



## Catecholamine depletion in first-degree relatives of individuals with mood disorders: An [<sup>18</sup>F]fluorodeoxyglucose positron emission tomography study<sup>☆</sup>



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### ABSTRACT

Catecholamine depletion with alpha-methylparatyrosine (AMPT) has previously been shown to induce depressive symptoms in currently remitted patients with major depressive disorder (MDD) but not healthy controls. Thus sensitivity to catecholamine depletion has been hypothesized to be an endophenotype of MDD. Here we tested this hypothesis in the context of a randomized, double-blinded, placebo-controlled design by measuring changes in mood in a group of psychiatrically-healthy individuals at risk of mood disorders by virtue of family history (high-risk subjects, HRs). In addition, we tested whether HRs differed from healthy controls with no family-history of mood disorders (low-risk controls, LRs) in their cerebral metabolic response when undergoing catecholamine depletion. Eight healthy LRs (6 males, mean age =  $34.1 \pm 7.1$ ) and 6 healthy HRs (3 males, mean age =  $29.3 \pm 4.6$ ) participated in two, 3-day-long identical sessions during which they completed standardized measures of depression, anxiety and fatigue and an [<sup>18</sup>F]fluorodeoxyglucose (FDG) positron emission tomography (PET) scan. On one occasion participants received 4 weight-adjusted doses of AMPT and on the other occasion participants received 4 doses of placebo. The LR and HR groups did not differ from each other in their mood during sham depletion. However, during the period of peak catecholamine depletion, the HR group reported significantly more depression, anxiety and fatigue than the LR group. A region-of-interest analysis showed that during catecholamine depletion versus placebo the combined LR and HR groups displayed a significant increase in cerebral metabolic rate in the left and right ventral striata, left and right amygdalae, and left and right hippocampi (FWE-corrected  $p < 0.05$ ). Whole brain voxel-wise analyses indicated significantly increased glucose metabolism in the left and right putamina (FWE-corrected  $p < 0.05$ ) in the combined LR and HR groups in the AMPT versus the placebo session. In the LR group, alone, no significant elevation in glucose metabolism was observed in the regions-of-interest in the catecholamine depletion versus placebo condition. In the HR group, alone, the region-of-interest analysis showed a significant increase in cerebral metabolic rate in the left and right ventral striata (FWE-corrected  $p < 0.05$ ). No regions-of-interest showed significantly different metabolism in the HR group versus the LR group in the placebo condition, however compared with the LR group, the HR group displayed nominally increased glucose metabolism in the left amygdala during catecholamine depletion (SVC-corrected  $p = 0.05$ ). A region-of-interest analysis for the interaction contrast confirmed that catecholamine depletion had differential effects on HR and LR participants. Compared with the LR group, the HR group displayed significantly increased glucose metabolism in the left ventral striatum, left amygdala, and left lateral orbitofrontal cortex (OFC) (FWE-corrected  $p < 0.05$ ). Our results suggest that sensitivity to catecholamine depletion may be a phenotypic marker of vulnerability to mood disorders that is characterized

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at the neurophysiological level by disinhibition of the striatum and its efferent projections comprising the limbic–cortical–striatal–pallidal–thalamic circuitry.

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## 1. Introduction

We and others have previously shown that currently remitted subjects with major depressive disorder (MDD) demonstrate significant depressive symptoms when undergoing catecholamine depletion with alpha-methylparatyrosine (AMPT), a tyrosine hydroxylase inhibitor which blocks the conversion of tyrosine to dihydroxyphenylalanine (DOPA), the rate-limiting step in the synthesis of catecholamines (Bremner et al., 2003; Hasler et al., 2008). The mood-lowering effect of AMPT in remitted MDD patients is robust with average increases of 21 points on the Hamilton Depression Rating Scale (HDRS) (Berman et al., 1999) and 10 points on the Montgomery–Asberg Depression Rating Scale (MADRS) (Hasler et al., 2008) previously reported. In contrast, never-depressed healthy subjects without a family history of mood disorders in first-degree relatives (low-risk control subjects, LRs) are asymptomatic or show only minor changes in mood when administered with AMPT (Hasler et al., 2008; McCann et al., 1995; Ruhe et al., 2007; Salomon et al., 1997). Given these data, sensitivity to catecholamine depletion has been proposed as an endophenotype for MDD (Berman et al., 1999). Here we conduct the first test of this hypothesis by measuring changes in mood in a group of psychiatrically-healthy individuals at risk of mood disorders by virtue of family history (high-risk subjects, HRs) in the context of a randomized, double-blind, placebo-controlled design.

[<sup>18</sup>F]Fluorodeoxyglucose (FDG) PET has been previously used to evaluate the cerebral metabolic correlates of the effects of AMPT on mood. Bremner et al. (2003) reported that MDD patients who remitted on antidepressant treatment and subsequently relapsed after catecholamine depletion, showed decreased metabolism in the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex and thalamus but increased metabolism in the middle frontal gyrus, hippocampus and amygdala in their baseline scans (i.e. prior to receiving AMPT). More recently, we showed that AMPT administration resulted in increased metabolism of the striatum in both unmedicated, remitted MDD patients and healthy controls, while during catecholamine depletion, metabolism increased in the MDD subjects, but decreased in controls, in a number of regions associated with the medial prefrontal (visceromotor) network such as the ventromedial prefrontal cortex, thalamus, ventral striatum, and infralimbic cortex (Hasler et al., 2008). Here we test whether HRs differ from LRs in their cerebral metabolic response when undergoing catecholamine depletion.

Dopamine projections from the substantia nigra and ventral tegmental area (VTA) to the striata, amygdalae, and prefrontal cortex (PFC) act to inhibit neurotransmission from afferent, glutamatergic neurons synapsing onto the striata, amygdalae, and PFC (e.g. Bunney and Aghajanian, 1976; Goldman-Rakic et al., 1989). Similarly, noradrenergic projections from the locus ceruleus exert a hyperpolarizing effect on hippocampal, thalamic, and PFC neurons, inhibiting the activity of glutamatergic neurons (Samuels and Szabadi, 2008). Because cerebral glucose metabolism is primarily reflective of glutamatergic neurotransmission (Shulman et al., 2004), we therefore hypothesized that catecholamine depletion would disinhibit glutamatergic transmission to the striatum and its efferent projections comprising the limbic–cortical–striatal–pallidal–thalamic circuits via the depletion of dopamine and norepinephrine. Thus due to the combined depletion of dopamine and norepinephrine, we expected to find increased metabolism in the striatum, amygdala, hippocampus, OFC, ventromedial PFC, and the medial thalamus. We additionally hypothesized that this effect would be more pronounced in HR subjects than LR subjects

and that the magnitude of this response would be associated with symptoms of depression, anxiety and fatigue.

## 2. Methods

### 2.1. Participants

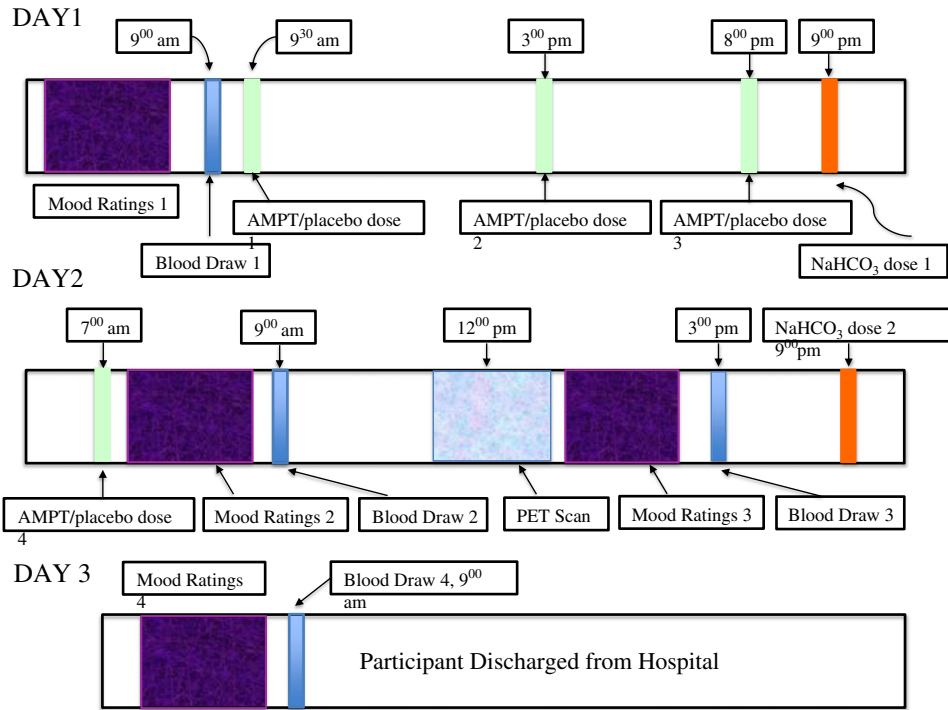
Eight healthy, “low-risk” subjects with no family history of psychiatric illness (LRs, 6 males, mean age =  $34.1 \pm 7.1$ ) and 6 healthy “high-risk” subjects with at least one first-degree relative and/or multiple second relatives with either bipolar disorder or recurrent MDD (HRs, 3 males, mean age =  $29.3 \pm 4.6$ ) participated in the study after giving informed consent as approved by the NIMH IRB. Two HR subjects had a family history of bipolar disorder and 4 subjects had a family history of MDD (see Supplementary data file for more detailed information). Diagnosis was established using the Structured Clinical Interview for the DSM-IV (SCID-IV) (First et al., 1995) and confirmed by an unstructured interview with a psychiatrist. Family history was established using the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992). Subjects were recruited through advertisements in local newspapers and posters placed at the NIH campus, local colleges, fitness centers, and libraries.

The following exclusion criteria applied: inability to provide informed consent, current pregnancy (as documented by pregnancy testing at screening or at days of the challenge studies), age <18 and >45, personal history of psychiatric illness (or family history of psychiatric illness in the case of the LRs), cigarette smoking, use of alcohol (>4 oz/day for men and >3 oz/day for women), lactose intolerance, significant medical or neurological disorders likely to affect physiology or anatomy, (e.g. hypertension, cardiovascular disorders, and diabetes), past head injury with loss of consciousness, general MRI and PET exclusion criteria (e.g. metallic implants, exposure to more than 5 rem of radiation within the past year), abnormalities on the Chem-20, including electrolyte disturbance and anemia, or positive drug, hepatitis or HIV screen.

