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Dopamine-Related Deficit in Reward Learning After Catecholamine Depletion in Unmedicated, Remitted Subjects with Bulimia Nervosa

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Disturbances in reward processing have been implicated in bulimia nervosa (BN). Abnormalities in processing reward-related stimuli might be linked to dysfunctions of the catecholaminergic neurotransmitter system, but findings have been inconclusive. A powerful way to investigate the relationship between catecholaminergic function and behavior is to examine behavioral changes in response to experimental catecholamine depletion (CD). The purpose of this study was to uncover putative catecholaminergic dysfunction in remitted subjects with BN who performed a reinforcement-learning task after CD. CD was achieved by oral alpha-methyl-para-tyrosine (AMPT) in 19 unmedicated female subjects with remitted BN (rBN) and 28 demographically matched healthy female controls (HC). Sham depletion administered identical capsules containing diphenhydramine. The study design consisted of a randomized, double-blind, placebo-controlled crossover, single-site experimental trial. The main outcome measures were reward learning in a probabilistic reward task analyzed using signal-detection theory. Secondary outcome measures included self-report assessments, including the Eating Disorder Examination-Questionnaire. Relative to healthy controls, rBN subjects were characterized by blunted reward learning in the AMPT—but not in placebo—condition. Highlighting the specificity of these findings, groups did not differ in their ability to perceptually distinguish between stimuli. Increased CD-induced anhedonic (but not eating disorder) symptoms were associated with a reduced response bias toward a more frequently rewarded stimulus. In conclusion, under CD, rBN subjects showed reduced reward learning compared with healthy control subjects. These deficits uncover disturbance of the central reward processing systems in rBN related to altered brain catecholamine levels, which might reflect a trait-like deficit increasing vulnerability to BN.
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INTRODUCTION

Bulimia nervosa (BN) has been associated with behavioral and neural abnormalities in response to rewarding stimuli (Harrison *et al*, 2010; Wagner *et al*, 2010). Impairments in processing reward-related stimuli might reflect a dysregulation of the central catecholaminergic neurotransmitter system. Catecholamines, particularly dopamine (DA), are involved in diverse aspects of reward processing, including the evaluation of rewarding proprieties of food (Fulton, 2010; Schienle *et al*, 2009), reinforcement learning (Montague *et al*, 1996; Schultz, 2002), and in the development of addictions (Koob and Volkow, 2010), which are likely associated with the pathogenesis of BN (Kaye, 2008).

Reward learning, defined as the ability to make stimulus-reward associations and subsequently modulate behavior to optimize the likelihood of obtaining future rewards, constitutes a key component of the reward system (Berridge *et al*, 2009) and is of interest when investigating the relationship between impairments in processing reward-related stimuli and central dopaminergic function in BN. To this end, in the current study, we applied a well-validated probabilistic reward task based on a differential reinforcement schedule that allowed us to objectively assess participants' propensity to modulate behavior as a function of reward (Pizzagalli *et al*, 2005). Critically, reward learning—as assessed by the probabilistic reward task utilized in the present study—has been found to be sensitive to pharmacological challenges targeting DA

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transmission (Pizzagalli *et al*, 2008) and correlated with striatal responses to rewards (Santesso *et al*, 2008).

To assess the relationship between catecholaminergic function and behavior, a useful technique has involved evaluating behavioral responses to catecholamine depletion (CD) achieved by oral administration of alpha-methyl-para-tyrosine (AMPT) (Berman *et al*, 1999; Hasler *et al*, 2004). AMPT is a competitive inhibitor of the rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (Nagatsu *et al*, 1964) and decreases catecholamine transmission by depleting central DA and norepinephrine stores. Its efficacy is evidenced by reduced concentrations of catecholamines and their metabolites in plasma, urine, and cerebrospinal fluid (Mignot and Laude, 1985; Stine *et al*, 1997), and decreased occupancy of striatal DA receptors by DA (Verhoeff *et al*, 2003).

The purpose of the study was to assess, we believe for the first time, differential responses of the brain reward system to CD in remitted female subjects with BN (rBN) and healthy female controls using a probabilistic reward task. We hypothesized that rBN subjects and controls would be equally responsive to rewards in the placebo condition. Following CD, we hypothesized that rBN subjects would be less able to modulate behavior in response to rewards than controls, reflecting a possible trait-like deficit in BN.

