

Dietary Tyrosine/Phenylalanine Depletion Effects on Behavioral and Brain Signatures of Human Motivational Processing

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Dopamine (DA) neurotransmission is critical for motivational processing. We assessed whether disruption of DA synthesis in healthy controls using an amino-acid beverage devoid of catecholamine precursors (tyrosine–phenylalanine depletion (TPD)) would blunt recruitment of the nucleus accumbens (NAcc) by rewards. Sixteen controls ingested each of a tyr/phe-depleting beverage (DEP) or a tyr/phe-balanced (BAL) control beverage in two laboratory visits. Five hours after consumption of each drink, subjects underwent functional magnetic resonance imaging while they viewed anticipatory cues to respond to a target to either win money or avoid losing money. TPD did not exert main effects on mood or on task behavior, but affected brain activation. In right NAcc, TPD blunted activation by anticipation of high rewards. In left NAcc, recruitment anticipating high rewards was modulated by individual differences in mood change across the DEP drink day, where subjects whose mood worsened following TPD (relative to within-day mood change under BAL conditions) also showed lower activation under DEP conditions relative to BAL conditions. Exploratory analysis indicated that TPD qualitatively blunted the voxel-wise spatial extent of suprathreshold activation by reward anticipation. Finally, loss outcomes activated anterior insula under DEP conditions but not under BAL conditions. These data indicate that: (1) dietary depletion of catecholamine precursors will blunt dopaminergic mesolimbic activity, and (2) in controls, synthetic pathways of this neurocircuitry maintain sufficient buffering capacity to resist an effect on motivated behavior. Additional studies are needed to determine if clinical populations would show similar resistance to behavioral effects of TPD.

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INTRODUCTION

Understanding the role of dopamine (DA) in human incentive-motivational behavior has considerable clinical importance. First, impaired DA functioning is thought to mediate anhedonia and motor symptoms in depression (Stein, 2008). Second, reward deficiency syndrome in addiction has been attributed to impaired DA function (Blum *et al*, 2000). A key region to interrogate is the ventral striatum (VS), including nucleus accumbens (NAcc) (Haber and Knutson, 2010; Schultz, 2007). Reward-related signaling involves phasic activity of dopaminergic neurons that project to the VS (Hernandez *et al*, 2007; Stuber *et al*,

2008). Blood oxygen level-dependent (BOLD) signal increases in VS have been frequently elicited by reward-predictive signals or reward deliveries during functional magnetic resonance imaging (fMRI), and this has been inferred as a marker of phasic dopaminergic activity (Knutson *et al*, 2001). Notably, individual differences in VS recruitment by reward anticipation (assessed with fMRI) during a monetary incentive delay (MID) task correlated with individual differences in displacement of ¹¹C raclopride in the VS by MID task rewards themselves (Schott *et al*, 2008), and with displacement of ¹⁸F fallypride by amphetamine (Buckholtz *et al*, 2010).

Inhibition of catecholamine synthesis with α -methyl-*para*-tyrosine blunted caudate activation during reward anticipation in the MID task (da Silva Alves *et al*, 2011). An alternative intervention, tyrosine–phenylalanine depletion (TPD), entails ingestion of an amino-acid beverage lacking catecholamine precursor L-tyrosine (Tyr) as well as L-phenylalanine (Phe), which is peripherally converted into Tyr by phenylalanine hydroxylase. Large neutral amino acids (LNAA) compete with Tyr for CNS uptake

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(Oldendorf, 1973) and stimulate hepatic protein synthesis to incorporate extant Tyr into proteins (Harper *et al*, 1970). This markedly lowers concentrations of Tyr in the plasma (Moja *et al*, 1996; Palmour *et al*, 1998) as well as catecholamine metabolites in the CSF (Palmour *et al*, 1998) and striatum (Biggio *et al*, 1976) within hours. Perturbations of Tyr/Phe availability have altered responses to DA-mediated pharmacological or environmental challenges (reviewed in Milner and Wurtman, 1986; Tam and Roth, 1997). In humans, TPD blunted mood effects (thought to be DA-dependent) but not anorexic effects (thought to be NE-dependent; Silverstone, 1983) of amphetamine challenge (McTavish *et al*, 1999c). TPD also increased plasma prolactin (Harmer *et al*, 2001) by impairing dopaminergic lactotrophs of the tuberoinfundibular system.