### 2.2. Experimental design

In a randomized, double-blind, placebo-controlled design, subjects participated in two identical sessions separated by at least one week (in order to avoid carry-over effects) in which they received either AMPT or placebo. Subjects were admitted to the inpatient unit at the NIH Clinical Center for 3 days per session (6 in total) where they received psychological assessments and underwent a PET scan and blood draws to measure serum prolactin levels (Fig. 1). Serum prolactin levels ( $\mu\text{g/L}$ ), which were obtained to confirm catecholamine depletion, were measured using an electrochemiluminescent immunoassay (Boehringer, Mannheim, Germany). The PET images were obtained ~27 h after the first dose of AMPT or placebo corresponding to the time of maximum catecholamine depletion reported in previous studies (Berman et al., 1999; Bremner et al., 1997). Patients were placed on a low-monoamine diet for the duration of their stay in the inpatient unit in order to minimize dietary effects on monoamine levels.

In order to mitigate the risk of adverse reactions such as akathisia, dystonia or postural hypotension, a weight-adjusted AMPT dose of 40 mg/kg up to a maximum of 4 g was administered orally over a 24-hour period (Hasler et al., 2008) (Fig. 1). Adverse effects were reported in some studies in response to doses of AMPT >4 g (de



**Fig. 1.** Flow-chart showing the experimental procedure for a study session. Each participant completed 2 identical sessions, on one occasion receiving AMPT, and on the other occasion receiving placebo. Each participant was given 3 weight-adjusted doses of AMPT/placebo on day 1, and one dose of AMPT/placebo on day 2 so that the period of maximum depletion coincided approximately with the PET scanning session on day 2. Each participant also completed four clinical/behavioral interviews to capture changes in mood and fatigue over the course of the catecholamine depletion procedure. The blue bars represent the blood draws. The green bars represent the times of AMPT or placebo administration. The orange bars show the times at which sodium bicarbonate was administered to the subjects (to prevent crystalluria). The purple blocks represent the behavioral/clinical assessment sessions, and the mottled blue block represents the PET scanning session.

Haan et al., 2005), however, no serious adverse effects occurred in our participants. To minimize the risk of crystaluria during AMPT administration, subjects received 650 mg sodium bicarbonate on evenings one and two (Fig. 1), and drank at least 2 L of water daily. In addition, urine analysis was performed twice daily to screen for the presence of crystals.

**2.3. Clinical ratings**

Both clinician-administered and self-rating scales were administered to the participants. The 24-item version of the Hamilton Rating Scale for Depression (HDRS) (Hamilton, 1960) and the MADRS (Montgomery and Asberg, 1979) were administered by raters (research nurses, 2 physicians, and a clinical psychologist), who trained together to establish interrater reliability with an intra-class correlation coefficient of ~0.8. Self-rating scales, which were used to evaluate changes in mood, anxiety, and drowsiness, consisted of the Beck Depression Inventory (BDI) (Beck and Steer, 1993), Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970), the Profile of Mood States (POMS) (Pollock et al., 1979), the Visual Analog Scales (VAS) (Stern, 1997), and the Stanford Sleepiness Scale (SSS) (Hoddes et al., 1972).

**2.4. Clinical data analysis**

Differences in behavioral ratings between the groups and across the placebo and AMPT sessions were assessed using repeated measures ANOVA. We were primarily interested in the interaction between group (HR versus LR), session (AMPT versus placebo) and time (0 h, 24 h, 30 h, and 48 h post initial dose of drug/placebo). In the case of a significant interaction, post-hoc *t*-tests were used to perform specific contrasts. In order to confirm the results of the repeated measures ANOVA, we also analyzed the data with the nonparametric Mann-Whitney test with a  $\chi^2$  approximation. Because we hypothesized a

priori that AMPT would induce greater mood symptoms in the HR group, and that the behavioral ratings obtained 24 h and 30 h post initial dose of AMPT would be most sensitive to catecholamine depletion, all *p*-values obtained from *t*-tests and  $\chi^2$  tests are one-tailed, with the significance threshold set at  $p < 0.05$ .

Correlations between normalized glucose metabolism at the peak voxel coordinates representing the left and right striata and the clinical measures described above were measured using the nonparametric Spearman's Rho test. All correlation analyses were conducted on the data derived from the catecholamine depletion condition only.

**2.5. Imaging**

Regional cerebral glucose metabolism was measured with a High-Resolution-Research Tomograph PET scanner (HRRT, Siemens ECAT: 207, 1.22 mm slices, 3D resolution ~2.5 mm<sup>3</sup>). Ten mCi of <sup>18</sup>F<sup>18</sup>FDG was administered by i.v. slow bolus injection (over 2 min) using a Harvard pump. In order to quantitatively model the cerebral glucose utilization non-invasively, an initial emission scan was acquired over the heart on a whole body GE Advance Scanner (GE Medical Systems, Waukesha, WI) during the initial 35 min period following <sup>18</sup>F<sup>18</sup>FDG administration (Moore et al., 2003). In addition, a transmission scan with <sup>68</sup>Ge was acquired on the GE Advance Scanner for attenuation correction of the cardiac emission scan during the tracer uptake period.

After completion of the cardiac emission scan the participant was subsequently moved to the HRRT scanner. Using the HRRT, a 20 min emission scan was acquired over the brain as subjects rested with eyes-closed, beginning 45 min after the <sup>18</sup>F<sup>18</sup>FDG administration. Following the emission scan, a transmission scan of the head was obtained using <sup>137</sup>Cs for attenuation correction of the cerebral emission scan.

Whole brain structural MRI scans with a spatial resolution of ~1 mm<sup>3</sup> were acquired separately using a 3 T scanner (Signa, GE Medical Systems) and T1-weighted magnetization prepared rapidly

acquired gradient echo (MPRAGE) pulse sequence in order to facilitate localization and co-registration of the functional PET data.

## 2.6. Imaging data analysis

We were primarily interested in answering 2 questions. Firstly, how does regional cerebral glucose metabolism differ between drug and placebo sessions across all subjects, and secondly, does catecholamine depletion have a differential impact on regional cerebral glucose metabolism in the LR and HR groups? We tested these hypotheses with repeated measures ANOVA using Statistical Parametric Mapping software (SPM5; Wellcome Department of Imaging Neuroscience, London, England). The PET images were co-registered with the MRI images and spatially normalized to the Montreal Neurological Institute (MNI) T1 template. Images were smoothed using a 10 mm Gaussian kernel to compensate for individual differences in neuroanatomy and misalignment errors in spatial normalization. Note that one LR individual was omitted from the PET analysis due to a normalization failure.

Regional metabolism was compared across groups and conditions in predefined regions-of-interest in the ventral striatum, amygdala, hippocampus, mediodorsal thalamus, OFC, ventromedial prefrontal cortex, and subgenual anterior cingulate cortex (sgACC). The ROIs were selected on the basis of our previous report of changes in regional cerebral glucose metabolism during catecholamine depletion (Hasler et al., 2008) as well as the model of Drevets et al. (1992), which in turn was based on the study of Swerdlow and Koob that discussed the effects of dopamine depletion on limbic-cortical-striatal-pallidal-thalamic neurocircuitry (Swerdlow and Koob, 1987). A sphere of 8 mm radius (4 mm in the case of the relatively smaller amygdala) was defined around the spatial coordinate that best represented the stereotaxic center of each ROI (these coordinate sets are listed in the legend for Table 2). The spatial coordinates were selected on the basis of the neuroanatomical atlases of Mai et al. (2004) and Talairach and Tournoux (1988) except in the case of the ventral striatum where we followed the methodology of Nusslock et al. (2012) and the ventromedial frontal polar cortex where we used the locus where Hasler et al. (2008) found a significant positive correlation between the change in metabolism and the severity of depression under AMPT challenge in their sample (Tables 2 and 3). Using the small volume correction (SVC) option within SPM5, each ROI was searched for voxel *t*-values corresponding to  $p_{\text{corrected}} < 0.05$  using the family-wise error (FWE) correction for multiple testing. A cluster of at least 10 contiguous voxels that yielded a corrected *p*-value  $< 0.05$  was considered to be statistically significant.

Whole brain voxel-wise analyses were conducted post-hoc in order to explore whether metabolic changes occurred in other brain regions. To assess the presence of other metabolic differences the significance threshold was set at a corrected *p*-value  $< 0.05$  after applying the false discovery rate (FDR) error correction across the entire brain. In addition, this image was evaluated to ensure that the peak voxel *t*-values identified in the ROI analyses actually were encompassed within the target ROI (albeit at lower significance thresholds).

We were unable to accurately calculate the input function necessary to model regional cerebral glucose utilization for 6 of the 28 scans because clotting in the intravenous line prohibited some blood draws needed to complete the input function. Consequently, all the data were analyzed using the tissue radioactivity concentrations, which were globally normalized by the whole brain  $^{18}\text{F}$ FDG uptake.

## 3. Results

### 3.1. Prolactin measurements

Both LR and HC groups showed a significant increase in prolactin levels indicative of catecholamine depletion 24–48 h after administration of the first dose of AMPT (Fig. 2,  $F = 25.2$ ,  $p < 0.001$ ). Neither the basal prolactin levels nor the change in prolactin levels under AMPT

differed between the groups during either the placebo or AMPT sessions ( $F = 0.832$ ,  $p = 0.371$ ). Fig. 2 indicates the time points at which prolactin levels are significantly elevated in the AMPT session relative to the placebo session.

### 3.2. AMPT-associated changes in mood

Relative to the LR group, the HR group showed significant elevations in self-reported depression, anxiety and fatigue 24 h (time 2) and 30 h (time 3) after the first dose of AMPT (Tables 1 and 2, Figs. 3–11). With the exception of the MADRS (Fig. 7), the LR and HR groups did not differ from each other during sham depletion. The red stars on the figures indicate a statistically significant difference between the LR and HR groups for the relevant time point according to the Mann–Whitney test.

### 3.3. FDG PET data analyses

#### 3.3.1. Region of interest analyses

**3.3.1.1. Effect of AMPT versus placebo in the combined sample.** Consistent with our hypothesis, the combined HR and LR samples showed significant (FWE-corrected) increases in normalized glucose utilization in the right ventral striatum, the left and right amygdalae, and the left and right hippocampi (Table 3, Fig. 12).

**3.3.1.2. Differential effects of AMPT versus placebo across groups.** In the placebo condition, no significant difference in the normalized (regional-to-global) metabolism was observed in the LR group compared with the HR group. Compared with the LR participants, the HR participants displayed a nominally significant elevation in regional-to-global metabolism in the left amygdala (Table 4) during catecholamine depletion.