MATERIALS AND METHODS

Participants

In all, 19 women who had previously met DSM-IV criteria for BN (mean age = 25.2 years (SD = 3.5), range = 19–31 years) and 28 healthy control women (mean age = 25.8 years (SD = 3.6), range = 21–32 years) without a history of any psychiatric disorder and no major psychiatric conditions in first degree relatives were included in the study. Both groups were recruited by advertisements in local newspapers and announcements at the University of Zurich and the Swiss Federal Institute of Technology Zurich (ETH). The screening visit included a diagnostic interview with a psychiatrist, the Structured Clinical Interview for DSM-IV, (First *et al*, 2001) and a physical examination. Exclusion criteria were lifetime diagnosis of psychosis, major medical or neurological illness, psychoactive medication exposure in the past 6 months, lifetime history of substance dependence, pregnancy, suicidal ideation, and history of suicide attempts. Remitted subjects with a history of BN (rBN) had been in remission for at least 6 months (mean time in remission from BN = 28.8 months (SD = 24.8), range: 6–84 months) at the time of study participation. Six rBN subjects had a history of antidepressant use, including SSRIs and TCAs (mean time medication free: 40 months (SD = 3.5), range: 12–96 months). Five rBN subjects had a history of mild-to-moderate anorexia nervosa (AN) (AN: mean lowest weight: 42.5 kg (SD = 7.3), range: 29–49 kg; mean time in remission from AN: 101 months (SD = 39.5), range: 36–144 months) and four rBN had been diagnosed with major depressive disorder preceding or during BN. All subjects had a body mass index (BMI) within the normative range (19–24 kg/m^2). rBN subjects had a mean BMI of 21.7 kg/m^2 (SD = 2.9), range: 18.3–32 kg/m^2. Healthy controls had a BMI of 22.1 kg/m^2 (SD = 2.1), range: 18.6–26.5 kg/m^2. All subjects provided written informed consent before participation. The study protocol was approved by the ethics committee of the Canton of Zurich (Kantonale Ethikkommission Zürich).

Experimental Design

Using a randomized, double-blind, placebo-controlled, crossover design, participants underwent two identical sessions separated by at least 7 days in which they received either AMPT or placebo. Each session included 2 days on an inpatient eating disorder unit at the Department of Psychiatry and Psychotherapy of the University Hospital of Zurich. CD was induced by oral administration of a body weight-adjusted AMPT dose of 40 mg/kg body weight, to a maximum of 4 g, over 22 h (on day 1 at 0900 h, 1200 h, and 1900 h; on day 2 at 0700 h). During sham depletion, subjects received inactive placebo on day 1 at 0900 h and 1200 h and 25 mg diphenhydramine orally on day 1 at 1900 h and on day 2 at 0700 h because AMPT frequently induces mild sedation. To prevent the formation of urinary crystals during AMPT administration, subjects were instructed to drink at least 2 L of water per day, starting on day 1. Possible adverse reactions were assessed regularly during the hospitalization (26, 30, 54, 78, and 102 h after the first AMPT/placebo administration) through medical examination including blood pressure measurement, and during the subsequent 3 days after discharge by daily telephone interviews.

During hospitalization (0, 26, and 30 h after the first AMPT/placebo administration) and on the 3 subsequent days (54, 78, and 102 h after the first AMPT/placebo administration), participants completed various self-report ratings, which were administered repeatedly (at each time point). Self-report ratings included the Eating Disorder Examination-Questionnaire (EDE-Q) (Fairburn and Beglin, 1994), Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979), Young Mania Rating Scale (YMRS) (Young *et al*, 1978), Beck Anxiety Inventory (BAI) (Beck *et al*, 1988), Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith *et al*, 1995), and Stanford Sleepiness Scale (SSS) (Hoddes *et al*, 1973).

To estimate the depth of CD, blood samples were drawn 26 h after the first AMPT/placebo administration to measure serum prolactin levels.