Subjects performed the MID task after consuming a Tyr/Phe-deficient amino-acid beverage (DEP), and also after consuming a balanced (BAL) amino-acid beverage containing proportional Tyr and Phe. We hypothesized that TPD would blunt VS activation by reward-predictive cues (Nagano-Saito *et al*, 2012). Because NAcc recruitment during reward anticipation has correlated with self-reported positive affect (Bjork *et al*, 2004; Knutson *et al*, 2001), we further hypothesized that blunted NAcc recruitment by rewards following TPD would be specific to those subjects who showed worsened mood. Finally, because mesolimbic DA neurons show phasic decreases in response to omissions of expected reward (Schultz, 2007), we hypothesized that occasional replacement of reward outcomes with a demand to repeat the trial would deactivate the NAcc (Bjork *et al*, 2008), but that this would be blunted following TPD owing to reduction in DA tone.

MATERIALS AND METHODS

Subjects

Procedures were approved by the Institutional Review Board of the National Institute on Alcohol Abuse and Alcoholism. Subjects ($n=16$, 10 males; age 22–41, mean 29.2 ± 5.2 years) provided written informed consent and underwent physical examination and a structured clinical interview for DSM-IV. The exclusion criteria were as follows: history of an Axis I psychiatric disorder, left-handedness, pregnancy, current or past use of psychotropic medications, chronic medical conditions, or history of significant head injury or neurological disorder. Illicit drug abstinence was confirmed with urinalysis.

Experiment Schedule

Each subject was tested in two sessions at least 1 week apart. Female subjects were tested during the follicular phase of the menstrual cycle. Each subject was instructed not to eat after 2200 hours before the test day. Subjects underwent baseline venipuncture at 0930 hours. At 0945 hours, subjects completed a baseline mood rating with the Profile of Mood States (POMS) (McNair *et al*, 1992), and then ingested one of the two amino-acid beverages (described below) at 1000 hours. At 1430 hours, subjects underwent postdrink venipuncture and POMS readministration, and then were scanned at 1500 hours to correspond with plasma

nadir levels of Tyr and Phe that occur 5–6 h after ingestion of the DEP beverage (Leyton *et al*, 2000; Moja *et al*, 1996). Subjects were scanned during three runs of the MID task, and then were administered a high-resolution structural scan for colocalization of functional images.

Amino-Acid Administration

Amino-acid mixtures were administered in a randomized, double-blind manner. On one visit, the subject ingested the Tyr/Phe-depleting beverage (DEP condition). On the other visit (order counterbalanced), the subject consumed a beverage containing balanced amounts of Tyr and Phe (BAL condition) (see Supplementary Methods). The amino acids were mixed with water and either chocolate or orange flavoring to a volume of 500 ml. Methionine was administered in capsules. The dosage for female subjects was reduced by 20% for all amino acids.

MID Task

Stimuli were back-projected on a screen at the foot of the scanner bed and viewed using a head coil mirror. In the MID task used here, some reward trial outcomes were replaced by a demand to repeat the trial (Bjork *et al*, 2008). Trials consisted of: cue presentation, target presentation, and either success-dependent feedback or notification of the requirement to repeat the trial (Figure 1). All task stimuli were separated by presentation of a fixation crosshair for a jittered, uniformly distributed interval of either 2, 4, or 6 s.

First, a cue shape was presented for 250 ms, which signaled what the subject would win for hitting a target: low

