

Neural response to catecholamine depletion in remitted bulimia nervosa: Relation to depression and relapse



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Abstract

Bulimia nervosa has been associated with a dysregulated catecholamine system. Nevertheless, the influence of this dysregulation on bulimic symptoms, on neural activity, and on the course of the illness is not clear yet. An instructive paradigm for directly investigating the relationship between catecholaminergic functioning and bulimia nervosa has involved the behavioral and neural responses to experimental catecholamine depletion. The purpose of this study was to examine the neural substrate of catecholaminergic dysfunction in bulimia nervosa and its relationship to relapse. In a randomized, double-blind and crossover study design, catecholamine depletion was achieved by using the oral administration of alpha-methyl-paratyrosine (AMPT) over 24 h in 18 remitted bulimic (rBN) and 22 healthy (HC) female participants. Cerebral blood flow (CBF) was measured using a pseudo continuous arterial spin labeling (pCASL) sequence. In a follow-up telephone interview, bulimic relapse was assessed. Following AMPT, rBN participants revealed an increased vigor reduction and CBF decreases in the pallidum and posterior midcingulate cortex (pmCC) relative to HC participants showing no CBF changes in these regions. These results indicated that the pallidum and the pmCC are the functional neural correlates of the dysregulated catecholamine system in bulimia nervosa. Bulimic relapse was associated with increased depressive symptoms and CBF reduction in the hippocampus/parahippocampal gyrus following catecholamine depletion. AMPT-induced increased CBF in

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this region predicted staying in remission. These findings demonstrated the importance of depressive symptoms and the stress system in the course of bulimia nervosa.

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1. Introduction

Bulimia nervosa (BN) is a severe psychiatric disorder defined by recurrent binge eating episodes accompanied by inappropriate compensatory behavior like purging or excessive exercise. Understanding the pathophysiology of BN could guide the development of new and improved treatments for this disorder. Positron emission tomography (PET) and pharmacological challenge studies have implicated aberrant serotonin signaling in BN (Bailer and Kaye, 2011; Kaye, 2008). PET imaging revealed increased binding of the 5-HT_{1A} receptor tracer WAY100635 in ill and recovered BN (Kaye, 2008; Tiihonen et al., 2004), whereas the binding of the 5-HTT tracer 11C-McN5652 did not differ between recovered persons with BN and control participants (Bailer et al., 2007).

Acute tryptophan depletion was followed by increased sadness, body shape concerns, and subjective loss of control of eating in remitted BN (Smith et al., 1999). Monoamine systems interact in a reciprocal manner, such that aberrant serotonin functioning suggests alterations in catecholamine functioning in BN (Tremblay and Blier, 2006). Importantly, abnormal serotonin and dopamine functioning might contribute to different symptoms in BN, as demonstrated in major depression (MDD) (Homan et al., 2015). Whereas tryptophan depletion induced significantly more sadness, hopelessness, and depressed mood, catecholamine depletion induced lassitude, concentration difficulties, inactivity, and somatic anxiety in subjects with remitted MDD (Homan et al., 2015). Indeed, a central role has been proposed for the dopamine system in eating disorders (Frank, 2016): BN is related to a desensitized, and anorexia nervosa (AN) to a sensitized dopaminergic system (Frank, 2013). This thesis is supported by the finding that individuals with BN displayed a reduced activation of the ventral striatum and insula after unexpected delivery of a sucrose solution while participants with AN revealed increased activation in these regions (Frank et al., 2012, 2011). Further evidence for the implication of dopamine in the psychopathology of BN stems from the finding that higher frequency of binge eating is related to lower concentrations of the dopamine metabolite homovanillic acid in the cerebral spinal fluid (Jimerson et al., 1992). An experimental pharmacological challenge study with methylphenidate measuring the binding potential of the dopamine type 2 (D₂) receptor with PET revealed reduced dopamine reactivity in the striatum in individuals with BN (Broft et al., 2012), indicating a deficient dopamine activity, as suggested by Frank (2016). Importantly, experimental catecholamine depletion induced mild eating disorder symptoms, mild depressive symptoms and reward learning deficits in fully remitted bulimia nervosa (rBN) (Grob et al., 2012, 2015). These findings provide causative evidence for the exacerbating action of reduced dopamine activity on psychiatric symptoms linked to BN. Nevertheless,

studies relating the behavioral effects of catecholamine depletion to measures of brain functioning are still missing. Therefore, in the present study, we focused on the functional neuroanatomical role of the dysfunctional dopamine system in BN and on its impact on relapse.

Based on our previous findings (Grob et al., 2012, 2015; Homan et al., 2015), we hypothesized that catecholamine depletion will induce lassitude, inactivity, mood and eating disorder symptoms in rBN participants and that this induction will be associated with reduced CBF in basal ganglia and insula in rBN relative to healthy control (HC) participants. In addition, we assumed that the dopamine-related dysfunction revealed by catecholamine depletion will be associated with later relapses in rBN participants.

By using a pseudo-continuous arterial spin labeled (pCASL) perfusion functional magnetic resonance imaging (fMRI) we aimed to examine the influence of catecholamine depletion on resting brain cerebral blood flow (CBF) in rBN and HC participants. This method provides a direct and absolute quantification of CBF, representing neural activity indirectly through the binding between blood flow and neural activity (Detre et al., 2012; Wang et al., 2011). Arterial spin labeling (ASL) fMRI methods are sensitive to assess different conditions of psychological stress (Wang et al., 2005). Moreover, pharmacological manipulation of the central dopamine system was found to influence CBF in dopamine-rich brain regions: A single dose of haloperidol was reported to increase CBF in the striatum, midcingulate cortex, and motor cortex, and decrease CBF in the inferior temporal gyrus in healthy individuals (Handley et al., 2013). In addition, metoclopramide, a dopamine D₂ receptor antagonist, increased CBF in the pallidum, putamen, and thalamus and decreased CBF in the insula and anterior temporal lobes (Fernández-Seara et al., 2011). For investigating our hypotheses, we analyzed the perfusion imaging data using a region of interest (ROI) approach to assess specifically the effect of catecholamine depletion in the basal ganglia and insula. We furthermore conducted a voxel-wise analysis, as we may assume that catecholamine depletion has a high likelihood to induce CBF alterations in brain regions beyond these ROIs.

2. Experimental procedures

2.1. Participants

Eighteen female participants in remission from BN (rBN), and 22 female healthy volunteers (HC) with no history of any psychiatric disorder and no major psychiatric condition in first-degree relatives participated in this study. We included only females in the study because previous studies had reported a higher prevalence of BN in women and had described gender differences in the pathogenesis of BN (Hoek and Hoeken, 2003; Hudson et al., 2007; Nagl et al., 2016; Weltzin et al., 2005). All rBN participants had previously met the DSM-IV criteria for BN, and had been in remission without any binge eating

Table 1 Characteristics and clinical ratings at the screening.