Task and Procedure

Thirty hours after the first AMPT/placebo administration (day 2, 1400 h) subjects participated in a 25-min probabilistic reward task presented on a PC using E-prime software (Psychology Software Tools, Pittsburgh, Pennsylvania). The task is based on signal-detection theory and allows analysis of subjects' performance with respect to signal discriminability, response bias, and reaction time. Participants were instructed that the goal of that task was to win as much money as possible. The task included 300 trials, divided into 3 blocks of 100 trials. Blocks were separated by a 30-s break. Each trial started with the presentation of a fixation cross for 500 ms followed by a mouthless schematic face. After 500 ms, either a short mouth (11.5 mm) or a long mouth (13 mm) appeared on the face for 100 ms. The mouthless face remained on the screen until participants made a key

response. Participants' goal was to identify which stimulus (short mouth or long mouth) was presented and to press the corresponding 'z' key or '/' key on the keyboard (counterbalanced across subjects and between conditions) (Figure 1). To produce a response bias, an asymmetric reinforcement ratio was utilized. (McCarthy and Davison, 1979; Tripp and Alsop, 1999) Subjects received a reward for correct identification of either the short or long mouth ('Correct!! You won 5 cents') three times more frequently for correct identification of one stimulus ('rich stimulus') than for correct identification of the other stimulus ('lean stimulus'). In each session (AMPT and placebo), the same stimulus type (short *vs* long) was rewarded three times more frequently than the other stimulus. In each block, an equal number of short and long mouths were presented and only 40 correct trials (30 rich and 10 lean) were rewarded. Stimulus presentation was pseudo-randomized, with the constraint that no more than three repetitions of the same mouth were allowed. In the event of an incorrect identification on a trial scheduled to be rewarded, the reward feedback was delayed until the next correct response of the same stimulus type. As a result, each participant was exposed to the same reward ratio. Participants were informed that not all correct responses would be rewarded. For the entire task subjects earned ~6 Swiss francs. More detailed information regarding task validation in various independent samples is available elsewhere (Bogdan and Pizzagalli, 2006; Pizzagalli *et al*, 2009; Pizzagalli *et al*, 2005).

Statistical Analysis

Participants' performance in the probabilistic reward task was evaluated with respect to response bias, discriminability, and reaction time. Response bias was the main variable of interest and refers to subjects' preference for the stimulus coupled with the more frequent reward. High rates of correct identification (hits) for the rich stimulus and high miss rates for the lean stimulus yield a high response bias. Response bias (log *b*) was computed as:

Response Bias:

$$\log b = \frac{1}{2}\log\left(\frac{(Rich_{correct}+0.5)\times(Lean_{incorrect}+0.5)}{(Rich_{incorrect}+0.5)\times(Lean_{correct}+0.5)}\right)$$

Discriminability indexes participants' ability to differentiate between the two stimuli (short *vs* long mouths). Discriminability (log *d*) was computed as:

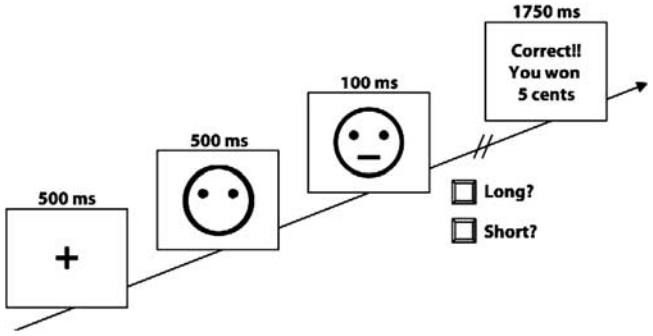

Figure 1 Schematic diagram of the signal-detection task design (Pizzagalli *et al*, 2005).

Discriminability:

$$\log d = \frac{1}{2}\log\left(\frac{(Rich_{correct}+0.5)\times(Lean_{correct}+0.5)}{(Rich_{incorrect}+0.5)\times(Lean_{incorrect}+0.5)}\right)$$

Reaction time was assessed in milliseconds (ms) and refers to response speed.

According to recommendations described elsewhere (Hautus, 1995; Pizzagalli *et al*, 2009), 0.5 was added to each cell of the detection matrix to permit the computation in cases that contain a zero in one cell of the formula. Response bias, discriminability, and reaction time values were computed after removing outlier responses (eg, trials with responses shorter than 150 ms), following previously established procedures (Pizzagalli *et al*, 2005).