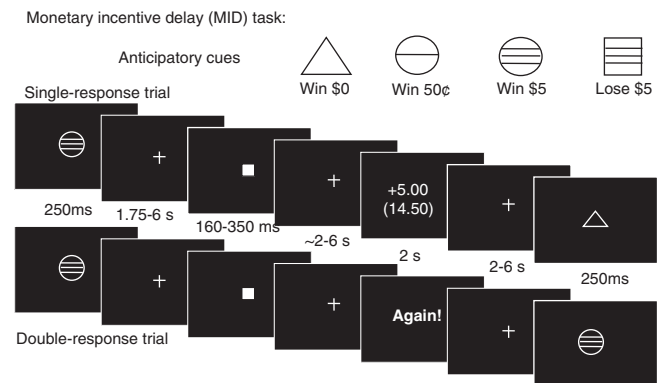


Figure 1 Monetary incentive delay (MID) task. In each trial, subjects were initially presented with one of four anticipatory cues followed by a fixation crosshair for 1.75–6 s, and a target followed by a post-target fixation crosshair for ~2–6 s. Subjects were required to respond during target presentation ('hit') to either win money, avoid losing money, or for no consequence. In single-response trials, subjects next viewed notification of whether or not the target was hit, followed by a 2–6 s intertrial interval with fixation crosshair. In double-response trials (one-third of each of win \$0, win 50¢, and win \$5 magnitudes), trial outcome notifications were pseudorandomly replaced with the word 'Again!' This notified the subject that he or she must repeat the trial to obtain its outcome. The 'Again!' notification was followed by: a 2–6 s fixation crosshair, re-presentation of the same incentive cue, jittered (1.75–6 s) postcue fixation, target presentation of the same duration as the trial's initial target, followed by ~2–6 s fixation crosshair, and final outcome notification. In double-response trials, hits on both targets were required for trial success.

reward (50¢; 27 trials), high reward (\$5; 27 trials), or no reward (\$0; 27 trials). Second, the cue was replaced by a crosshair for a variable interval (2, 4, or 6 s). Third, a white target square was presented for 180–450 ms. Subjects were instructed to respond to every target. To succeed, the subject was required to press a button on a button box while the target was on-screen. After a 2–6 s delay following the target, the trial concluded with a 2 s presentation of either: (a) feedback of whether the subject hit the target (single-response trials; $n=18$) or (b) the word ‘Again!’, which notified the subject that the cue and target for the trial would be re-presented before an outcome would be delivered (double-response trials; $n=9$). In each double-response trial, the same cue and target duration were repeated, and then were followed by feedback, where hits on both the first and second target were required to obtain reward. In loss-avoidance trials ($n=18$), a striped square cue (250 ms) signaled the possibility of losing \$5 if the subject failed to hit the target. Loss-avoidance trials were single-response only.

Before scanning, subjects were shown an envelope containing the cash they could win, and were read an instruction script. Subjects were briefed that in some trials (double-response trials) they would be required to hit the target on two presentations to win. Later, during a practice session, reaction times (RTs) to targets were covertly measured, and the distribution of target presentation durations was rigged so that each participant would succeed on ~67% of trials during the scan. Immediately after scanning, subjects rated on four-point scales how ‘excited’, ‘happy’, ‘fearful’, and ‘unhappy’ they felt when they saw each type of anticipatory cue or the ‘Again!’ demand.

Behavior and Mood Analyses

RTs were analyzed with repeated-measures analyses of variance, with mixture (two levels: BAL, DEP), incentive (four levels: non-incentive, win 50¢, win \$5, and lose \$5), and time (three levels: scan runs 1–3) as nested within-subject factors. Analysis of double-response trial types added target attempt (initial vs repeated) as a within-subject factor. Task hit rates were also analyzed with repeated-measures analyses of variance, with mixture and incentive amount as within-subject factors. Analysis of POMS used raw scores and was restricted to the total mood disturbance (TMD) composite score to reduce comparisons. TMD ratings were compared before and 5 h after ingestion of each of the DEP and BAL conditions, using a 2×2 ANOVA. Postscan affective ratings were also compared across incentive magnitudes and between the two mixture conditions using repeated-measures ANOVA.

Plasma Amino-Acid Analysis

Free plasma amino-acid concentrations were measured with the EZ:faast kit (Phenomenex, Torrance, CA) and analyzed as the concentration of tyrosine relative to the sum of concentrations of LNAAs administered in the drink (Tyr/LNAA ratio).

FMRI Acquisition

Imaging was performed using a 3 T MRI scanner (General Electric, Milwaukee, WI) with an eight-channel head coil. Functional scans were acquired using a T2*-sensitive echoplanar sequence with TR = 2000 ms, echo time = 30 ms, and flip = 90°. We collected 24 4.0-mm-thick sagittal slices. In-plane resolution was $3.75 \times 3.75 \text{ mm}^2$. Structural scans were acquired using a T1-weighted sequence for coregistration of functional data. Each subject’s head was restrained with a deflateable head cushion.

FMRI Analysis

Preprocessing. Data sets were time-shifted to compensate for non-simultaneous slice acquisition, warped out into Talairach space as 3.75 mm isotropic voxels, corrected for head motion, and spatially smoothed to a uniform 6 mm full-width half-maximum. Time series were modeled with gamma variate hemodynamic responses time-locked to anticipatory cues, targets, presentation of ‘Again!’ commands, or outcome notifications. Activations were detected by five linear contrasts (hereafter ‘contrasts’): (1) Anticipation of responding for all rewards (50¢ and \$5) vs for no incentive (\$0). For this contrast, signal in single-response trials was pooled with the first cue of double-response trials. (2) Anticipation of responding to avoid losses (\$5) vs for no incentive (\$0). (3) Notification of successful vs unsuccessful outcomes in single-response reward trials. (4) Notification of losses vs avoided losses in the loss-avoidance trials. Finally, to approximate brain activation elicited by omission of expected reward, ‘Again!’ notifications were contrasted with notification of non-wins in single-response trials.