In the LR group, alone, no significant elevation in glucose metabolism was observed in the catecholamine depletion versus placebo condition. In the HR group, alone, there was a significant increase in metabolism in the left and right ventral striata (FWE-corrected  $p < 0.05$ ) in the catecholamine depletion versus placebo condition (Table 5).

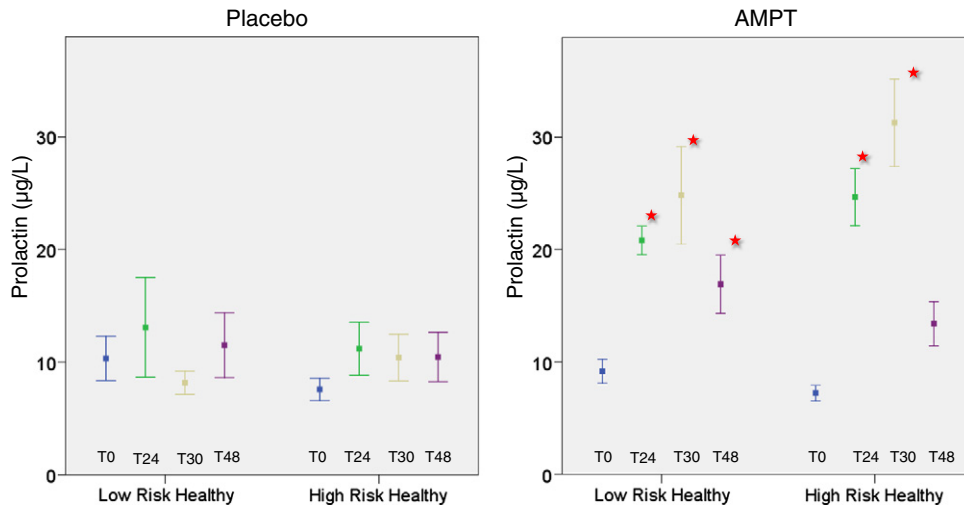
Compared with the LR participants, the HR participants displayed significantly elevated regional-to-global metabolism in the left striatum, left amygdala, and left OFC in the catecholamine depletion versus placebo condition (interaction contrast, Table 6, Fig. 12).

#### 3.3.2. Post-hoc whole brain voxel-wise analyses

**3.3.2.1. Effect of AMPT versus placebo in the combined HR and LR samples.** During catecholamine depletion, a significant increase in metabolism was found in the bilateral ventral striatum, with the peak voxel *t*-value localizing specifically to the left anteroventral putamen (cluster size = 137, FWE-corrected  $p = 0.028$ ) and the right anteroventral putamen (cluster size = 328, FWE corrected  $p = 0.038$ , Table 7, Fig. 13). No brain regions showed a significant decrease in metabolism in the AMPT versus the placebo contrast after applying the conservative FWE correction for multiple comparisons across the whole brain.

**3.3.2.2. Differential effects of AMPT versus placebo across groups.** In the LR group, alone, no significant difference in glucose metabolism was observed in the catecholamine depletion versus placebo condition after applying the FWE-correction for multiple comparisons. Similarly, in the HR group, alone, no significant difference in glucose metabolism was observed in the catecholamine depletion versus placebo condition. No brain regions showed a significant difference between the HR and LR groups after applying the FWE-correction for multiple comparisons in either the placebo condition or the AMPT condition. No brain region showed a significantly different AMPT-induced change





**Fig. 2.** This figure shows the mean plasma prolactin levels in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline measure prior to administration of AMPT/placebo (blue color), T24 represents the prolactin levels of 24 h post administration of AMPT/placebo (green color), T30 represents the prolactin levels of 30 h post administration of AMPT/placebo (gold color), and T48 represents the prolactin levels of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show the time points at which the prolactin levels in each subject group differed (Mann–Whitney test,  $p < 0.05$ ) between the placebo and AMPT conditions. Dopamine neurons project from the arcuate nucleus of the hypothalamus to the pituitary gland where they exert an inhibitory effect on prolactin secretion. Thus an increase in prolactin levels is expected during catecholamine depletion.

in metabolism between groups after applying the FWE correction for multiple comparisons (interaction contrast).

**3.4. Correlations between striatal cerebral glucose metabolism and behavioral ratings**

Within the combined LR and HR groups there were no significant correlations between the normalized cerebral glucose metabolism under catecholamine depletion in the left and right ventral striata and the clinical measures of mood, anxiety and fatigue (Table 8). In the HR group, alone, however, the normalized metabolism of the right striatum

correlated positively with scores on the POMS tension factor ( $r = 0.98$ ,  $p = 0.002$ ), the POMS fatigue factor ( $r = 1$ ,  $p < 0.001$ ), and the Stanford Sleepiness Scale scores ( $r = 0.82$ ,  $p = 0.044$ ).

**4. Discussion**

The previously observed depressive and anhedonic responses precipitated by catecholamine depletion in remitted patients with MDD have raised the possibility that dysfunction of the dopaminergic/noradrenergic system constitutes a potentially latent characteristic of MDD. Following this line of reasoning, we hypothesized that disrupting central

**Table 1**  
Means and standard deviations of prolactin levels and scores on the psychometric rating scales across AMPT and placebo sessions.

Scale	N	Placebo				AMPT			
		LR				LR			
		T0	T24	T30	T48	T0	T24	T30	T48
Prolactin (µg/L)	8	9.4 ± 4.7	12.0 ± 9.6	7.4 ± 3.0	10.3 ± 6.6	8.9 ± 2.6	18.7 ± 5.4	22.3 ± 10.9	15.8 ± 6.5
	6	7.8 ± 2.4	11.2 ± 5.8	10.4 ± 5.1	10.5 ± 5.4	7.2 ± 1.7	24.7 ± 6.2	31.3 ± 9.5	13.4 ± 4.8
BDI	5	0.5 ± 1.0	0.5 ± 1.0	0 ± 0	0.2 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	5	1.0 ± 1.3	0.5 ± 0.8	0.7 ± 1.0	0.3 ± 0.8	1.2 ± 1.9	10.8 ± 9.4	7.1 ± 9.5	1.8 ± 4.5
POMS depression	7	0.3 ± 0.8	0.4 ± 1.1	0.6 ± 1.1	0.4 ± 1.1	0 ± 0	0.4 ± 0.9	0 ± 0	0 ± 0
	6	0.7 ± 1.6	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.8	1.6 ± 1.8	5.6 ± 6.3	4.6 ± 6.6	0.6 ± 1.3
VAS sadness	7	0.5 ± 0.8	0.1 ± 0.2	0.1 ± 0.4	0.3 ± 0.5	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.3
	6	0.3 ± 0.8	0.3 ± 0.8	0.3 ± 0.8	0.3 ± 0.8	0.5 ± 0.6	2.4 ± 2.3	2.0 ± 1.4	1.0 ± 1.2
HAM-D	8	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.4	0 ± 0	0 ± 0	3.5 ± 4.7	1.3 ± 2.2	1.5 ± 3.7
	6	1 ± 1.3	1.2 ± 1.8	1.3 ± 2.0	0.3 ± 0.8	0.2 ± 0.4	7.2 ± 5.4	4.8 ± 4.9	1.8 ± 4.0
MADRS	8	0.3 ± 0.7	0.1 ± 0.4	0 ± 0	0.1 ± 0.3	0 ± 0	4.8 ± 7.1	1.7 ± 3.2	1.7 ± 4.1
	6	2.2 ± 2.6	1.8 ± 1.2	1.2 ± 0.4	0.3 ± 0.5	1.2 ± 1.5	8.8 ± 8.3	7.8 ± 8.4	2.8 ± 5.1
STAI (State)	7	22.6 ± 3.4	25.0 ± 5.3	24.3 ± 5.4	24.4 ± 4.4	23.0 ± 5.7	24.4 ± 7.1	23.0 ± 6.2	23.3 ± 4.0
	6	24.5 ± 5.0	25.3 ± 7.5	24.2 ± 6.9	24.3 ± 7.3	27.4 ± 8.4	42.6 ± 12.0	36.8 ± 8.2	26.0 ± 7.4
POMS tension	7	0.7 ± 1.5	1.1 ± 1.9	0.6 ± 1.1	0.7 ± 1.5	0 ± 0	0.8 ± 1.3	0.2 ± 0.4	0 ± 0
	6	1.3 ± 1.0	0.3 ± 0.8	0.3 ± 0.8	1.0 ± 1.3	3.6 ± 3.0	8.8 ± 7.5	5.2 ± 6.7	0.4 ± 0.5
POMS fatigue	7	1.5 ± 2.4	1.6 ± 2.4	1.3 ± 2.4	1.1 ± 0.4	3.4 ± 5.5	3.0 ± 3.7	1.0 ± 1.7	0.3 ± 0.5
	6	2.7 ± 4.7	1.7 ± 2.7	0.5 ± 0.5	1.2 ± 1.8	1.4 ± 0.9	14.2 ± 2.0	10.6 ± 7.7	1.8 ± 1.8
Sleepiness (SSS)	7	1.7 ± 0.8	1.7 ± 1.0	1.3 ± 0.5	1.4 ± 0.5	2.0 ± 1.0	2.6 ± 0.5	1.8 ± 0.4	1.0 ± 0
	6	1.7 ± 0.8	1.7 ± 0.8	1.3 ± 0.5	1.5 ± 0.5	1.5 ± 1.0	5.2 ± 1.9	3.6 ± 1.1	1.4 ± 0.5

Note: The sample size varied slightly across questionnaires because not all subjects completed the full battery of scales across all 8 time points. T0, T24, T30, and T48 = 0 h, 24 h, 30 h, and 48 h post first dose of AMPT/placebo, respectively.

Abbreviations: BDI = Beck Depression Inventory; POMS = Profile of Mood States; VAS = Visual Analog Scale; HAM-D = Hamilton Depression Rating Scale; MADRS = Montgomery–Asberg Depression Rating Scale; STAI (State) = The state subscale of the State-Trait Anxiety Scale; Sleepiness (SSS) = Stanford Sleepiness Scale.

**Table 2**  
Statistical analysis of AMPT-associated changes in mood, anxiety, and fatigue in low-risk versus high-risk participants.