To analyze the effects of *Condition* (AMPT, placebo), *Diagnosis* (rBN, HC), and *Block* (1,2,3) on response bias and discriminability, full factorial linear mixed models with restricted maximum likelihood estimation were utilized. For reaction time scores, the factor *Stimulus* (rich and lean) was added to the model. Based on Akaike's Information Criterion (AIC), a first-order factor analytic covariance structure with heterogeneous diagonal offsets (FAH1) was applied to the model for computing response bias. For the analyses of discriminability and reaction time, an identity (ID) covariance structure was fitted to the models. For control analyses, discriminability was entered as covariate in the model. Estimated marginal means regarding the interaction between *Diagnosis* and *Condition* allowed for analysis of the diagnostic groups separately.

To evaluate the influence of BN individuals with AN histories ($n=5$) on the results, control analyses were run considering only BN individuals without AN histories ($n=14$).

To evaluate whether CD-induced changes in response bias correlated with CD-induced changes in clinical symptoms (as assessed by EDE-Q, MADRS, YMRS, BAI, SHAPS, and SSS), Pearson correlations were computed across groups and separately for each group. As in prior studies (Bogdan and Pizzagalli, 2006; Pizzagalli *et al*, 2009, 2005), reward learning was defined as the difference in response bias between Block 1 and Block 3 (ΔRB = Block 3−Block 1). CD-induced changes in reward learning were obtained by subtracting the response bias in the AMPT condition from the response bias in the placebo condition. Similarly, for each clinical scale, the change score of the placebo condition was subtracted from the change score of the AMPT condition.

To test study hypotheses, we calculated one model to estimate the effects of *Block*, *Condition*, and *Diagnosis* on response bias. Statistical tests on discriminability, reaction time, and clinical symptoms were not corrected for multiple testing since we considered them secondary analyses aimed to test the specificity of putative response bias results, and to further elucidate differences in task performance as a function of *Condition* and *Diagnosis*.

Analyses were performed using SPSS 18.0 statistical software (SPSS, Chicago, Illinois). The statistical significance level was set at $\alpha = 0.05$.

RESULTS

Response Bias

Figure 2a shows response bias scores as a function of block, diagnosis, and condition. Across diagnosis and conditions, a main effect of *Block* emerged ($F_{1,104.5} = 4.45$, $P < 0.05$). Response bias significantly increased from Block 2 to Block 3 ($P < 0.01$). Moreover, there was a significant *Block* × *Diagnosis* interaction ($F_{1,104.5} = 7.10$, $P < 0.01$). rBN subjects showed significantly less response bias in Block 3 than healthy controls ($F_{1,59.5} = 12.53$, $P < 0.01$). Critically, and consistent with our hypotheses, this latter effect was qualified by the triple *Diagnosis* × *Condition* × *Block* interaction ($F_{1,103.3} = 4.00$, $P < 0.05$). In Block 3, rBN subjects showed reduced reward learning compared with controls in the AMPT condition ($F_{1,45.1} = 12.92$, $P < 0.05$). Control analyses confirmed that this effect remained significant when excluding rBN individuals with AN history ($n = 5$) ($F_{1,39.9} = 7.15$, $P < 0.05$). The main effects of Block ($F_{2,94.4} = 5.05$, $P < 0.01$) and Block × Diagnosis ($F_{2,94.4} = 4.40$, $P < 0.05$) remained unchanged when running the analyses only within the subgroup of BN without AN histories. The triple interaction Diagnosis × Condition × Block showed a statistical trend ($F_{2,91.0} = 2.82$, $P < 0.10$) considering only the subgroup of BN without AN histories. In the placebo condition, no difference in reward learning between diagnostic groups and blocks emerged ($F_{1,50.1} = 0.42$, $P = 0.52$). Moreover, results comparing the two subgroups: BN individuals with AN histories (BN-AN, $n = 5$) and BN individuals without AN histories (BN, $n = 14$), indicated no significant difference ($F_{1,14.2} = 2.86$, $P = 0.11$) between the two subgroups regarding the main outcome variable (response bias). The main findings remained when excluding subjects with a history of MDD ($n = 4$; $F_{1,98.5} = 3.70$, $P < 0.05$).