Characteristics/clinical ratings	HC participants	rBN participants	T-statistic	p-value
Age, mean \pm SD, years <i>range, years</i>	27.1 \pm 9.2 20-53	28.1 \pm 8.1 20-49	$T_{37.8} = -0.36$	$p = 0.73$
Years of education, mean \pm SD, years	15.1 \pm 2.3	15.3 \pm 2.7	$T_{33.4} = -0.23$	$p = 0.83$
Body mass index (BMI), mean \pm SD, kg/m ² , <i>range, kg/m²</i>	24.2 \pm 3.2 19.3-32.2	21.6 \pm 2.2 18.4-27.6	$T_{36.9} = 2.91$	$p < 0.01$
Age at onset of BN, mean \pm SD, years, <i>range, years</i>	NA	17.9 \pm 3.5 12-29		
Time in remission from BN, mean \pm SD, months, <i>range, months</i>	NA	44.2 \pm 46.1 4-146		
Major depression during or after BN, <i>n</i> , Time in remission, mean \pm SD, months, <i>range, months</i>	NA	11 53.2 \pm 65.7 6-228		
Mild to moderate anorectic symptoms preceding BN, <i>n</i> , Time in remission, mean \pm SD, months, <i>range, months</i>	NA	7 87.4 \pm 68.1 48-240		
Previous psychoactive medication (SSRI, SNRI, TCA), <i>n</i> Time medication free, mean \pm SD, months, <i>range, months</i>	NA	7 51.0 \pm 41.2 10-112		
EDE-Q global score - past (4 weeks during acute phase with most severe bulimic symptoms), mean \pm SD, scores	NA	4.20 \pm 1.00		
EDE-Q global score - screening (4 weeks before screen- ing), mean \pm SD, scores	0.57 \pm 0.49	1.11 \pm 0.82	$T_{26.4} = -2.46$	$p < 0.05$
MADRS, mean \pm SD, scores	1.41 \pm 2.20	3.00 \pm 3.77	$T_{26.1} = -1.58$	$p = 0.13$

Clinical ratings and characteristics at the screening visit. Differences between the remitted bulimic (rBN) and healthy control (HC) participants were calculated using two-tailed *t*-tests.

Abbreviations: BMI, body mass index; BN, bulimia nervosa; EDE-Q, Eating Disorder Examination-Questionnaire; HC, healthy control participants; MADRS, Montgomery-Åsberg Depression Rating Scale; *n*, number; NA, not applicable; rBN, remitted bulimic participants; SD, standard deviation; SNRI, serotonin and norepinephrine reuptake inhibitors; SSRI, selective serotonin reuptake inhibitors; TCA, tricyclic antidepressants.

and purging episode. Seven rBN participants reported mild to moderate anorectic symptoms before the onset of the bulimic symptoms. Major depressive episodes during or after their acute BN phases were described by 11 rBN participants (10 and 3 rBN participants, respectively). None of the participants fulfilled the diagnostic criteria for an anxiety disorder in their past or of any psychiatric disorder during study participation. Detailed information on the characteristics of both diagnostic groups are presented in Table 1.

All participants were recruited by advertisement in local newspapers, and by announcements and e-mail at the University of Bern. Before the participants provided written informed consent the study had been fully explained to them. The protocol and the written informed consent were approved by the local ethics committee of

Canton Bern, Switzerland, and were performed in accordance with the principles of the Declaration of Helsinki. During the screening visit, all participants underwent the Structural Clinical Interview for DSM-IV (First et al., 2002), a physical examination, a diagnostic interview with a psychiatrist, and filled out clinical questionnaires. These clinical scales included the Eating Disorder Examination-Questionnaire (EDE-Q) (Hilbert and Tuschen-Caffier, 2006), a self-report questionnaire measuring cognitive and behavioral features of eating disorders, and the Montgomery-Åsberg Depression Rating Scale (MADRS) (Schmidtke et al., 1988), assessing depressive symptoms. Exclusion criteria for both groups included current Axis I psychiatric disorder, a lifetime diagnosis of psychosis, major medical or neurological illness, psychoactive medication exposure in the past 6 months, lifetime history of substance

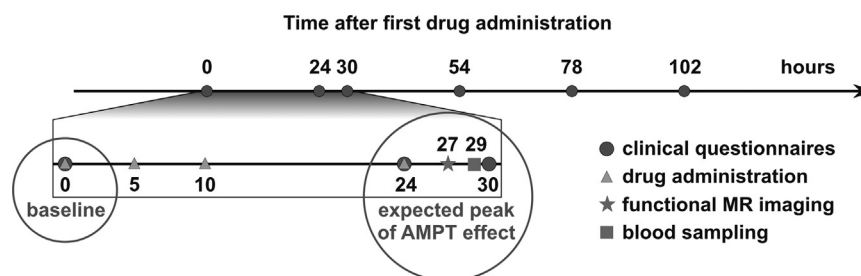


Figure 1 Time schedule of the experiment procedure. Drug administration at 4 time points in 24 h (0, 5, 10 and 24 h). Alpha-methyl-paratyrosine (AMPT) was given in a body-weight adjusted dose, administered at the 4 time points. During sham depletion, the participants received 25 mg diphenhydramine at the first time point (0 h) and placebo at 5, 10 and 24 h after the first drug administration. The functional and structural magnetic resonance (MR) imaging started 27 h after the first drug administration. Blood samples were collected after MR imaging (29 h after first drug administration). The participants filled out clinical questionnaires at 0, 24, 30, 54, 78 and 102 h after the first drug administration. Each session took 5 days.

dependency, pregnancy, suicidal ideations within the last 4 weeks before and during study participation, and a history of suicide attempts.

In a follow-up telephone interview, we assessed bulimic relapse defined as at least 1 binge eating or purging episode in rBN participants. The interview took place with a latency varying between 18 and 42 months after study participation.

2.2. Procedure

The whole procedure of the study included a screening visit, which took place at the University Hospital of Psychiatry in Bern, and 2 identical experimental sessions, performed at the Inselspital, University Hospital of Bern. The experimental sessions comprised an MR imaging, blood sampling, and clinical ratings. During these 2 sessions, the participants received once catecholamine depletion induced by alpha-methyl-paratyrosine (AMPT) and once sham depletion, in a randomized order, using a double-blind, crossover study design. We used a body-weight adjusted dose (40 mg/kg body weight, to a maximum of 4 g) of AMPT, which was administered at 4 time points over 24 h (time schedule in Figure 1) to avoid any adverse reactions. This weight-adjusted dose of AMPT was already used in previous studies (Grob et al., 2015; Hasler et al., 2008). These studies revealed that this dose of AMPT was sufficient to induce eating disorder and depressive symptoms in rBN and depressive participants, respectively, without causing severe aversive reactions. During sham depletion, the participants received 25 mg diphenhydramine at the first and placebo at the remaining time points. Diphenhydramine was chosen for sham depletion because it induces similar sedation, but no symptoms compared to AMPT (Bremner et al., 2003; Lam et al., 2001; Neumeister et al., 1998). To avoid any crossover effects, we separated the 2 experimental sessions by at least 7 days. On average, the second session took place 27.4 days after the first session (SD = 27.5, range = 7–112 days). Possible adverse reactions were assessed regularly at 6 time points (Figure 1). Additionally in each session, the induced eating disorder and depressive symptoms were examined using the MADRS and an adapted version of the EDE-Q. To measure the response to AMPT the time frame of the EDE-Q was set to past 12 h and a visual analog scale with a length of 60 mm was used for answering each question instead of a seven-point rating scale. Further, the subscales vigor and fatigue of the Profile of Mood States (POMS) (McNair et al., 1981) were used to assess the reduction of activity and increase of lassitude following AMPT as reported in a previous study (Homan et al., 2015). Blood samples were taken in each session to measure serum prolactin levels as a proxy of the depth of catecholamine depletion. A comparison study on the effect of AMPT administered over 2 different intake durations revealed that only after the longer challenge session over 24 h the striatal dopamine D₂ receptor binding potential increased (Boot et al., 2008).