Scale	Repeated measures ANOVA DX * session * time	Post-hoc <i>t</i> -test (significant results)	Mann–Whitney with $X^2$ approximation (significant results)	Interpretation
BDI	F = 2.4, p = 0.015	P = 0.029 –	$X^2 = 4.4$ , p = 0.036 $X^2 = 4.2$ , p = 0.040	During AMPT session, HR > LR at times: 2 3
POMS depression	F = 2.8, p = 0.050	P = 0.042 P = 0.054 –	– $X^2 = 4.1$ , p = 0.044 $X^2 = 3.7$ , p = 0.054	During AMPT, HR > LR at times: 1 2 3
VAS sadness	F = 2.2, p = 0.010	P = 0.029 P = 0.009	$X^2 = 4.5$ , p = 0.034 $X^2 = 4.6$ , p = 0.032	During AMPT, HR > LR at times: 2 3
HAM-D	F = 1.2 p = 0.322	–	$X^2 = 2.8$ , p = 0.097	During AMPT, a trend for HR > LR at time 3
MADRS	F = 1.0, p = 0.394	– –	$X^2 = 3.6$ , p = 0.058 $X^2 = 3.8$ , p = 0.050	During AMPT, HR > LR at times: 1 3
		– –	$X^2 = 4.1$ , p = 0.042 $X^2 = 9.9$ , p = 0.002 $X^2 = 6.7$ , p = 0.010	During placebo, HR > LR at times: 1 2 3
STAI (State)	F = 3.7, p = 0.016	P = 0.010 P = 0.009	$X^2 = 4.9$ , p = 0.029 $X^2 = 4.5$ , p = 0.034	During AMPT, HR > LR at times: 2 3
POMS tension	F = 3.0, p = 0.039	P = 0.015 P = 0.023	$X^2 = 5.5$ , p = 0.019 $X^2 = 7.0$ , p = 0.008	During AMPT, HR > LR at times: 1 2
POMS fatigue	F = 8.9, p < 0.001	P < 0.001 P = 0.013	$X^2 = 7.0$ , p = 0.008 $X^2 = 5.9$ , p = 0.015	During AMPT, HR > LR at times: 2 3
Sleepiness (SSS)	F = 5.6, p = 0.002	P = 0.010 P = 0.006	$X^2 = 5.7$ , p = 0.017 $X^2 = 5.5$ , p = 0.019	During AMPT, HR > LR at times: 2 3

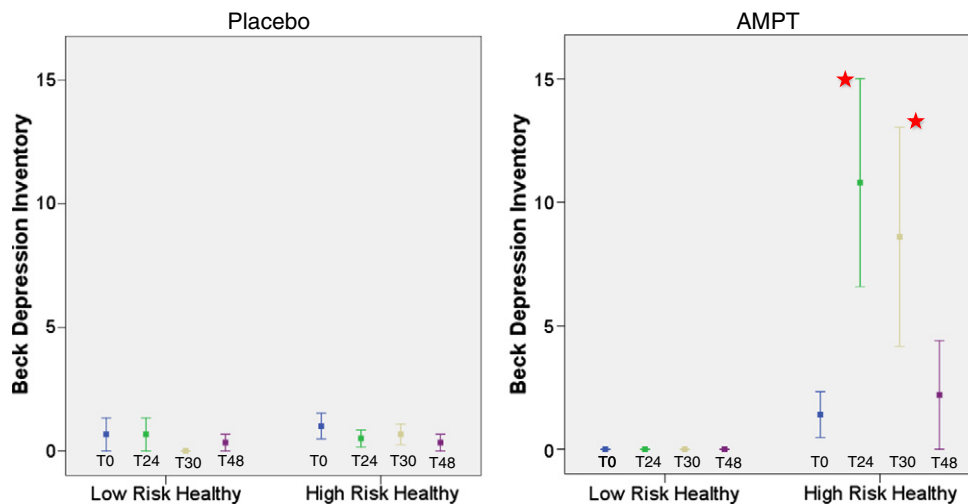
Note: p-values are from one-tailed statistical tests. DX = diagnosis; Session = AMPT/Placebo; Time = time points within a session – 0 h, 24 h, 30 h, and 48 h post first dose of AMPT/placebo, where 0 = 1, 24 = 2, 30 = 3, and 48 = 4.

Abbreviations: BDI = Beck Depression Inventory; POMS = Profile of Mood States; VAS = Visual Analog Scale; HAM-D = Hamilton Depression Rating Scale; MADRS = Montgomery–Asberg Depression Rating Scale; STAI (State) = The state subscale of the State-Trait Anxiety Scale; Sleepiness (SSS) = Stanford Sleepiness Scale.

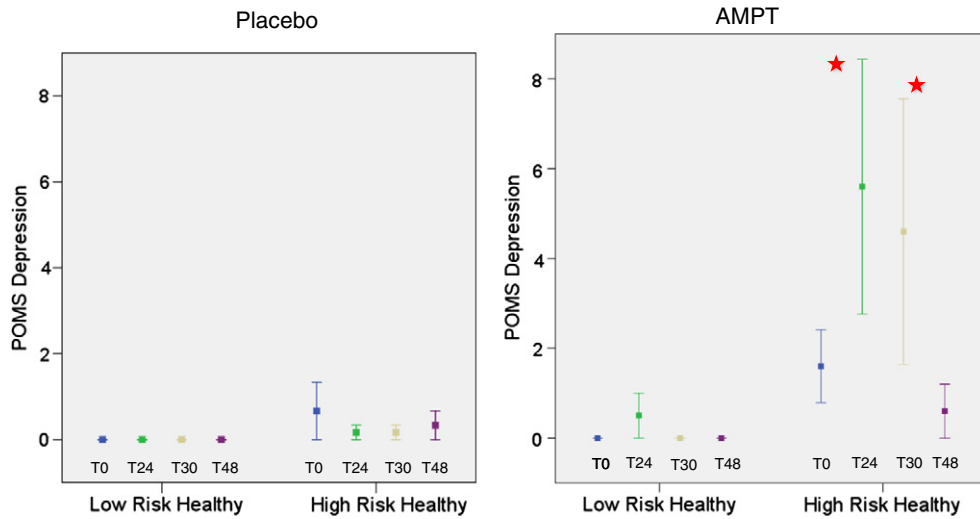
catecholamine function by administration of AMPT would induce depressive symptoms along with changes in regional cerebral glucose metabolism in a subset of unaffected relatives who are at genetic risk for developing a mood disorder by virtue of their family history.

As hypothesized, compared with LR participants, HR participants showed significant increases in symptoms of depression, anxiety and

fatigue during catecholamine depletion. Because activation of the locus ceruleus is closely correlated with level of arousal and wakefulness (Samuels and Szabadi, 2008), the increased levels of fatigue and sleepiness observed in the HR group during the AMPT condition suggest that people with a genetic predisposition to mood disorders may conceivably be more sensitive to perturbations in the level of intrasynaptic



**Fig. 3.** This figure shows the change in Beck Depression Inventory scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the BDI scores of 24 h post administration of AMPT/placebo (green color), T30 represents the BDI scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the BDI scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the BDI scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann–Whitney test,  $p < 0.05$ ).

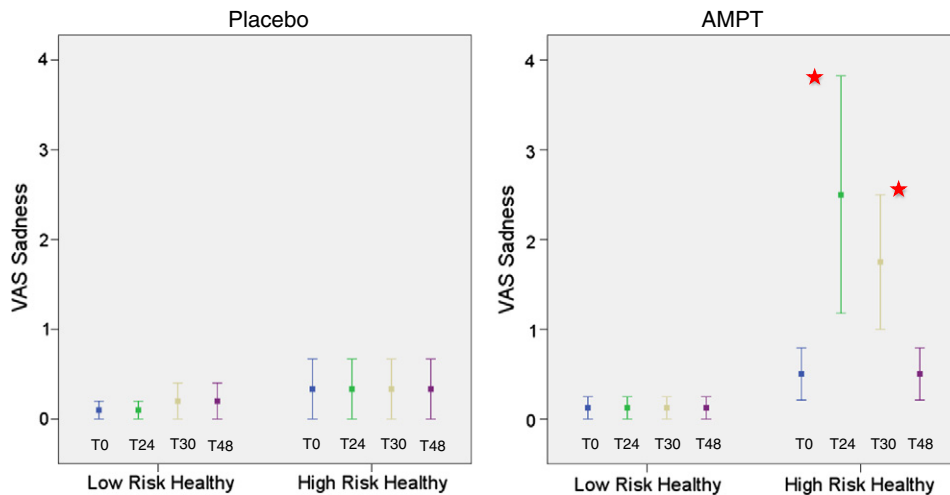


**Fig. 4.** This figure shows the change in the Profile of Mood States (POMS) depression scale scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the POMS depression scores of 24 h post administration of AMPT/placebo (green color), T30 represents the POMS depression scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the POMS depression scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the POMS depression scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann–Whitney test,  $p < 0.05$ ).

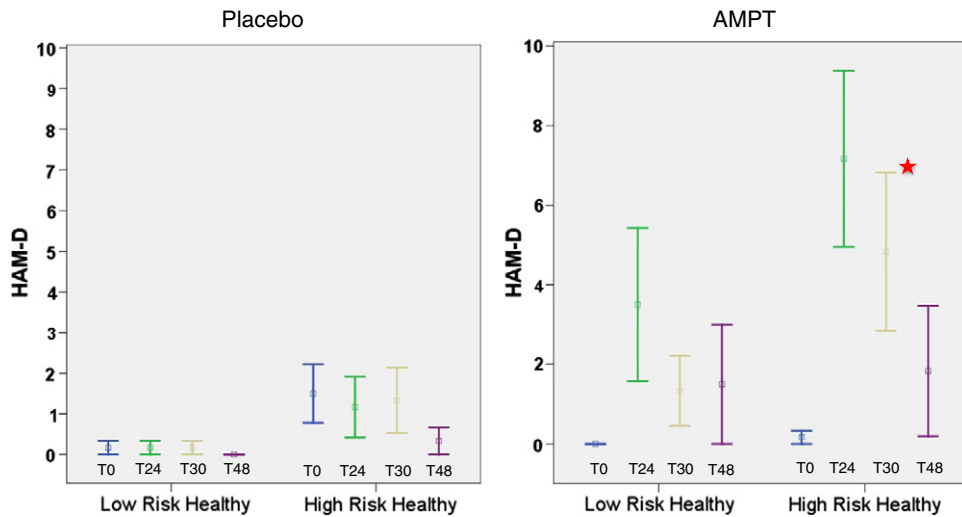
and/or extrasynaptic norepinephrine. Potentially consistent with this hypothesis, norepinephrine reuptake inhibitors such as reboxetine are sometimes used as antidepressant medications and may also be used to treat depression with high levels of fatigue (Bould et al., 2012). The AMPT-associated increase in fatigue, anxiety, and depression is also potentially consistent with reports of decreased expression of norepinephrine transporters in the locus ceruleus of patients with a history of depression (Klimek et al., 1997). The data are also potentially consistent with increased levels of tyrosine hydroxylase in the locus ceruleus of suicide cases (Ordway et al., 1994; Zhu et al., 1999), increased mRNA expression of  $\alpha_2$ -adrenoceptors in the frontal cortex of patients who had committed suicide (Valdizan et al., 2010), and increased binding of an  $\alpha_2$ -adrenoceptor agonist in the locus ceruleus of patients with a history of depression at *postmortem* (Ordway et al., 2003). Conceivably, the decreased expression of norepinephrine transporters together with

the increased expression of  $\alpha_2$ -adrenoceptors reflects a compensatory response to decreased levels of norepinephrine. In support of this hypothesis, depletion of norepinephrine has been shown to upregulate  $\alpha_2$ -adrenoceptors in rodents.

