multiple comparisons. To address the question of how the AMPT-induced depressive symptoms might have influenced the hemodynamic response to the face-paradigm, we calculated additional voxelwise correlational analyses in SPM using the MADRS, HAMD, and BAI scores as covariates, and did not find any significant results at a significance threshold of $p<0.05$ and a familywise error correction for multiple comparisons. This suggests that the hemodynamic response to the face-paradigm was not associated with the AMPT-induced depressive symptoms. Finally, the AMPT-effect on the regional CBF response to emotional stimulus processing was relatively subtle, and no association with mood symptoms was evident in the corresponding brain regions. It is noteworthy, however, that the reversal of altered neural responses to affective face expression in unmedicated individuals with MDD occurs early in the course of antidepressant treatment, before symptom changes are evident (Harmer et al., 2009), suggesting that clinically relevant neural responses precede overt changes in symptoms.

The current study had several strengths that merit comment. First, the [^{15}O]H_2O PET method enabled us to study the AMPT-effect on normalized (i.e. regional/global) CBF, which is coupled to normalized glucose metabolism. We showed that the administration of AMPT resulted in a CBF increase in the left ITG and a decrease in the anterior lobe of the right cerebellum. Second, the use of NF as the control-condition allowed us to specifically assess the processing of facially expressed fear by controlling for nonspecific aspects of processing face stimuli. Third, the fact that we induced a transient depressive relapse with AMPT in RMDD allowed us to interpret our findings as risk-factors for a depressive relapse.

Finally the participants were selected in the unmedicated, remitted phase of MDD, so that nonspecific effects of antidepressant drugs or of depressive episodes (e.g. related to chronic stress or sleep deprivation) were excluded.

In conclusion, the current study provided evidence for a catecholamine-related modulation of the neural responses in the left PCC and the left DLPFC to FF expressions in subjects with RMDD that might constitute a persistent, trait-like abnormality in MDD.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institutes of Mental Health and the University Hospital and Hermann Klaus Foundation, Zurich. We are grateful to Y. Morishima for advice on the PET imaging analysis.

Statement of Interest

None. WCD is currently an employee of Janssen Pharmaceuticals of Johnson & Johnson, Inc.

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