Discriminability

Overall, a main effect of AMPT on discriminability was evident ($F_{1,225} = 8.94$, $P < 0.01$). In the AMPT condition, subjects across diagnostic groups showed lower discriminability

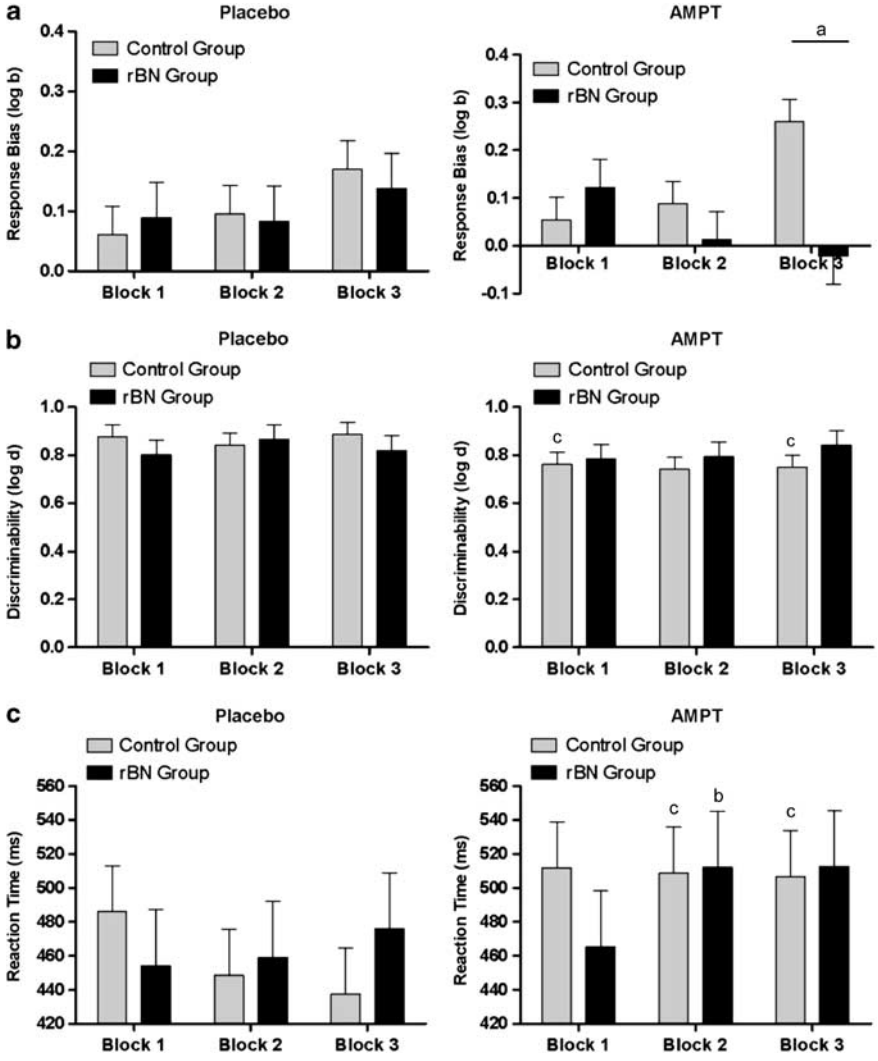

Figure 2 Mean (a) response bias, (b) discriminability, and (c) reaction time (across 'rich' and 'lean' condition) for unmedicated subjects with Bulimia Nervosa in remission (rBN group; $n = 19$) and healthy control subjects (control group; $n = 28$). Error bars represent standard errors. Alphabetic characters denote significant findings in *post-hoc* analyses. A denotes significant diagnosis effect (rBN *vs* controls, $P < 0.05$); B denotes significant condition effect for rBN; C denotes significant condition effect in controls.

compared with the placebo condition. In addition, a *Diagnosis* × *Condition* interaction reached significance ($F_{1,225} = 4.14$, $P < 0.05$). Control subjects showed lower discriminability in the AMPT condition compared with the placebo condition ($F_{1,225} = 15.61$, $P < 0.001$), while rBN subjects did not show a significant difference in discriminability between AMPT and placebo conditions ($F_{1,225} = 0.38$, $P = 0.54$). Diagnostic groups did not differ in the AMPT ($F_{1,59.0} = 0.72$, $P = 0.40$) nor in the placebo ($F_{1,59.2} = 0.38$, $p = 0.54$) condition (Figure 2b).

Critically, including discriminability scores in the model on response bias did not change the results, suggesting that group differences in response bias were not affected by participants' ability to differentiate between the two stimuli.