Changes in the expression and/or sensitivity of dopamine receptors have also been found in patients with a history of depression at *post-mortem* (Savitz and Drevets, 2012). For example, the percentage of D1-expressing neurons as well as D1 mRNA expression was increased by 25% in the CA3 region of the hippocampus in patients with BD (Pantazopoulos et al., 2004) and increased D2 receptor binding was found in the amygdalae of depressed patients (Klimek et al., 2002). In addition, two PET studies have reported a reduction in the binding potential of the dopamine transporter in MDD (Meyer et al., 2001) and BD (Anand et al., 2011), respectively. We previously hypothesized that one interpretation of the decrease in dopamine transporter binding



**Fig. 5.** This figure shows the change in the Visual Analog Scale (VAS) sadness scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the VAS sadness scores of 24 h post administration of AMPT/placebo (green color), T30 represents the VAS sadness scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the VAS sadness scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the VAS scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann–Whitney test,  $p < 0.05$ ).

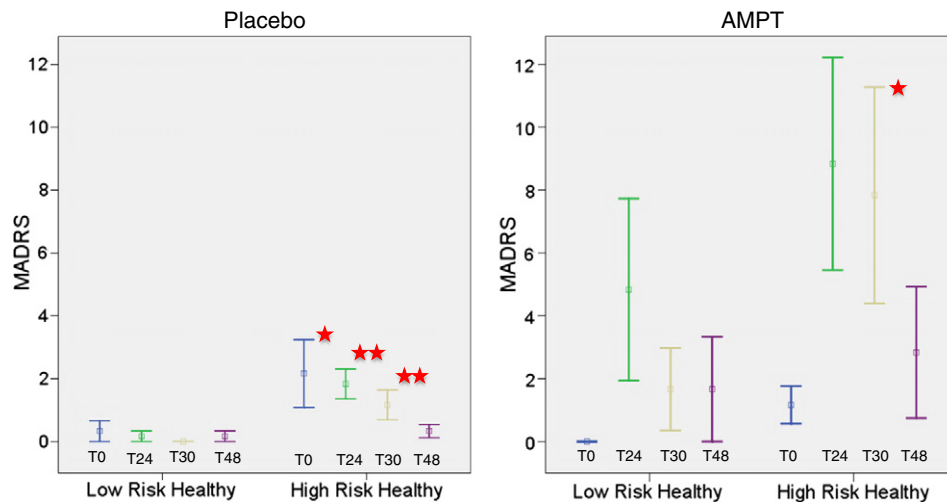


**Fig. 6.** This figure shows the change in the Hamilton Depression scale (HAM-D) scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the HAM-D scores of 24 h post administration of AMPT/placebo (green color), T30 represents the HAM-D scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the HAM-D scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red star indicates that the HAM-D scores at T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann–Whitney test,  $p < 0.05$ ).

potential is that it reflects a compensatory response to decreased dopaminergic signaling in the ventral striatum (Savitz and Drevets, 2012). In support of this hypothesis, dopamine transporter density decreases after chronic dopamine depletion (Gordon et al., 1996), suggesting that at least some types of depression may be associated with low basal levels of intrasynaptic dopamine (Savitz and Drevets, 2012). Consistent with these data, both currently depressed and remitted MDD subjects have markedly decreased levels of the dopamine catabolite, homovanillic acid (HVA) in the cerebrospinal fluid (Asberg et al., 1984; Kaddurah-Daouk, 2012). Our results raise the possibility that some HR subjects also have low basal levels of intrasynaptic dopamine and norepinephrine rendering them sensitive to the mood-lowering effects of AMPT, which putatively induces reductions in plasma MHPG and HVA in healthy controls (Salomon et al., 1997), patients with MDD (Berman et al., 1999; Miller et al., 1996), and patients with

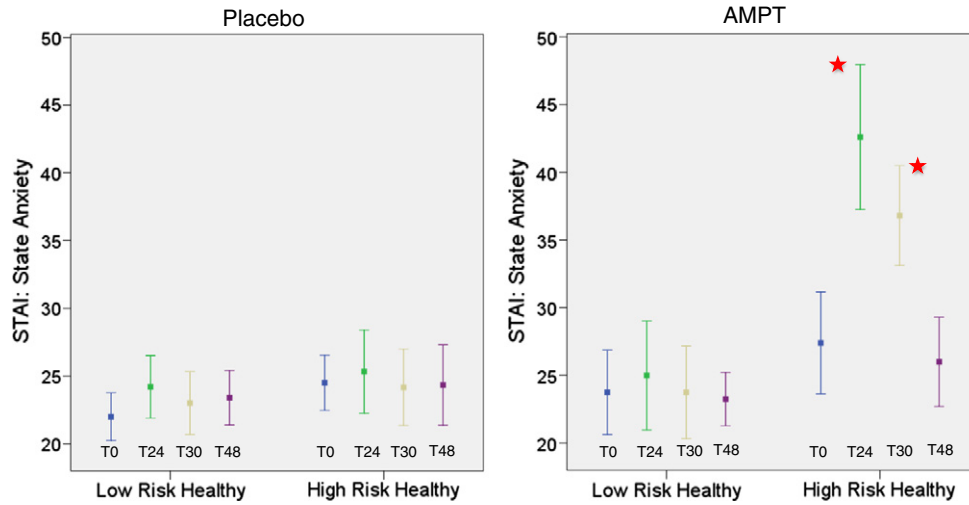
seasonal affective disorder (Lam et al., 2001). However, because we did not obtain measures of central dopamine or norepinephrine function, our study design does not allow us to definitely address this hypothesis.

Consistent with a previous study (Hasler et al., 2008) there was no main effect of diagnosis (HR versus LR) and no diagnosis by treatment interaction on serum prolactin levels, an arguably counterintuitive finding if a depressive diathesis is indeed associated with lower basal levels of dopamine. One possibility is that the putative reduction in dopaminergic signaling is specific to particular dopaminergic pathways (e.g. the mesolimbic dopaminergic pathway); i.e. MDD and HR subjects may not differ from LR subjects in terms of dopaminergic signaling within the tuberoinfundibular dopaminergic pathway. There is a precedent for pathway-specific abnormalities in dopamine function in the psychiatric literature. Schizophrenia has been hypothesized to



**Fig. 7.** This figure shows the change in the Montgomery–Asberg Depression Rating Scale (MADRS) scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the MADRS scores of 24 h post administration of AMPT/placebo (green color), T30 represents the MADRS scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the MADRS scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars indicate that during the placebo condition, the HR group had significantly higher MADRS scores than the LR group at T0, T24, and T30. In the AMPT condition, the HR group had higher MADRS scores than the LR group at T30. Two red stars:  $p < 0.01$ , one red star  $p < 0.05$ . Refer to Table 2 for more detail.





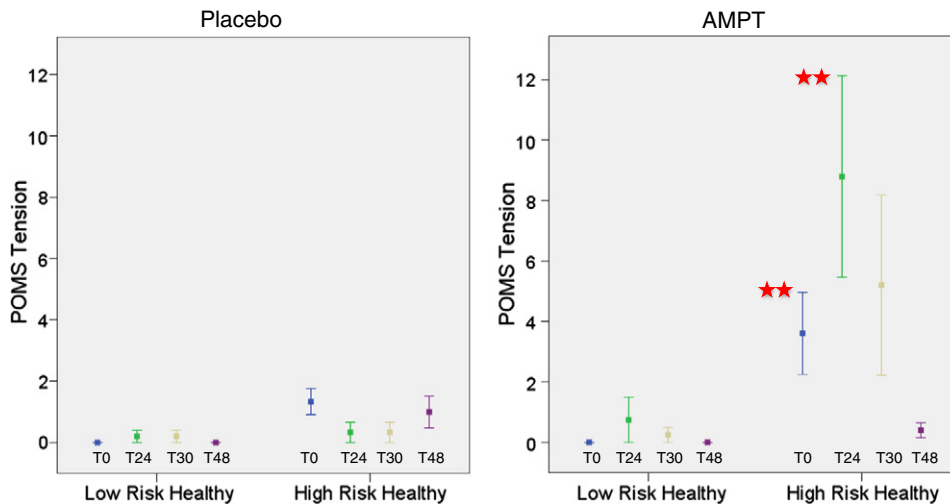
**Fig. 8.** This figure shows the change in the State subscale of the State-Trait Anxiety Inventory (STAI) scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the STAI scores of 24 h post administration of AMPT/placebo (green color), T30 represents the STAI scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the STAI scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the VAS scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann-Whitney test,  $p < 0.05$ ).

be characterized by a hyperactive mesolimbic dopaminergic pathway, resulting in stimulation of D2 receptors and positive disease symptoms, but a hypoactive mesocortical dopaminergic pathway, resulting in negative disease symptoms (Abi-Dargham and Moore, 2003; Weinberger, 1987).