2.3. MR imaging

In each session, functional and anatomical MR images were acquired on a 3 T Siemens Magnetom Trio Scanner (Erlangen, Germany) with a 12-channel regular head coil. For the measurement of the cerebral blood flow (CBF), a pseudo continuous arterial spin labeling (pCASL) sequence (Dai et al., 2008; Wu et al., 2007) with the following parameter was used: repetition time (TR) = 4000 ms, echo time (TE) = 18 ms, field of view (FoV) = 230 mm², voxel size = 3.6 × 1.8 × 6.0 mm³, balanced labeling with mean Gz of 0.6 mT/m and 60 Hanning window-shaped RF pulses (RF duration 600 μs with 900 μs gap, flip angle (FA) = 25°, bandwidth = 752 Hz/pixel). After the labeling (duration = 1720 ms), a delay of 1500 ms was applied and the isocenter of the readout slice was set 90 mm above the labeling plane. Sixteen ascending slices were acquired during each of the 100 images (50 pairs of interleaved measured labeled and control images) and were oriented along the anterior-posterior commissure (AC-PC) line. The total acquisition time was 7 min and 4 s.

For anatomical reference, high-resolution T1-weighted anatomical images were acquired using a magnetization prepared rapid gradient-echo (MP-RAGE) sequence (TR = 1480 ms, TE = 2.2 ms, inversion time (TI) = 900 ms, FA = 9°, 256 × 256 matrix size, FoV = 256 mm², 176 slices, and voxel size = 1 × 1 × 1 mm³).

2.4. Analysis of the behavioral data and serum prolactin level

The clinical ratings were analyzed with linear mixed effect models using the “lmer” method of the “lme4” package (Bates et al., 2015), and the “lmerTest” package (Kuznetsova et al., 2016), providing p-values, in R (version 3.3.2) (R Core Team, 2016). Group (rBN versus HC participants), drug conditions (AMPT versus sham depletion), and time (6 time points) were included in the model as fixed effects, and the random effect term modelled a random intercept and slope for the drug conditions for each participant. The statistical significance level was set at $\alpha = 0.05$. The serum prolactin levels were analyzed accordingly. This analysis included group and drug as fixed effects and a random effect term modelling a random intercept for each participant.

2.5. CBF quantification

The pCASL time series and the structural images were analyzed using Statistical Parametric Mapping (SPM8, Wellcome Trust Center for Neuroimaging, University College London, <http://www.fil.ion.ucl.ac.uk/spm/>). The pCASL time series first were spatially realigned to correct for movement artifacts. The structural images were coregistered to the mean image of the realigned pCASL times

series and then segmented into gray matter, white matter and cerebrospinal fluid using SPM routines. To obtain mean CBF maps in absolute units of ml/100g/min the realigned ASL images were quantified by using the following equation implemented in an in-house MATLAB (MATLAB and Statistics Toolbox Release 2012a, The MathWorks, Inc., Natick, Massachusetts, United States) script (Federspiel et al., 2006):

$$CBF = \left(\frac{\lambda \cdot \Delta M}{2 \cdot \alpha \cdot M_0 \cdot T_{1b}} \right) \cdot \left(\frac{1}{e^{-\omega/T_{1b}} - e^{-(\tau+\omega)/T_{1b}}} \right)$$

For the CBF quantification the difference between the control images and labeled images (ΔM) is multiplied by the blood tissue partition coefficient ($\lambda = 0.9$ g/ml) and divided by inversion efficacy ($\alpha = 85\%$), the equilibrium magnetization images (M_0) and the double decay time for labeled blood in a 3.0 T MR scanner ($T_{1b} = 1.65$ s). This division is multiplied with inverse exponential functions including the post-labeling delay time ($\omega = 1.5$ s), the decay time for labeled blood (T_{1b}) and the labeling duration ($m = 1.72$ s).

After the CBF quantification, the mean CBF maps were spatially normalized to Montreal Neurological Institute space and smoothed using an 8 mm full width at half maximum Gaussian kernel using SPM routines.

2.6. fMRI analysis

For region of interest (ROI) analyses, we extracted the mean CBF of 4 regions, the anteroventral striatum, putamen, pallidum, and insula, separately for each participant and in each drug condition. The regions were defined by the Wake Forest University (WFU) Pick Atlas Tool (version 3.0.5) (Maldjian et al., 2003; Tzourio-Mazoyer et al., 2002). The definition of the anteroventral striatum is based on the description of this region of Drevets et al. (2001). The mask for the anteroventral striatum includes the nucleus accumbens, and the anteroventral caudate and putamen. The posterior border of the mask was defined by the anterior commissure and the ventral tip of the frontal horn of the lateral ventricle described the dorsal boundary. These mean CBF values were analyzed with mixed effect models as described above. The models included group, drug and laterality (right versus left hemisphere) as fixed effects, and a random effect term modelling intercept and slope for the drug conditions for each participant.

In addition to the ROI analyses, we conducted whole brain voxel-wise analyses. For analyzing group differences under sham depletion, we included the smoothed CBF maps assessed during sham depletion in a second level two-sample *t*-test. To correct for systematic effects of AMPT, the mean global CBF values following sham depletion were entered as a covariate into the analysis. The mean global CBF values were obtained by averaging the CBF in the gray matter separately for each condition and participant. A voxel-level threshold of $p < 0.001$ (uncorrected) and a minimum cluster size of 17 voxels were determined for this analysis. The cluster size criterion was based on the “expected voxels per cluster” threshold on the SPM output file. To analyze group-by-drug interactions, the smoothed CBF maps were entered into a further second level analysis using a flexible factorial design with group as a between-subject, drug as a within-subject factor, and a random factor for each subject. The mean CBF values were included as a covariate in the analysis. A voxel-level threshold of $p < 0.001$ (uncorrected) and a minimum cluster size of 15 voxels according to the “expected voxels per cluster” threshold on the SPM output file were determined for this analysis.