Reaction Time

As expected, reaction times were shorter in response to the rich stimulus than the lean stimulus, as reflected in the main effect of *Stimulus* ($F_{1,495} = 7.88$, $P < 0.01$). Subjects in the AMPT condition showed slower reaction times compared with the placebo condition ($F_{1,495} = 32.94$, $P < 0.001$). Moreover, a significant *Diagnosis* × *Block* interaction emerged ($F_{1,495} = 6.16$, $P < 0.01$). While reaction times generally increased in rBN subjects from Block 1 to Block 3 ($P < 0.05$), they decreased in controls ($P < 0.05$). There was no significant *Diagnosis* × *Condition* × *Block* interaction, indicating that slowing in rBN was not restricted to one particular stimulus type ($F_{2,495} = 0.26$, $P = 0.77$) (Figure 2c).

Correlations Between Clinical Ratings and Changes in Response Bias ($n = 47$)

CD-induced eating disorder symptoms assessed by the EDE-Q did not correlate with CD-induced changes in response bias in any of the three blocks (all $rs < 0.07$, all $ps > 0.61$) or reward learning (ΔRB) ($r = 0.13$, $P > 0.35$). Among rBN subjects, CD-induced anhedonia as assessed by the SHAPS was negatively correlated with corresponding CD-induced changes in response bias in Block 1 ($r = -0.67$, $P < 0.05$) and revealed a negative trend in Block 3 ($r = -0.41$, $P = 0.08$). CD-induced changes on the SHAPS revealed a trend with CD-induced changes in reward learning (ΔRB) ($r = 0.40$, $P = 0.09$). In control subjects, CD-induced changes on the SHAPS did not correlate with CD-induced changes in response bias in any of the three blocks (Block 1 $r = 0.28$, $P = 0.16$; Block 2 $r = 0.11$, $P = 0.58$; Block 3 $r = 0.22$, $P = 0.26$) or reward learning (ΔRB) ($r = -0.07$, $P = 0.74$). Fisher tests for independent correlations indicated that correlations for the rBN and control groups were significantly different for Block 1 ($Z = -3.43$, $P < 0.01$) and Block 3 ($Z = -2.06$, $P < 0.05$) but not ΔRB ($Z = 1.54$, ns). Further highlighting the specificity of the link between anhedonic symptoms and response bias, no correlations emerged across groups between the self-report ratings MADRS, YMRS, BAI, and SSS. Finally, among the rBN group, correlations between responses bias and time in remission from BN were not significant (Block 1: $r = -0.31$, $P = 0.23$; Block 2: $r = -0.12$, $P = 0.65$; Block 3: $r = 0.06$, $P = 0.83$).

As expected, serum prolactin levels were significantly higher in the AMPT condition *vs* the placebo condition (mean (SD), 42.0 (2.5) *vs* 29.5 (2.6) μg/l; $F_{1,36.5} = 20.93$, $P < 0.001$). There was no *Diagnosis* effect ($F_{1,39.3} = 0.095$, $P = 0.76$) and no *Diagnosis* × *Condition* interaction ($F_{1,36.5} = 0.16$, $p = 0.69$) regarding serum prolactin concentration.

DISCUSSION

The current study is the first to examine the effects of CD on reinforcement learning in subjects with a history of BN and controls. The findings indicate that subjects with BN in remission and healthy controls did not differ with respect to reward learning in the placebo condition. However, following CD, rBN subjects (but not controls) showed reduced responsiveness to rewards leading to an inability to modulate behavior as a function of reinforcement history. This DA-mediated deficit was not associated with time in remission from BN, suggesting that reduced reinforcement learning might represent a stable, trait-like feature of BN. This novel finding provides important empirical evidence for catecholamine's role in impaired reward processing in eating disorders. Control analyses confirmed that history of AN did not modulate the findings. The triple interaction *Diagnosis* × *Condition* × *Block* showed a statistical trend when excluding rBN individuals with AN history ($n = 5$). A loss of power from 19 BN individuals (BN and BN-AN) to 14 BN individuals (BN without BN-AN) seems to be responsible for the *P*-value increase regarding the triple interaction. Several studies investigating the neurobiology of BN indicate that altered DA activity in reward-related brain structures such as mesolimbic regions are involved in aberrant reward processing (Bencherif *et al*, 2005; Frank *et al*, 2006; Kaye *et al*, 2001). The literature is in disagreement, however, with respect to whether BN is associated with increased sensitivity to reward or with blunted reward responsiveness. Few studies report that binge-eating and purging behaviors are associated with elevated sensitivity to reward (Farmer *et al*, 2001; Harrison *et al*, 2010; Loxton and Dawe, 2001). A study investigating reward sensitivity and brain activation to images of food in a sample of 14 patients with BN reported greater arousal in affective ratings of food pictures, as well as exaggerated anterior cingulate cortex and insula activation compared with healthy controls, binge-eating patients and overweight subjects without eating disorder (Schienle *et al*, 2009). Another behavioral study showed that BN participants were more sensitive to financial rewards than healthy controls (Farmer *et al*, 2001) (Kane *et al*, 2004).