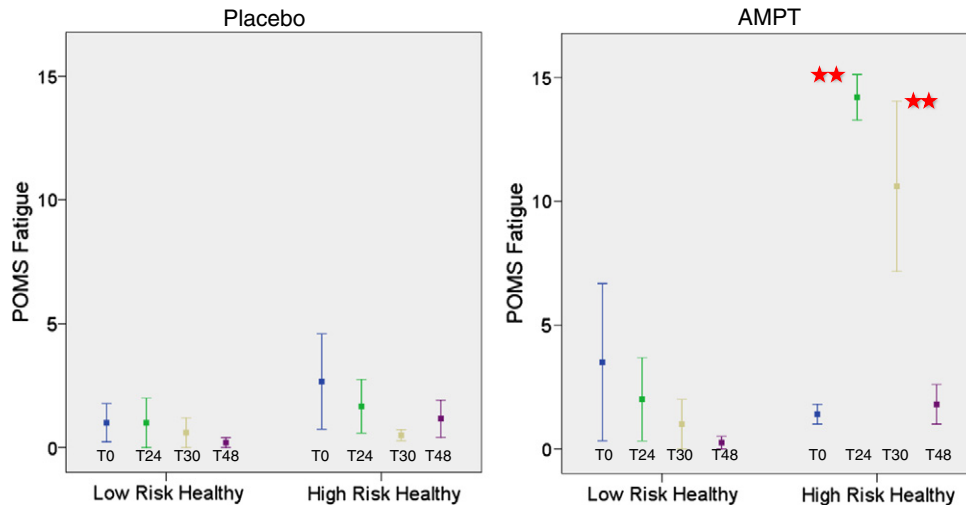
We hypothesized above that catecholamine depletion would disinhibit the limbic-cortical-striatal neural circuits previously implicated in depression (Savitz and Drevets, 2009a, 2009b) resulting in an increase in cerebral metabolic rate in these structures. Shulman et al. (2004) reviewed the magnetic resonance spectroscopy literature and showed that the activity of glutamatergic neurons consumes about 85% of the total energy consumed by the brain. Because dopamine projections from the substantia nigra and ventral tegmental area synapse onto afferent glutamatergic projections of the striatum, amygdala, and PFC and inhibit neuronal activity, depletion of dopamine would be expected to disinhibit the efferent projections of the

striatum, amygdala, and PFC; an effect that has previously been demonstrated (Bunney and Aghajanian, 1976; Goldman-Rakic et al., 1989; Kerkerian et al., 1987; Wooten and Collins, 1981). Nevertheless, we note that the signal derived from  $^{18}\text{F}$ -FDG may also be reflective of activity in other neuronal circuits and should not be regarded as mechanistically selective.

Given the extensive interaction between the dopaminergic, noradrenergic and serotonergic systems, the increase in anxiety and depressive symptoms in the HR group with AMPT depletion is potentially consistent with a number of studies that assessed the effect of serotonin depletion on mood in healthy individuals with a family history of mood disorders (defined as at least one first degree relative with depression). In one study, 6 out of 20 healthy males have a family-history of MDD or bipolar disorder but 0 out of 19 male controls displayed a lowering of mood in response to tryptophan depletion (defined as an increase of  $\geq 10$  points on the POMS depression scale) (Benkelfat et al., 1994).



**Fig. 9.** This figure shows the change in the POMS tension scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the POMS tension scores of 24 h post administration of AMPT/placebo (green color), T30 represents the POMS tension scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the POMS tension scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the POMS tension scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann-Whitney test,  $p < 0.01$ ).



**Fig. 10.** This figure shows the change in the POMS fatigue scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline score prior to administration of AMPT/placebo (blue color), T24 represents the POMS fatigue scores of 24 h post administration of AMPT/placebo (green color), T30 represents the POMS fatigue scores 30 h of post administration of AMPT/placebo (gold color), and T48 represents the POMS fatigue scores 48 h of post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the POMS fatigue scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann–Whitney test,  $p < 0.01$ ).

Similarly, 8 out of 16 subjects with a positive family history of bipolar or unipolar depression showed a tryptophan depletion-associated increase in depressive symptoms (defined by at least 1 point increase on the POMS depression scale) compared with one out of 11 subjects without a family history of depression (Klaassen et al., 1999). Healthy individuals with a family-history of bipolar disorder also showed a modest decline in mood compared with low-risk healthy controls during tryptophan depletion (Quintin et al., 2001). More recently, van der Veen et al. (2007) reported a greater lowering of mood concomitant with a stronger amygdala response to fearful faces in healthy individuals with a family history of unipolar depression compared with healthy controls without a family history of depression during tryptophan depletion.

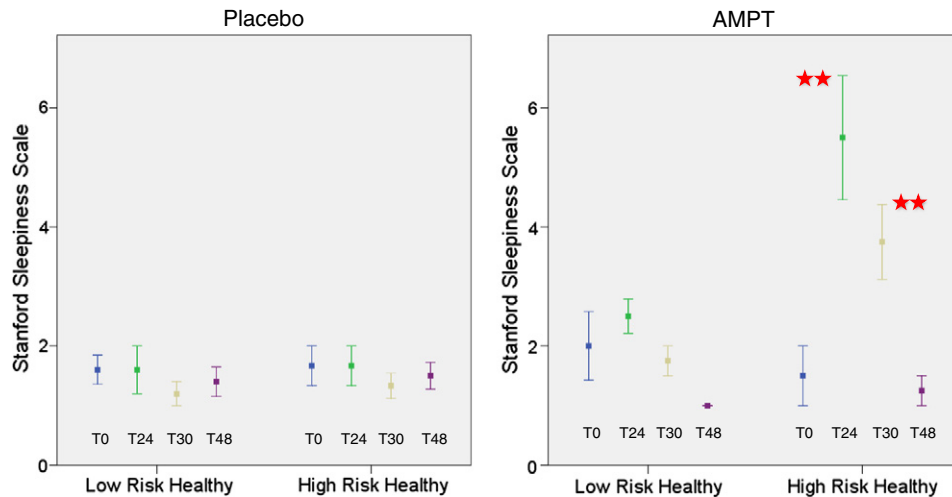
The variable mood response to AMPT in the high-risk group (Figs. 3–11) suggests that the vulnerability for developing anxiety, depression, and/or fatigue in response to catecholamine depletion is

either absent in some HR subjects (since some subjects may actually be resilient given that they have remained healthy) or that dysregulation of the catecholaminergic system is only one of many pathophysiological mechanisms underpinning the development of mood disorders.

## 5. PET imaging

An additional aim of the study was to examine the neurophysiological correlates of catecholamine depletion across the entire sample, and to test whether HR subjects show differences in cerebral glucose utilization compared with LR subjects when subjected to catecholamine depletion. Our most salient results were as follows.

A significant increase was found in regional cerebral glucose metabolism in the ventral striatum, bilaterally, during catecholamine depletion in the combined HR and LR groups (Tables 3 and 5, Figs. 12a, 13).



**Fig. 11.** This figure shows the change in the Stanford Sleepiness Scale (SSS) scores in both the LR and HR groups across the placebo (figure to the left) and AMPT sessions (figure to the right) for all 4 session time points. That is, T0, T24, T30, and T48 where T0 represents the baseline SSS score prior to administration of AMPT/placebo (blue color), T24 represents the SSS scores of 24 h post administration of AMPT/placebo (green color), T30 represents the SSS scores of 30 h post administration of AMPT/placebo (gold color), and T48 represents the SSS scores of 48 h post administration of AMPT/placebo (purple color). The error bars represent the standard error of the mean. The red stars show that the SSS scores at T24 and T30 differed significantly between the placebo and AMPT sessions in the HR but not the LR group (Mann–Whitney test,  $p < 0.01$ ).

**Table 3**  
Results of ROI analysis showing regions of increased metabolic rate in the AMPT versus the placebo condition for all subjects.

ROI Analysis								
Increased metabolism in the AMPT versus the placebo condition								
ROI	Cluster size	T score	Uncorrected p-value	FWE p-value	Peak coordinates			
					X	Y	Z	
Left ventral striatum	173	3.05	0.003	0.054	-12	11	0	
Right ventral striatum	246	5.46	<0.001	<0.001	13	9	-4	
Left amygdala	24	3.37	0.001	0.012	-22	-4	-10	
Right amygdala	33	3.32	0.002	0.013	21	-4	-10	
Left hippocampus	215	3.51	0.001	0.033	-27	-23	-6	
Right hippocampus	120	3.68	0.001	0.025	30	-18	-6	

Coordinates correspond to the stereotaxic array of Talairach and Tournoux (1988) and denote the distance in millimeters from the anterior commissure, with positive x = right of midline, positive y = anterior to the anterior commissure, and positive z = dorsal to a plane containing both the anterior and the posterior commissures.

The significance threshold was set at >10 contiguous voxels at  $P < 0.05$  (FWE-corrected) computed using the small volume correction provided within SPM5 software.

The ROIs were defined by an 8 mm sphere (4 mm in the case of the amygdala) drawn around the following coordinates: Ventral striatum =  $\pm 9, 9, -8$ ; amygdala =  $\pm 22, -6, -14$ ; OFC =  $\pm 20, 40, -20$ ; subgenual ACC =  $0, 30, -2$ ; mediadorsal thalamus =  $\pm 5, -15, 4$ ; hippocampus =  $\pm 28, -20, -10$ , ventromedial PFC =  $0, 54, -8$ .

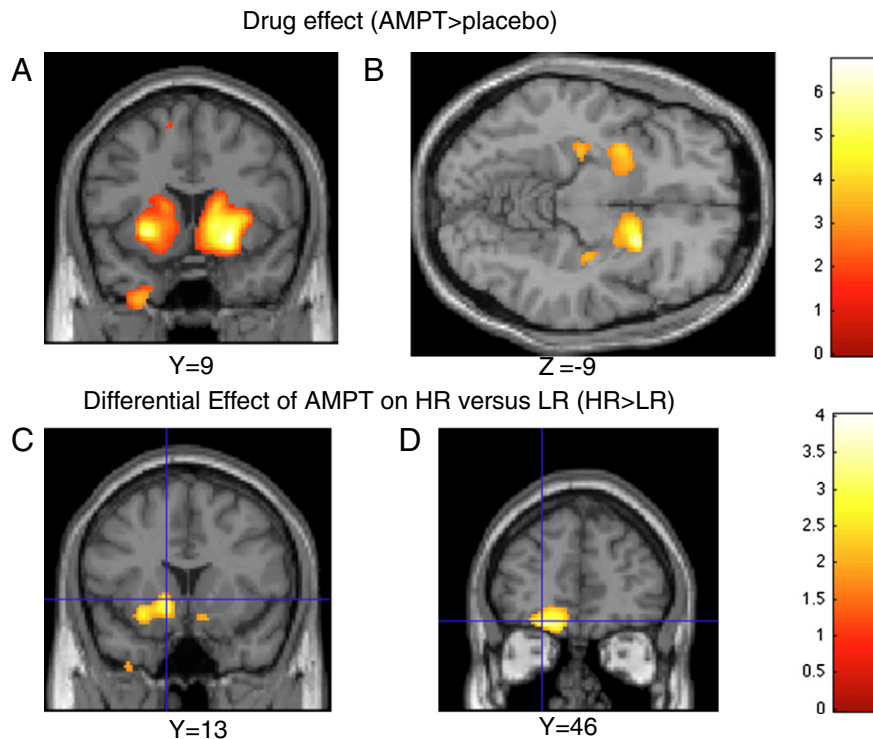
The ROI coordinates were obtained from atlases of Mai et al. (2004) and Talairach and Tournoux (1988) except for the ventral striatum where we followed the methodology of Nusslock et al. (2012) and the ventromedial frontal polar cortex where used the peak coordinate that Hasler et al. (2008) reported to correlate positively with severity of depression in their sample.