Additionally, we tested for associations between AMPT-induced symptoms and neural activity. Hence, we included the CBF changes in the ROIs and the clinical rating changes of all participants in a Spearman's rho rank correlation analyses by using the “rcorr” method of the “Hmisc” package (Harrell, 2016) of R (R Core Team, 2016). The within-session clinical rating changes were calculated by subtracting the maximum deviation score at the time point when the peak of

catecholamine depletion is expected (24 or 30 h after first drug administration) from the baseline in each session, as described in our previous study (Grob et al., 2015). Then these clinical rating changes following sham depletion were deducted from the rating changes following AMPT administration. The CBF changes were obtained by the subtraction of the mean CBF following sham depletion from the AMPT-induced mean CBF in each ROI. We additionally calculated a voxel-wise multiple regression analyses involving the CBF changes in all voxels and the induced within-session clinical rating changes. The CBF changes in all voxels were calculated by subtracting the smoothed CBF maps following sham depletion from the maps following AMPT administration. For this voxel-wise analysis, we used a voxel-level threshold of $p < 0.001$ (uncorrected) and a minimum cluster size of 18 voxels according to the “expected voxels per cluster” threshold on the SPM output file.

As reported in the literature (Komatsu et al., 2010; Willeumier et al., 2011), the body mass index (BMI), which is expected to differ between BN and healthy individuals, may be related to CBF. To check for a possible confounding between BMI and CBF in our study we carried out several analyses. First, we tested whether including BMI as an additional factor would explain more CBF variance in the sham condition. For this purpose, the goodness of fit of our original model was compared to the goodness of fit of an extended model containing BMI as an additional factor in a voxel-wise manner in the brain regions, where both groups differed in CBF under sham treatment. The significance of the improvement of goodness of fit was averaged for all voxels belonging to each cluster of significant between-group differences. Second, in a similar manner, we checked whether including BMI as an additional predictor would better explain the variance in the effects of AMPT. Finally, we calculated direct correlations of BMI with CBF in the sham condition and with AMPT-induced CBF changes in each predefined ROIs separately, and in a whole-brain voxel-wise manner. For the latter, a voxel-level threshold of $p < 0.001$ (uncorrected) and a minimum cluster size of 25 and 18 voxels was determined for the sham condition and the AMPT-induced CBF changes, respectively, based on the “expected voxels per cluster” threshold on the SPM output files. The analyses were carried out with the “lm” and “anova” methods of the “stats” package, the “lmer” method of the “lme4” package (Bates et al., 2015), and with the “rcorr” method of the “Hmisc” package (Harrell, 2016) in R (R Core Team, 2016), and by using SPM8 for the voxel-wise analyses.

To identify prognostic biomarkers for the course of BN, the rBN participants were separated into 2 groups according to the results of the follow-up telephone interview. A voxel-wise two-sample *t*-test including the CBF changes in all voxels was calculated (voxel-level threshold of $p < 0.001$ (uncorrected), minimum cluster size of 14 voxels as determined by the SPM output file).

3. Results

3.1. Behavioral ratings and serum prolactin level

3.1.1. Screening

In the screening visit, the participants filled out the EDE-Q and were interviewed using the MADRS to examine residual eating disorder and depressive symptoms (Table 1). RBN participants scored higher on the EDE-Q global score mainly due to residual exaggerated eating and body shape concerns. The groups revealed no significant difference regarding depressive symptoms.

3.1.2. Behavioral response to catecholamine depletion

In both sessions over all 6 time points, rBN participants showed higher EDE-Q global scores than HC participants

Table 2 Behavioral response to catecholamine depletion.

Questionnaire		HC participants		rBN participants		interaction group-by-drug	
		sham depletion	AMPT	sham depletion	AMPT	$F_{1,38}$	p -value
EDE-Q	baseline (0 h)	3.33 \pm 5.1	3.69 \pm 4.4	9.87 \pm 8.0	8.51 \pm 6.3	0.24	0.63
	+ 24 h	-0.51 \pm 1.6	-0.46 \pm 0.8	+0.24 \pm 5.1	-0.59 \pm 3.2		
	+ 30 h	-0.29 \pm 2.1	-0.27 \pm 1.25	-1.95 \pm 4.7	+0.24 \pm 3.4		
MADRS	baseline (0 h)	0.27 \pm 0.9	0.32 \pm 0.8	0.83 \pm 1.7	0.50 \pm 0.9	0.60	0.44
	+ 24 h	+0.14 \pm 1.3	+0.55 \pm 1.1	+0.50 \pm 2.0	+1.50 \pm 1.7		
	+ 30 h	+0.27 \pm 1.4	+1.55 \pm 1.7	+0.78 \pm 1.9	+2.39 \pm 2.2		
POMS - vigor	baseline (0 h)	22.71 \pm 5.9	20.86 \pm 7.0	22.00 \pm 7.6	22.97 \pm 8.0	4.71	< 0.05
	+ 24 h	-1.12 \pm 4.7	-2.73 \pm 4.9	-1.22 \pm 6.9	-9.53 \pm 7.7		
	+ 30 h	-1.26 \pm 6.9	-5.95 \pm 5.6	-2.39 \pm 6.7	-10.97 \pm 6.9		
POMS - fatigue	baseline (0 h)	4.64 \pm 4.9	7.27 \pm 7.1	6.22 \pm 5.5	5.28 \pm 4.2	5.40	< 0.05
	+ 24 h	+0.95 \pm 6.7	+3.91 \pm 9.4	-1.11 \pm 5.6	+10.22 \pm 11.1		
	+ 30 h	+2.77 \pm 5.0	+7.36 \pm 7.1	+1.78 \pm 5.7	+11.79 \pm 10.3		

Behavioral response to catecholamine depletion and sham depletion. Mean, standard deviation and the results of the group-by-drug interaction are presented. Decreased scores from baseline at 24 or 30 h after the first medication intake are marked by '-', and increases from baseline were indicated by '+'. Abbreviations: AMPT, alpha-methyl-paratyrosine; EDE-Q, Eating Disorder Examination-Questionnaire; HC, healthy control participants; MADRS, Montgomery-Åsberg Depression Rating Scale; POMS, Profile of Mood States; rBN, remitted bulimic participants

($F_{1,38} = 11.77$, $p < 0.01$), but no significant effect of drug condition and no interaction was found. RBN participants reported more depressive symptoms measured using the MADRS than HC participants in both sessions ($F_{1,38} = 13.58$, $p < 0.001$). In POMS, AMPT induced fatigue ($F_{1,38} = 22.48$, $p < 0.001$), and reduced vigor ($F_{1,38} = 5.30$, $p < 0.05$) in both groups. Detailed behavioral responses to catecholamine depletion are presented in Table 2.

We investigated the induced changes in POMS rating scales 24 and 30 h after the first AMPT and sham drug administration in relation to its baseline in separate mixed model analyses, as we have done in our previous study (Grob et al., 2015). These analyses revealed that AMPT induced fatigue and reduced vigor significantly more in rBN than in HC participants (group-by-drug interaction: POMS vigor: $F_{1,38} = 4.71$, $p < 0.05$; POMS fatigue: $F_{1,38} = 5.40$, $p < 0.05$).