In contrast, most studies in BN subjects in remission showed reduced reward responsiveness relative to controls. Blind administration of glucose revealed reduced responsiveness within brain reward pathways to nutrients in rBN subjects, possibly making them vulnerable to overeating (Frank *et al*, 2006). A recent study designed to investigate reward processing in response to monetary wins and losses assessed specifically the activity of the anterior ventral striatum, a region involved in motivational responses to stimuli. rBN subjects showed difficulty in discriminating between positive ('win') and negative ('loss') feedback compared with healthy control subjects, indicating an inability to modulate responses to reward-relevant stimuli in rBN individuals. Furthermore, rBN subjects were unable to distinguish between negative and positive feedback in the

dorsal caudate nucleus, a region implicated in linking action to outcome (Wagner *et al*, 2010). Critically, response bias as measured by the current task has been found to correlate with reward-related activation in striatal regions (Santesso *et al*, 2008; Wacker *et al*, 2009) and was modulated by dopaminergic challenges (Pizzagalli *et al*, 2008). Together with these prior findings, the current data highlight an impaired, dopaminergic-mediated tendency to modulate behavior as a function of prior reinforcements in individuals with a history of BN.

The correlation between CD-induced increases in the EDE-Q global score and CD-induced changes in response bias was not significant. This argues against a direct, immediate relationship between reward learning and eating disorder symptoms, at least in the current asymptomatic sample. In rBN subjects, CD-induced anhedonia as measured with the SHAPS was negatively correlated with corresponding CD-induced changes in response bias. This finding confirms the relationship between anhedonia and CD-induced impairments of the brain reward system (Hasler *et al*, 2009). This pattern is also in line with several studies suggesting anhedonia as an important clinical feature of BN (Davis and Woodside, 2002; Eiber *et al*, 2002; Harrison *et al*, 2010).

The signal-detection task can be best conceptualized as a measure of reinforcement learning. In prior independent studies, up to 30% of healthy participants could not verbalize at the end of the experiment which stimulus was rewarded more frequently and yet, their response bias scores clearly showed a preference for the rich stimulus (Pizzagalli *et al*, unpublished observation). These data indicate that conscious awareness of the reinforcement contingency is not necessary to elicit a response bias, and that, at least for some participants, response bias captures implicit reinforcement learning. This is consistent with independent findings indicating that response bias correlates with striatal responses (Santesso *et al*, 2008). As a result, we believe that decreased responsiveness to fluctuating rewards and difficulty integrating reinforcement history over time contributes to clinical anhedonia. The lack of a correlation between AMPT-induced response bias and AMPT-induced anhedonia in controls may be due to the small AMPT effects. In addition, the experiment was specifically related to monetary rewards, whereas the anhedonia scale assessed a broad range of natural rewards, which likely reduces the correlation between these two measures.

To date, the effects of CD on reward learning have been studied exclusively in mood disorders (Hasler *et al*, 2009). Studies considering CD and reward learning in other psychiatric conditions are missing. Because of the substantial comorbidity of BN and depression (Wade *et al*, 2004), findings from studies in affective disorders may also be relevant for BN. Unmedicated patients with major depressive disorder have demonstrated impairment in integrating reinforcement history over time and developing a response bias toward a more frequently rewarded cue in the absence of immediate reward (Pizzagalli *et al*, 2009, 2005). Moreover, a trait-like deficit in reward learning has been observed in remitted subjects with major depressive disorder participating in a reward processing task under CD (Hasler *et al*, 2009). Critically, the present finding of reduced response bias toward a more frequently rewarded stimulus remained when excluding rBN subjects with a history of MDD. Taken together, these findings indicate that a DA-related blunting of reward learning may represent a transdiagnostic risk factor for various psychiatric conditions including affective and eating disorders.