Because of a lack of an a priori hypothesis, no reverse contrast was conducted.

This finding replicates the results of our previous PET-FDG study that used the same AMPT dose in individuals who had developed MDD and healthy controls (Hasler et al., 2008). Bremner et al. (2003) studied medicated subjects with MDD in remission and reported that AMPT-induced depressive symptoms were associated with decreased activity in regions such as the OFC, thalamus, and dorsolateral PFC. However, the ventral striatum was not specifically assessed by these researchers. In addition, we report for the first time that compared with LR subjects,

HR subjects showed a greater increase in regional cerebral glucose metabolism in the left ventral striatum during catecholamine depletion (Table 6, Fig. 12c).

Compared with LR subjects, HR subjects showed a greater increase in regional cerebral glucose metabolism in the left OFC during catecholamine depletion (Table 6, Fig. 12d). Bremner et al. (2003) reported that AMPT-induced depressive symptoms were associated with decreased glucose metabolism in the OFC in remitted MDD subjects. Further, our



**Fig. 12.** This figure shows selected SPM image sections indicating changes in normalized cerebral glucose metabolism measured using [ $^{18}\text{F}$ ]fluorodeoxyglucose PET. In order to visually demonstrate the existence of separate peak clusters in the striatum, the hippocampi, and the OFC the significance thresholds for the figures were set at  $p < 0.005$  uncorrected. Figure A is a coronal section showing significant increases in normalized glucose utilization in the left and right ventral striata in the AMPT versus placebo condition in the combined HR and LR samples. Figure B is an axial image showing the significant bilateral increases in normalized glucose utilization in both the striata (anteriorly) and the hippocampus, (posteriorly), in the AMPT versus placebo condition in the combined HR and LR samples. Figure C is a coronal section showing that compared with LR subjects, the HR subjects displayed a greater increase in regional cerebral glucose metabolism in the left ventral striatum during catecholamine depletion. Figure D is a coronal section showing that compared with LR subjects, HR subjects displayed a greater increase in regional cerebral glucose metabolism in the left OFC during catecholamine depletion. The significance threshold is set at a voxel level  $p_{\text{corrected}} < .05$  using the FDR test to correct for comparisons in the regional analyses. Stereotaxic coordinates provided beneath each section image denote the distance z in mm from the stereotaxic origin (anterior commissure), with positive x indicating right of midline (for sagittal sections), positive y indicating anterior (for coronal sections), and positive z indicating dorsal (for horizontal sections).

**Table 4**

Results of ROI analysis showing regions of significantly increased metabolic rate in the HR versus the LR groups in the AMPT condition.

ROI analysis							
ROI	Cluster size	T score	Uncorrected P	FWE p-value	Peak coordinates		
Left amygdala	31	2.86	0.007	0.052	−21	−8	−8

Note: No regions showed significantly increased metabolism in the HR Versus the LR Group in the placebo condition.

The interpretation of the stereotaxic coordinates and the definition of the regions-of-interest are as described in the legend for Table 3.

The significance threshold was set at > 10 contiguous voxels at  $P < 0.05$  (FWE-corrected) computed using the small volume correction provided within SPM5 software.

Because of a lack of an a priori hypothesis, no reverse contrasts were conducted.

previous study showed that catecholamine depletion was associated with decreased OFC metabolism in both healthy controls and remitted patients with MDD as well as reduced OFC metabolism in the healthy control group versus the MDD group under both placebo and AMPT (Hasler et al., 2008). The discrepancy in results reported here potentially reflects differences in subject group (HR versus remitted MDD) or differences in sex ratio. The majority of subjects in the Hasler et al. (2008) study were female while 9 out of 14 subjects in this study were males.

We found an increase in regional cerebral glucose metabolism in the amygdala, bilaterally, during catecholamine depletion in the combined HR and LR groups (Table 3). In addition, compared with LR subjects, HR subjects showed a greater increase in regional cerebral glucose metabolism in the left amygdala during catecholamine depletion (Table 6). The amygdala sends abundant efferent anatomical projections to the ventral striatum (Price and Drevets, 2010). In addition, axons from the medial VS (including from the cells that share connective features with the rodent accumbens shell region) terminate in the bed nucleus of the stria terminalis, indicating a direct striatal influence on the extended amygdala.

The neuroimaging literature has consistently reported that patients with MDD show increased amygdala metabolism, specifically on the left side, as well as exaggerated hemodynamic responses to negative emotional stimuli including words, images and emotionally-valenced faces (reviewed in Drevets, 2001; Savitz and Drevets, 2009b). Based in part on these data, we hypothesized that increased amygdala activity in response to negatively-valenced stimuli is a potential endophenotype for MDD (Savitz and Drevets, 2009b). The greater increase in cerebral glucose metabolism in the left amygdala in the HR group versus the LR group during catecholamine depletion provides support to the hypothesis that abnormalities of amygdala function may predispose to the development of mood disorders in genetically-vulnerable populations. Unfortunately, the size of our sample precludes us from testing whether members of the HR group were differentially vulnerable to catecholamine depletion on the basis of their genetic makeup. For example,

the “short” allele of an insertion/deletion promoter polymorphism (5-HTTLPR) in the serotonin transporter gene (SLC6A4) has been previously associated with increased amygdala activity in response to negatively-valenced faces or decreased amygdala activity in response to neutral stimuli (Canli et al., 2005; Hariri et al., 2002).

We found an increase in regional cerebral glucose metabolism in the hippocampus, bilaterally, during catecholamine depletion in the combined HR and LR groups (Table 3, Fig. 12b). This result is partially consistent with Hasler et al. (2008) who reported an increase in cerebral glucose metabolism during catecholamine depletion in the right hippocampus/parahippocampal gyrus in a combined group of healthy subjects and remitted MDD subjects. Bremner et al. (2003) reported that MDD patients who remitted on antidepressant treatment and subsequently relapsed after catecholamine depletion displayed increased glucose metabolism in the hippocampus prior to receiving AMPT. We thus raise the possibility that the catecholamine depletion-associated increase in hippocampal activity may be an analog of the changes in hippocampal activity associated with depression.

In an independent PET study, we found that unmedicated MDD patients scanned at rest with [ $^{15}\text{O}$ ]-H<sub>2</sub>O showed increased rCBF in the hippocampus compared with the healthy controls (Savitz et al., 2012). Further, in an fMRI study we recently reported a greater BOLD response in currently depressed MDD patients versus LR patients to masked sad faces in an amygdala-centric neural circuit which included the hippocampus (Victor et al., 2012). Moreover, an independent sample of HR subjects scanned using the same backward masking paradigm displayed a greater BOLD response to sad versus happy faces than LR subjects in the parahippocampal gyrus (unpublished data). The increased hippocampal activity associated with depression is conceivably consistent with the morphometric MRI literature which has consistently reported hippocampal volume reductions in MDD (Savitz and Drevets, 2009a, 2009b). Theoretically, reductions in hippocampal volume in MDD are reflective of a loss of neuropil (Stockmeier et al., 2004) following glutamate-induced excitotoxicity (McEwen and Magarinos, 1997).

**Table 5**

Results of ROI analysis showing regions of increased metabolic rate in the AMPT versus the placebo condition for the HR subjects only.

ROI analysis							
Increased metabolism in the AMPT Versus the Placebo Condition							
ROI	Cluster size	T score	Uncorrected p-value	FWE p-value	Peak coordinates		
					X	Y	Z
Left ventral striatum	64	4.12	0.001	0.045	−14	12	−2
Right ventral striatum	98	4.32	0.001	0.037	15	9	0

Note: No regions showed significantly increased metabolism in the AMPT > Placebo contrast in the LR subjects.

The interpretation of the stereotaxic coordinates and the definition of the regions-of-interest are as described in the legend for Table 3.

The significance threshold was set at > 10 contiguous voxels at  $P < 0.05$  (FWE-corrected) computed using the small volume correction provided within SPM5 software.

Because of a lack of an a priori hypothesis, no reverse contrasts were conducted.

**Table 6**

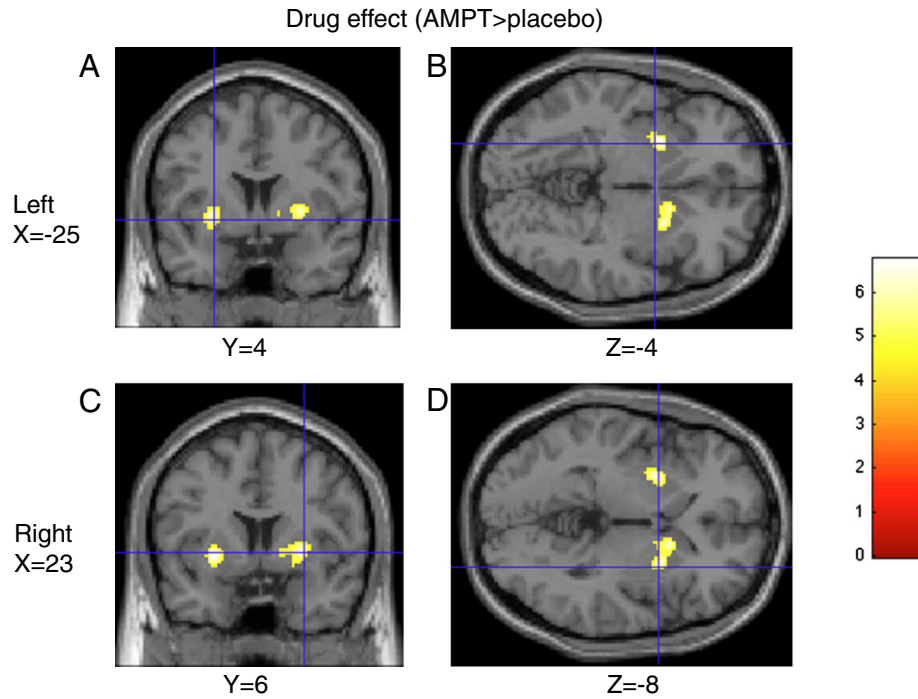
Results of ROI analysis showing regions of differential metabolism under catecholamine depletion in HR versus LR subjects (interaction contrast).