The serum prolactin level was not available for both condition in 1 rBN participant. A mixed effects model analysis on the serum prolactin level revealed a significant main effect for drug (sham depletion: mean = 9.07 ± 3.23 ; AMPT: mean = 49.2 ± 12.03 ; $F_{1,37} = 436.02$, $p < 0.001$), but no significant main effect for group ($F_{1,37} = 0.53$, $p = 0.47$) or interaction ($F_{1,37} = 1.78$, $p = 0.19$), suggesting that the depth of catecholamine depletion did not differ between groups.

3.2. Imaging results

The mean global CBF values showed no difference between the groups ($F_{1,38} = 0.001$, $p = 0.97$) and drug conditions

($F_{1,38} = 1.13$, $p = 0.30$). There was no significant drug-by-group interaction ($F_{1,38} = 0.004$, $p = 0.95$).

3.2.1. Regions of interest

The 2 groups revealed no significant main effect on CBF in any of the predefined ROIs (Table 3A). AMPT, however, influenced the mean CBF in the pallidum significantly and showed a trend towards a significant main effect ($p = 0.06$) on the CBF in the putamen (Table 3A). Both effects revealed an AMPT-induced reduction in CBF. In no other ROI, AMPT showed a significant main effect on the CBF. A significant drug-by-group interaction was found in 1 region, the pallidum (Table 3A). While AMPT did not alter CBF in HC participants, it led to reduced CBF in rBN participants in this region.

No significant correlations between BMI and CBF were found in any of the ROIs for the sham condition and for AMPT-induced CBF alterations.

3.2.2. Voxel-wise analysis

A between-group comparison in the sham depletion condition revealed a reduced CBF in the rBN group in the left rolandic operculum (MNI-coordinates: $x = -56$, $y = 0$, $z = 10$; peak- t -value: $T_{37} = 4.79$; $p < 0.001$; $k_E = 35$) and insula ($x = -40$, $y = 10$, $z = -4$; $T_{37} = 4.28$; $p < 0.001$; $k_E = 31$) relative to HC participants.

Detailed results of the drug-by-group interaction analysis are presented in Table 3B. We found drug-by-group interactions of the CBF in the right posterior midcingulate cortex

Table 3 Neural response to catecholamine depletion

(A) Mean cerebral blood flow (CBF) and standard deviation in the regions of interest (ROIs).

Region of interest	Mean \pm SD				interaction					
	HC participants		rBN participants		group		drug		group-by-drug	
	Sham	AMPT	Sham	AMPT	F _{1,38}	p-value	F _{1,38}	p-value	F _{1,38}	p-value
Left pallidum	33.2 \pm 7.7	34.1 \pm 7.6	37.6 \pm 9.3	30.9 \pm 8.4	< 0.01	0.94	5.60	< 0.05	5.60	< 0.05
Right pallidum	36.6 \pm 9.1	35.7 \pm 5.6	38.4 \pm 10.1	33.4 \pm 10.4						
Left putamen	40.3 \pm 7.9	40.2 \pm 7.8	41.7 \pm 8.1	38.0 \pm 8.3	0.04	0.84	3.89	0.06	0.52	0.48
Right putamen	42.4 \pm 7.2	40.0 \pm 6.8	41.7 \pm 7.1	39.8 \pm 7.4						
Left insula	47.0 \pm 8.9	45.5 \pm 8.4	44.3 \pm 8.3	45.6 \pm 6.4	0.11	0.75	0.37	0.55	0.50	0.49
Right insula	50.4 \pm 7.0	49.1 \pm 8.2	50.2 \pm 9.1	49.2 \pm 7.6						
Left anteroventral striatum	39.1 \pm 9.1	38.2 \pm 7.7	41.8 \pm 7.9	39.2 \pm 9.6	0.96	0.34	0.23	0.64	0.10	0.76
Right anteroventral striatum	38.6 \pm 5.6	39.2 \pm 8.0	40.5 \pm 7.4	41.6 \pm 6.5						

(B) Voxel-wise analysis of the group-by-drug interaction.

Region	BA	T ₃₇	p-value	No. of voxels	MNI - coordinates			Mean ± SD			
								HC participants		rBN participants	
					x	y	z	Sham	AMPT	Sham	AMPT
Group × drug condition interaction											
Interaction: rBN participants: catecholamine (AMPT) > sham depletion, HC participants: sham > catecholamine depletion (AMPT)											
Left posterior superior temporal gyrus	42	4.43	< 0.001	28	-60	-26	8	54.5 ± 11.1	46.7 ± 10.5	49.5 ± 6.1	55.5 ± 14.5
Right medial frontal gyrus	10	4.17	< 0.001	26	10	62	4	50.9 ± 9.3	42.8 ± 10.9	44.3 ± 13.1	47.7 ± 13.1
Right posterior superior temporal gyrus	42	4.09	< 0.001	24	68	-18	6	48.2 ± 10.1	41.1 ± 10.6	41.5 ± 12.3	49.5 ± 6.2
Left posterior middle temporal gyrus	22	4.08	< 0.001	28	-58	-44	8	55.5 ± 10.1	49.6 ± 9.7	49.5 ± 9.0	55.9 ± 12.7
Interaction: rBN participants: sham > catecholamine depletion (AMPT), HC participants: catecholamine (AMPT) > sham depletion											
Right posterior midcingulate cortex (pmCC)	24	4.40	< 0.001	41	6	-18	44	53.8 ± 12.4	55.7 ± 10.5	59.2 ± 13.6	45.6 ± 12.6

(A) Mean and standard deviation of cerebral blood flow (CBF) in the four regions of interest (ROIs), separately for each hemisphere. Results of the linear mixed model analyses are presented. (B) Voxel-wise analysis of the smoothed CBF maps in a flexible factorial design; $p < 0.001$, uncorrected; minimum cluster size of 15 voxels. CBF mean and standard deviation in the clusters are presented.

Abbreviations: AMPT, alpha-methyl-paratyrosine; BA, brodmann area; CBF, cerebral blood flow; HC, healthy control participants; MNI, Montreal Neurological Institute; No., Number; rBN, remitted bulimic participants; ROI, region of interest; SD, standard deviation; Sham, sham depletion.

Table 4 Correlations between neural and behavioral effects of catecholamine depletion.

(A) Correlation analyses of the regions of interest (ROIs).

Behavioral rating	Region of interest	rho	p-value
POMS vigor	Right putamen	0.47	< 0.01
	Left pallidum	0.55	< 0.001
POMS fatigue	Left pallidum	-0.47	< 0.01

(B) Voxel-wise correlation analyses.

Behavioral rating	Region	BA	T ₃₇	p-value	No. of voxels	MNI - coordinates		
						x	y	z
POMS vigor	Right posterior midcingulate cortex (pmCC)*	24	5.47	< 0.05*	180	4	-12	36
MADRS	Right hippocampus/parahippocampal gyrus		4.96	< 0.001	158	30	-34	-14
	Left posterior middle temporal gyrus	21/22	4.12	< 0.001	37	-60	-46	0

Correlation analyses with the various regions of interest (ROIs) and voxel-wise multiple regression analyses including catecholamine depletion-induced CBF differences and the induced within-session behavioral rating changes. (A) The Spearman's rho rank correlation was used to assess the correlations with the various ROIs: significant correlations ($p < 0.05$) were reported. (B) Multiple regression analyses: significance threshold: $p < 0.001$, uncorrected; minimum cluster size of 23 voxels; * peak is significant on a $p < 0.05$ FWE-corrected level.