Most prior studies using CD have been performed in subjects in the remitted phase of major depressive disorder, who were either medicated with norepinephrine reuptake inhibiting antidepressant drugs (Bremner *et al*, 2003; Delgado *et al*, 1993; Miller *et al*, 1996) or drug free (Berman *et al*, 1999; Hasler *et al*, 2008), and showed marked depressive responses following CD. In patients with obsessive-compulsive disorder, CD did not affect obsessive-compulsive symptoms (Longhurst *et al*, 1999). Administration of AMPT in healthy subjects usually has no behavioral effects (Ruhe *et al*, 2007; Salomon *et al*, 1997), although two previous studies reported a significant effect of AMPT on mood, alertness, and increased anxiety in healthy controls (Hasler *et al*, 2008; McCann *et al*, 1995). No study has used CD so far to evaluate the roles played by norepinephrine and DA in the pathophysiology of BN.

This study has notable strengths. First, we included an active placebo (diphenhydramine) to mimic the side effect of mild sedation of AMPT, thus providing an effective blinding of the study drugs. While in previous studies using AMPT doses >4 g, subjects experienced adverse reactions such as dystonic reactions (McCann *et al*, 1990), restlessness (Laruelle *et al*, 1997), crystals in urine, and decrease in blood pressure (Brogden *et al*, 1981), none of our participants reported any significant adverse reactions, probably due to the use of a low, body weight-adjusted AMPT dose. A potential pharmacological effect of the active placebo on task performance is unlikely since the last dose of the 25 mg diphenhydramine was administered 9 h before task administration. Second, the sample size was relatively large for a complex pharmacological challenge study. Third, to our knowledge, this is the first study investigating the effects of CD on reward learning in BN. Fourth, the fact that CD induced the same amount of prolactin in rBN subjects and healthy controls suggests that there was no difference of the CD effect on catecholamine synthesis between groups (Freeman *et al*, 2000).

Several limitations of this study also merit comment. First, the effects of CD using AMPT did not allow for differentiation of the specific effects of DA and norepinephrine, as CD reduces the synthesis of NE as well as DA. Of note, although DA is known to have an important role in learning (Schultz, 2010), norepinephrine depletion may also have contributed to the reward learning deficits in rBN. We believe this alternative interpretation is unlikely due to (1) theories linking norepinephrine to task performance accuracy rather than reward learning *per se* (Aston-Jones *et al*, 2000) and (2) the current findings that group differences in response bias remained when controlling for discriminability.

Second, only female subjects were included in the study, precluding generalization of the results to male subjects. Third, we did not reliably assess the phase of the menstrual cycle and women were tested in both the follicular and luteal phases, which may represent a potentially confounding factor. However, a previous study did not reveal any

effects of the menstrual cycle on CD-induced symptoms (Hasler *et al*, 2008). Finally, as a result of the inclusion criterion that rBN subjects had to be in remission and off medication, a selection bias may have been introduced, resulting in a sample with relatively mild forms of BN. Nevertheless, this approach avoided the potential confound of medication effects.

In conclusion, the present findings demonstrate CD-induced reward learning deficits in rBN. In particular, rBN subjects following CD were unable to integrate reinforcement history over time. Thus, an increased sensitivity of brain reward pathways to CD may represent a trait-like abnormality in BN. In light of the present findings, functional neuroimaging studies probing neuronal substrates of blunted reinforcement learning and evaluating the clinical predictive validity of impaired reward learning in larger samples are warranted. Finally, the current data encourage genetic association studies to elucidate the genetic underpinnings of this deficit given that catecholamine-related genes (eg, catecholamine-O-transferase (COMT) gene) have been associated with striatal processing of rewards (Schmack *et al*, 2008).

DISCLOSURE

SG and GH had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. During this project, Dr Pizzagalli was partially supported by NIMH grants (R01 MH68376, R21 MH078979). This research was supported by the Swiss National Science Foundation (NR 32003B-117763). In the past 3 years, Dr Pizzagalli received research support and consulting fees from Advanced Neuro Technology (ANT) and honoraria or consulting fees from AstraZeneca, Ono Pharma USA, Shire for studies unrelated to the current project. All other authors declare no conflict of interest.

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