ROI analysis								
Differential effects of catecholamine depletion in HR versus LR subjects: Metabolism increased in HR versus LR								
ROI	Cluster size	T score	Uncorrected p-value	FWE p-value	Peak coordinates			
					X	Y	Z	
Left ventral striatum	84	3.23	0.002	0.054	−12	13	−2	
Left amygdala	25	2.93	0.004	0.028	−23	−10	−6	
Left OFC	133	3.40	0.001	0.041	−22	40	−15	

The interpretation of the stereotaxic coordinates and the definition of the regions-of-interest are as described in the legend for Table 3.

The significance threshold was set at > 10 contiguous voxels for which the voxel p-value corresponded to  $p(\text{corrected}) < 0.05$ .

Because of a lack of an a priori hypothesis, no reverse contrast was conducted.



**Fig. 13.** Whole brain voxel-wise analyses showing a significant increase in normalized cerebral glucose metabolism in the ventral striatum, bilaterally, during catecholamine depletion in the combined HR and LR groups. Figures A (coronal section) and B (axial section) show the increases in cerebral glucose metabolism in the left anteroventral putamen and figures C and D show the increases in cerebral glucose metabolism in the right anteroventral putamen. The significance threshold is set at a voxel level  $p_{corrected} < .05$  using the FDR test to correct for comparisons across the whole brain. Stereotaxic coordinates provided beneath each section image denote the distance in mm from the stereotaxic origin (anterior commissure), with positive x indicating right of midline (for sagittal sections), positive y indicating anterior (for coronal sections), and positive z indicating dorsal (for horizontal sections).

**6. Limitations**

The small sample size may have led to type I and type II errors and did not allow for post-hoc analyses examining for example, the effect of sex on response to AMPT.

We did not examine absolute cerebral glucose metabolism here due to the input function failures. Nevertheless, we have previously demonstrated that absolute whole brain metabolism is not altered significantly by AMPT (Hasler et al., 2008). In the current sample, we also found no difference in absolute whole brain glucose metabolism between the placebo and AMPT conditions ( $t = 0.42, p = 0.680$ , 2-tailed test). In addition, all measurements of regional cerebral glucose metabolism were normalized by whole brain glucose metabolism.

The inclusion of a group of patients with remitted MDD or remitted bipolar disorder would have allowed us to better contextualize the behavioral and neurophysiological response of the HR group to catecholamine depletion.

**Table 7**  
Results of the voxel-wise analysis showing regions of increased metabolic rate in the AMPT versus the placebo condition for all subjects.

Whole brain analysis									
Increased metabolism in the AMPT versus the placebo condition									
Cluster size	T score	Z score	p-value	FWE p-value	FDR p-value	Stereotaxic coordinates			Region
						X	Y	Z	
137	6.76	4.92	<0.001	0.028	0.013	-25	4	-4	L Putamen
328	6.61	4.85	<0.001	0.038	0.013	23	6	-8	R Putamen
	6.28	4.70	<0.001	0.068	0.013	25	3	2	R Putamen
	6.03	4.58	<0.001	0.105	0.013	15	11	-2	R Putamen

The interpretation of the stereotaxic coordinates is as described in the legend for Table 3.

The threshold for statistical significance was set an FDR-corrected p-value of <0.05. After correcting for multiple-testing no results remained significant in the reverse contrast.

We did not obtain plasma or cerebrospinal fluid (CSF) measurements of monoamine metabolites such as HVA and 3-methoxy-4-hydroxyphenylglycol (MHPG), and thus could not evaluate whether the CSF concentrations of these metabolites were associated with catecholamine depletion-induced symptoms of depression and fatigue or catecholamine depletion-induced changes in glucose metabolism. Previous studies have reported reductions in plasma levels of MHPG and HVA in healthy controls (Salomon et al., 1997) and patients with MDD (Berman et al., 1999; Miller et al., 1996) after administration of AMPT. The PET D2/3 receptor ligand, <sup>11</sup>C-raclopride, which is sensitive to endogenous levels of dopamine could conceivably have been used to provide more definitive measures of the degree of intrasynaptic dopamine depletion induced by AMPT.

The HR group was defined by the presence of relatives with either BD or MDD and it is theoretically possible that the AMPT response would differ on the basis of family history of BD versus family history of MDD. However, family studies have demonstrated that MDD rather than BD is the most common illness in relatives of individuals with BD, and twin studies indicate that concordance rates for BD twin pairs are significantly increased when a co-twin of a BD index patient who is diagnosed with MDD is classified as concordant rather than discordant to the BD index patient: 75% versus 43% (Kieseppa et al., 2004).

A sedation-controlled sham condition (e.g. Berman et al., 1999) potentially could have allowed the effects of AMPT on fatigue to be separated from the effects of AMPT on mood. Although it is conceivable that the AMPT-induced sedation may have been interpreted as low mood by some subjects, this factor cannot account for the differences in fatigue experienced by the HR and LR groups. Moreover, the anxiogenic effect of AMPT is arguably difficult to attribute solely to the effects of sedation.

**7. Conclusion**

Here we show for the first time that healthy individuals who are at increased risk for MDD or BD on the basis of family history show greater



**Table 8**  
Correlations between normalized cerebral glucose metabolism at the peak ventral striatal coordinates and depression, anxiety, and fatigue ratings 24 h and 30 h post initial dose of AMPT.

Measure	L striatum T24 all	R striatum T24 all	L striatum T24 h	R striatum T24 h	L striatum T30 all	R striatum T30 all	L striatum T24 h	R striatum T24 h	L striatum T30 h	R striatum T30 h
BDI	r = -0.05 p = 0.453	r = -0.13 p = 0.384	r = -0.18 p = 0.482	r = -0.01 p = 0.491	r = -0.22 p = 0.359	r = 0.45 p = 0.225	r = -0.03 p = 0.478	r = -0.03 p = 0.478	r = -0.03 p = 0.478	r = -0.03 p = 0.478
POMS depression	r = -0.26 p = 0.253	r = -0.11 p = 0.390	r = 0.17 p = 0.333	r = 0.40 p = 0.146	r = -0.50 p = 0.196	r = 0.10 p = 0.436	r = 0.10 p = 0.436	r = 0.10 p = 0.436	r = -0.15 p = 0.402	r = 0.56 p = 0.161
VAS sadness	r = -0.18 p = 0.319	r = -0.04 p = 0.463	r = -0.15 p = 0.351	r = -0.06 p = 0.443	r = -0.30 p = 0.312	r = 0.30 p = 0.312	r = 0.30 p = 0.312	r = 0.30 p = 0.312	r = -0.11 p = 0.429	r = 0.22 p = 0.359
HAM-D	r = 0.04 p = 0.457	r = 0.08 p = 0.408	r = 0.12 p = 0.358	r = 0.06 p = 0.434	r = -0.17 p = 0.371	r = 0.46 p = 0.177	r = 0.46 p = 0.177	r = 0.46 p = 0.177	r = 0.03 p = 0.479	r = 0.37 p = 0.234
MADRS	r = 0.23 p = 0.253	r = 0.07 p = 0.415	r = 0.02 p = 0.473	r = -0.03 p = 0.462	r = 0.09 p = 0.436	r = 0.54 p = 0.133	r = 0.54 p = 0.133	r = 0.54 p = 0.133	r = -0.03 p = 0.478	r = 0.32 p = 0.269
STAI (state)	r = -0.10 p = 0.398	r = -0.09 p = 0.407	r = 0.00 p = 0.500	r = -0.14 p = 0.364	r = 0.05 p = 0.467	r = 0.21 p = 0.370	r = 0.21 p = 0.370	r = 0.21 p = 0.370	r = 0.00 p = 0.500	r = 0.30 p = 0.312
POMS tension	r = 0.08 p = 0.414	r = 0.02 p = 0.483	r = 0.17 p = 0.333	r = 0.40 p = 0.146	r = 0.30 p = 0.312	r = 0.80 p = 0.052*	r = 0.80 p = 0.052*	r = 0.80 p = 0.052*	r = 0.36 p = 0.276	r = 0.98 p = 0.002**
POMS fatigue	r = -0.01 p = 0.491	r = -0.08 p = 0.423	r = 0.15 p = 0.348	r = 0.10 p = 0.397	r = 0.47 p = 0.210	r = 0.16 p = 0.400	r = 0.16 p = 0.400	r = 0.16 p = 0.400	r = 0.70 p = 0.094	r = 1.0 p < 0.001**
Sleepiness (SSS)	r = -0.54 p = 0.068	r = -0.48 p = 0.097	r = -0.06 p = 0.438	r = 0.16 p = 0.343	r = -0.80 p = 0.052*	r = -0.70 p = 0.094	r = -0.70 p = 0.094	r = -0.70 p = 0.094	r = 0.410 p = 0.246	r = 0.82 p = 0.044*

Note: p-values are from one-tailed statistical tests.

The regional metabolic data for the striatum were extracted over the locus in the left and right ventral striata where the peak difference (voxel t-value) was located in the ROI analysis: left (X, Y, Z) = -12, 11, 0, and right (X, Y, Z) = 13, 9, -4 (see Table 3).

Abbreviations: All = combined low-risk and high-risk samples; HR = high-risk sample only; BDI = Beck Depression Inventory; POMS = Profile of Mood States; VAS = Visual Analog Scale; HAM-D = Hamilton Depression Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale; STAI (State) = The state subscale of the State-Trait Anxiety Scale; Sleepiness (SSS) = Stanford Sleepiness Scale.

\* Significant or nominally significant results (p < 0.06).

\*\* p < 0.01.

symptoms of depression, anxiety and fatigue during catecholamine depletion than healthy individuals with no family history of psychiatric illness. Moreover, this sensitivity to catecholamine depletion in the HR group was associated with increased cerebral glucose metabolism during AMPT in the left ventral striatum, left amygdala, and left OFC. These results should be treated as preliminary because of the small sample size. Nevertheless, our data raise the possibility that at least some individuals with a genetic vulnerability to mood disorders have constitutive abnormalities in central dopaminergic function that trigger the development of affective illness in the presence of other genetic risk factors or environmental insults.

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### Statement of interest

Wayne Drevets, M.D. is an employee of Johnson & Johnson, and previously served as a consultant to Myriad/Rules Based Medicine and Eisai. The other authors have no financial disclosures to declare.

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