Abbreviations: MADRS, Montgomery-Åsberg Depression Rating Scale; No., Number; MNI, Montreal Neurological Institute; POMS, Profile of Mood States; ROI, region of interest.

(pmCC), bilateral in the posterior temporal cortex, and in the right medial frontal gyrus. In the pmCC, CBF was decreased following AMPT relative to sham depletion in rBN, but remained unchanged in HC participants. In rBN participants AMPT induced increased CBF and in HC participants decreased CBF bilateral in the posterior temporal cortex.

Including BMI as an additional factor did not significantly improve the goodness of fit of the models for the sham condition and the group-by-drug interaction. Moreover, we found no significant correlations between BMI and CBF in the sham condition. Higher BMI, however, was related to higher AMPT-induced CBF reduction in the left rolandic operculum (MNI-coordinates: $x = -52$, $y = -6$, $z = 8$; peak- t -value: $T_{37} = 4.07$; $p < 0.001$; $k_E = 33$).

3.3. Relation between neural and behavioral AMPT effects

3.3.1. Correlations between AMPT-induced changes in symptoms and CBF

Across groups, the correlation analyses between AMPT-induced behavioral rating changes and CBF alterations in the different ROIs revealed that higher vigor reduction was associated with a stronger CBF decrease in the right putamen and left pallidum. The induced fatigue by AMPT correlated with CBF decreases in the left pallidum. In no ROI, the induced depressive symptoms correlated with CBF (Table 4A).

In a voxel-wise multiple regression analysis, AMPT-induced depressive symptoms correlated negatively with the induced CBF changes in the right hippocampus/parahippocampal gyrus and the left posterior middle temporal gyrus. The AMPT-induced vigor reductions correlated with

CBF decreases in the right pmCC ($p < 0.05$ family-wise error (FWE)-corrected) (Table 4B).

3.3.2. Follow-up assessment

The follow-up telephone interview revealed that 5 out of 16 rBN participants experienced relapse. Two participants denied their participation in the follow-up interview. The latency of the follow-up assessment varied between the participants. The latency, however, was not different between the participants reported a relapse and the participants stayed in remission ($T_{7,0} = 1.23$, $p = 0.26$). The following dopamine-related measures were associated with later relapse: AMPT induced increases in depressive symptoms ($T_{13,2} = -4.35$, $p < 0.001$; Figure 2B and C), CBF reduction in the right hippocampus/parahippocampus gyrus (peak t -value: $T_{14} = 6.41$; $p < 0.001$; Figure 2A,C and D) and CBF reduction in the right inferior parietal lobe (MNI-coordinates: $x = 58$, $y = -40$, $z = 36$; $T_{14} = 5.65$; $p < 0.001$; $k_E = 31$). In addition, relapse was associated with shorter time in remission ($T_{12,7} = -3.09$, $p < 0.01$, reporting relapse: mean = 12.8 ± 13.79 months; staying in remission: mean = 63.18 ± 50.03 months). Staying in remission was associated with AMPT-induced increase in CBF in the hippocampus/parahippocampal gyrus (Figure 2A,C and D).

4. Discussion

This present study showed that AMPT reduced vigor, and that this effect was stronger in remitted BN relative to healthy individuals. This behavioral finding was paralleled by CBF reduction in the pallidum and in the pmCC in rBN participants, while healthy individuals revealed no CBF alterations in these regions. In the posterior temporal cortex, the CBF changes following AMPT differentiated between rBN and healthy

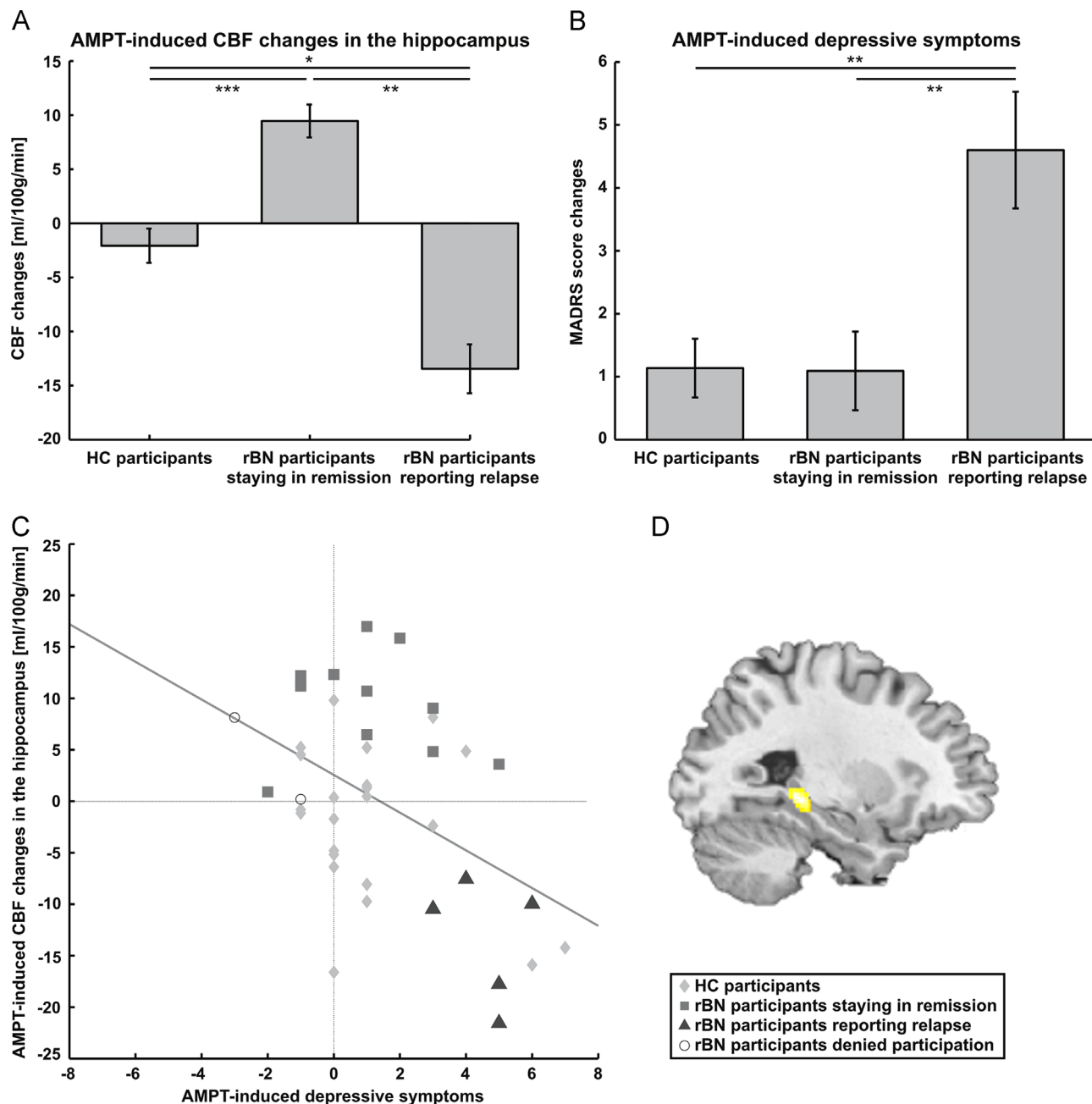


Figure 2 Predicting bulimic relapse: remitted bulimic (rBN) participants reporting bulimic relapse in the follow-up assessment revealed cerebral blood flow (CBF) reduction in the right hippocampus/parahippocampal gyrus and experienced increased depressive symptoms following AMPT. AMPT-induced CBF increase in the right hippocampus/parahippocampal gyrus predicted staying in remission. (A) AMPT-induced CBF changes in a cluster in the right hippocampus/parahippocampal gyrus. Separate bars representing the mean and standard error (error bars) of the AMPT-induced CBF changes in the following 3 groups: healthy control (HC, $N = 22$), rBN participants reporting bulimic relapse ($N = 5$), and rBN participants remaining in remission ($N = 11$). (B) AMPT-induced depressive symptoms. Bars representing the mean and standard error (error bars) of the AMPT-induced depressive symptoms separately for the 3 groups. (C) Scatter plot of the AMPT-induced CBF changes in the right hippocampus/parahippocampal gyrus cluster and the induced depressive symptoms. (D) Voxel-wise two-sample t -test comparing rBN participants remaining in remission and rBN participants reporting bulimic relapse after study participation: significant difference in the AMPT-induced CBF changes in a cluster in the right hippocampus/parahippocampal gyrus (MNI-coordinates: $x = 26$, $y = -36$, $z = -4$; cluster size: $k_E = 87$). Significance level: * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

individuals, showing increased and decreased CBF, respectively. In addition, we found catecholamine-associated biomarkers for BN relapse: higher AMPT-induced depressive symptoms and AMPT-induced CBF reduction in the hippocampus/

parahippocampal gyrus were related to later bulimic relapse. Reversely, AMPT-induced increase in CBF in the hippocampus/parahippocampal gyrus was associated with staying in remission, and appeared to reflect higher resiliency.

Our finding of AMPT-induced vigor reduction, that was more strongly pronounced in rBN than in HC participants, is well in line with the addiction model-based dopamine deficiency proposed for BN by Frank (2016). Addiction is associated with a desensitized dopamine system (Volkow et al., 2016). Drug withdrawal exacerbates this dopamine deficiency (Bailey et al., 2001) and is associated with drug craving, anhedonia, dysphoria, and sleep disturbances. Frank's (2016) theory yields similar predictions on the behavioral and neural level that have been supported by previous research. In healthy women, recurrent dieting and eating without restrictions periods over 4 weeks led to worse mood, increased fatigue, and enhanced caloric intake (Laessle et al., 1996). In a longitudinal study over three years in young female college students, vigor was found to be predictive for bulimic symptoms: in the first assessment in this study an increased vigor predicted bulimic symptoms (Cooley and Toray, 2001b). After three years, however, reduced vigor was associated with bulimic symptoms (Cooley and Toray, 2001a), probably induced by the desensitized dopamine system. Our current finding of AMPT-induced vigor reduction yields further support for the theory of Frank (2016).

Frank proposed in his model of eating disorders that the desensitized dopamine system in BN needs stimulation through binge eating behavior (Frank, 2016). The propensity for binge eating may be further exacerbated by experimental catecholamine depletion. Indeed, our previous study showed that AMPT increased eating disorder symptoms measured by the EDE-Q in remitted BN (Grob et al., 2015). In the present study we did not observe this effect, probably due to the important impact of the environment on eating behavior (Frank, 2016). The present study was conducted in an uncontrolled environment, whereas in our previous study, the administration of AMPT and the experiments were performed in a controlled environment without food cues and with regular, standardized meals (Grob et al., 2015). After leaving the controlled environment, rBN participants reported more eating disorder symptoms (Grob et al., 2015). Hence, we may not have observed an effect of AMPT on eating disorder symptoms in the rBN participants included in this study, because the effect of AMPT was overridden by environmental influences.

Based on our CBF measures, we found rBN-related AMPT-induced brain activity changes in the pallidum and the pMCC, which is consistent with Frank's model (Frank, 2016): reduced catecholamine neurotransmission led to CBF reduction in these regions in rBN participants. The pallidum is part of the brain reward system found to be desensitized in BN (Frank et al., 2011). HC participants in our study revealed no significant AMPT-induced CBF alterations in the pallidum and pMCC in contrast to the rBN participants. This is in line with previous pharmacological challenge studies using AMPT also revealing different effects of AMPT in control and experimental participants (Abi-Dargham et al., 2000; Hasler et al., 2008). Since rBN and HC participants, however, showed the same increase in prolactin levels, a lack of catecholamine depletion in HC participants is no possible explanation for these findings. The lack of response to catecholamine depletion in these regions suggests that the catecholaminergic system in healthy participants had enough reserve to compensate for the

partial catecholamine depletion by our low dose AMPT challenge.

Including BMI as an additional factor did not significantly improve the goodness of fit of the models for the sham condition and the group-by-drug interaction. Furthermore, CBF in the sham condition and AMPT-induced CBF alterations were not significantly related to BMI in the predefined ROIs. Moreover, the voxel-wise analysis revealed no significant association between BMI and CBF in the sham condition. The correlation between AMPT-induced CBF alterations and BMI, however, revealed a significant association in the left rolandic operculum: higher BMI was related to AMPT-induced CBF reduction in the left rolandic operculum. This finding might represent a floor effect, because a between-group comparison in the sham condition revealed a reduced CBF in the rBN group in the left rolandic operculum and the insula. These results, however, might also be relevant for the pathophysiology of BN in view of the report that the administration of milk shakes with varying fat and sugar contents in lean adolescents leads to increased activation of the rolandic operculum and the insula with increasing sugar content (Stice et al., 2013). Our results suggest that a neural mechanism regulating sugar intake that involves CBF in these regions is impaired in rBN individuals.

Contrary to our expectations, there was no AMPT effect on CBF in the anteroventral striatum and only a statistical trend for a decreased CBF in the putamen. In previous catecholamine challenge studies, glucose metabolism was altered following AMPT in these regions (Bremner et al., 2003; Hasler et al., 2008; Savitz et al., 2013). The glucose metabolism was reported to be increased following AMPT in the ventral striatum with the peak located in the putamen in both remitted MDD and healthy individuals (Hasler et al., 2008) and in participants with low and high risk for mood disorders (Savitz et al., 2013). Another study, however, associated metabolism decrease in the putamen with AMPT-induced relapse of depressive symptoms in remitted MDD, whereas increased metabolism following AMPT was found in remitted MDD participants experiencing no relapse (Bremner et al., 2003). These findings suggest that catecholamine deficiency may have a different impact on MDD and BN. The potentially different pathogenesis of "primary" MDD and MDD comorbid with BN may have important clinical implications.

Importantly, our study yields insights into the relation between catecholamine-driven brain activity and behavior in BN. Berridge, et al., described that the pallidum is involved in "wanting" for foods (Berridge et al., 2010). In an animal study, dopamine transporter (DAT)-knockdown mice having elevated extracellular dopamine revealed increased "wanting" for food-intake and vigorous behavior (Peciña et al., 2003). In our study, AMPT-induced CBF reduction in the pallidum was associated with vigor decrease. These findings in remitted BN revealed that the anhedonic behavioral response to catecholamine deficiency paralleled by reduced CBF in the pallidum might be involved in the dysfunctional eating behavior in BN.

Besides an altered brain reward system (Frank, 2013), eating disorders were also associated with emotion regulation difficulties (Harrison et al., 2010). Frank assumed that negative emotions and stress contribute to the

development and maintenance of eating disorder symptoms (Frank, 2016). The midcingulate gyrus (MCC) was described to be involved in the integration of negative emotions and motoric responses (Pereira et al., 2010). Different monetary incentives and therefore different states of vigor requiring motor responses activated the MCC differently (Pessiglione et al., 2007). In our study, the AMPT-induced vigor reduction was associated with induced CBF decrease in the pMCC. In contrast, AMPT increased glucose metabolism in the pMCC in remitted MDD, with higher induced glucose metabolism was associated with greater AMPT-induced reduction of hedonic capacity (Hasler et al., 2008). This finding points to important differences in the pathogenesis of depressive states in BN and MDD. Moreover, in our study, remitted BN was associated with increased CBF following AMPT in the posterior temporal cortex, which is involved in processing of social and emotional stimuli (Scharpf et al., 2010). These changes were negatively correlated with induced depressive symptoms measured by the MADRS, whereas in remitted MDD, AMPT-induced metabolism increase in this region revealed a positive association with depressive symptoms (Hasler et al., 2008). Taken together, depressive symptoms in MDD appear to be associated with catecholamine deficiency-related increase in neural activity in the emotional and social brain, whereas depression in BN is rather related to a catecholamine deficiency-induced decrease in neural activity.

Depressive syndromes in BN were associated with an unfavorable course with high chronicity (Keski-Rahkonen et al., 2013). Consistent with this finding from epidemiology, we demonstrated that increased AMPT-induced depressive symptoms were related to later bulimic relapse. This relationship was paralleled by reduced CBF in the hippocampus/parahippocampal gyrus, whereas increased CBF in this region was coupled with remaining in remission. These results are in agreement with a finding in MDD, revealing that the return of depressive symptoms following AMPT in remission was associated with a decreased metabolism in the hippocampus and other cortical regions, whereas increased metabolism in these brain regions were experienced by individuals reporting no relapse (Bremner et al., 2003). In an animal study, maternal separation and fasting/refeeding cycles led to binge eating behavior in rats (Ryu et al., 2008) resulting in reduced depression-like behavior and increased dopamine concentration in the hippocampus (Jahng et al., 2012). The anti-depressive and dopamine-elevating effect of binge eating might be responsible for the maintenance of this behavior. Anticipation and receive of food in a negative mood state was reported to result in increased activation in the parahippocampal gyrus and pallidum in emotional eaters (Bohon et al., 2009). Frank proposed in his model that stressful life events might result in a dysfunctional dopamine system in BN (Frank, 2016). Therefore, stressful events, dopamine deficiency, and a dysfunctional hippocampus reactivity might act in concert to trigger binge eating, to reduce distress, negative emotions and anhedonia.

Besides an AMPT-induced increase in depressive symptoms and CBF reductions in the hippocampus/parahippocampal gyrus, relapse was also associated with a shorter time in remission in our study. On the contrary, in rBN that remained

in remission, AMPT induced an increase in hippocampal/parahippocampal CBF, and they reported a longer duration of remission prior to the study. This complex of findings on the behavioral and neural level has important implications, suggesting that catecholamine-related hippocampus/parahippocampal gyrus CBF is a biomarker of susceptibility and resilience to BN relapse. This notion is consistent with reports on the relation between hippocampus integrity in food intake. Kanoski and Davidson (2011) claimed that the intake of high caloric food disrupts a neural inhibitory mechanism involving the hippocampus that controls food intake. In accordance with this theory, obese and previously obese individuals showed a reduced CBF in the hippocampus after food consumption to satiation whereas lean individuals revealed an increased CBF in this region (DelParigi et al., 2003). Furthermore, longitudinal data showed that higher consumption of unhealthy “Western” food was associated with smaller hippocampal volume (Jacka et al., 2015). Taken together with these findings, our results expand Frank’s model of eating disorders (Frank, 2016) by showing a catecholamine-related mechanism involving the hippocampus/parahippocampal gyrus that contributes to staying in remission or being susceptible to BN relapse.

Two limitation of this study merit comment. First, the large variation in the latency of the follow-up assessment might have had an influence on the group assignment of the rBN participants: we are not able to rule out that the participants who experienced no relapse by the time of the follow-up assessment will have a binge eating or purging episode in the future. Nonetheless, the latency of the follow-up assessment was at least 18 months. An earlier study showed that the risk of relapse was highest within the first 6-7 months (Richard et al., 2005). In addition, the latency of the follow-up assessments was not significantly different between the rBN participants experienced a relapse and the participants staying in remission. Therefore, we concluded that the latency had, if at all, only a minor impact on the group assignment in the follow-up assessment. Second, the duration between the two experimental sessions differed largely between the participants and therefore, might have had an effect on our results. Nonetheless, there was no significant difference in the duration between rBN and HC participants, and between the rBN participants experienced a relapse and the participants staying in remission. Hence, we assumed that the potential effect of differing durations on our findings is minor.

This study suggests that catecholamine depletion-induced reductions in CBF in the pallidum and the pMCC in remitted BN are the functional neuroanatomical correlates of a desensitized dopamine system in BN, as Frank proposed in his model of eating disorders (Frank, 2016). Most importantly, we were able to extend this model by revealing that later bulimic relapse was associated with catecholamine depletion-induced depressive symptoms paralleled by a decrease in hippocampal CBF, emphasizing the importance of depressive symptoms and the stress system in the course of BN. Our findings encourage clinical studies on the effect of interpersonal stress management in combination with drugs that enhance catecholaminergic neurotransmission on the course of BN. In addition, treatment of depression, including pharmacotherapy with selective serotonin, selective norepinephrine or serotonin-norepinephrine reuptake

inhibitors, may not be overlooked in order to facilitate treatment or prevent relapse of BN (Flament et al., 2012).

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Contributors

S.V. Mueller assisted in designing the study, acquired, analyzed and interpreted the data. Y. Mihov and A. Federspiel were involved in analyzing the data. A. Federspiel and R. Wiest were involved in designing the MRI sequences. G. Hasler conceptualized and designed the study, supervised data collection, obtained funding, and interpreted the data. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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