Volume-of-interest analysis of NAcc signal change. The primary data of this experiment were the patterns of reward-anticipatory signal change in the NAcc. Each subject’s hemodynamic responses were: (1) trial-averaged, (2) modeled for low-frequency baseline drifts, and (3) passed through a mask in gray matter at the ventromesial intersection of caudate and putamen at Talairach $Y=10$. The effect estimates (β -values) as measures of response (in percent signal change) to each of the task stimuli were analyzed using a combination of repeated-measures ANOVA and paired t -tests.

Groupwise and between-group statistical mapping. We also conducted a brain-wide search for task activations under each mixture condition, as well as the net difference in task activation (DEP conditions relative to BAL). Statistical maps were calculated in AFNI using 3dMEMA (<http://afni.nimh.nih.gov/sscc/gangc/MEMA.html>), with a linear mixed-effects multilevel model that incorporates both within- and cross-subject variability. The contrast between the two mixture conditions was analyzed with 3dMEMA. Group activations in each mixture condition were controlled by false discovery rate (FDR) correction. In the supplementary tables, these are reported at the maxima of activated voxel clusters. In the figures, FDR-surviving voxels are illuminated at a voxelwise significance threshold of $P < 0.001$.

RESULTS

Plasma Amino-Acid Levels

Blood samples were not available for four subjects. The BAL drink resulted in no significant change in the plasma-free Tyr/LNAA ratio (repeated-measures ANOVA $P=0.68$) across the day, whereas the DEP drink caused a substantial reduction in the Tyr/LNAA ratio $F(1,11)=94.635$, $P<0.0001$) in every subject (mean values in Figure 2a), resulting in a significant mixture \times time interaction effect ($F(1,11)=23.803$, $P<0.0001$).

Mood Responses Under Different Mixture Conditions

Subjects reported variable, but generally positive affect (Figure 2b). There were no main effects of either time (pre- vs postdrink) or of amino-acid mixture (BAL vs DEP) on TMD, nor was there a significant time \times mixture interaction effect on TMD. Self-reported excitement ($F(2,30)=135.870$, $P<0.000001$) and happiness ($F(2,30)=55.886$, $P<0.000001$) evoked by reward-anticipatory cues increased with incentive magnitude (\$0, 50¢, \$5). Similarly, self-reported fearfulness ($F(1,15)=198.818$, $P<0.000001$) and unhappiness ($F(1,15)=45.996$, $P<0.00001$) evoked by loss-anticipatory cues significantly increased with magnitude (\$0, \$5). In double-response trials, self-reported fearfulness ($F(2,30)=25.413$, $P<0.000001$) and unhappiness ($F(2,30)=6.530$, $P<0.01$) upon viewing the 'Again!' demand to repeat the trial also increased with incentive magnitude (\$0, 50¢, \$5) of the

trial. There were no significant main or interaction effects of amino-acid mixture, however, on MID task affective ratings.

MID Task Behavior

RT to the sole target of single-response trials (pooled with the initial target of double-response trials) showed main effects of incentive ($F(3,45)=16.422$, $P<0.00001$) but no main effects of mixture or time (Figure 2d). Subjects responded more quickly to gain or to avoid losing money than for no incentive. A significant time \times incentive interaction ($F(3,45)=3.531$, $P<0.01$) effect indicated that RT to incentivized targets became faster across task runs, but RT to non-incentive targets did not. Double-response trials also indicated a main effect of incentive on RT ($F(2,30)=17.016$, $P<0.0001$), but no main effect of response attempt on RT, nor any mixture \times attempt or incentive \times attempt interaction effects. Because target durations did not differ by incentive type, hit rates were accordingly lower for non-incentive targets relative to incentivized targets (main effect of incentive ($F(3,48)=44.973$, $P<0.000001$)). There were no significant main or interaction effects of mixture on hit rates.

Task-Elicited Signal Change in NAcc (VOI Analysis)

Responses to anticipatory cues increased with incentive amount under both mixture conditions (main effect of magnitude $F(2,30)=19.702$, $P<0.00001$), where non-incentive

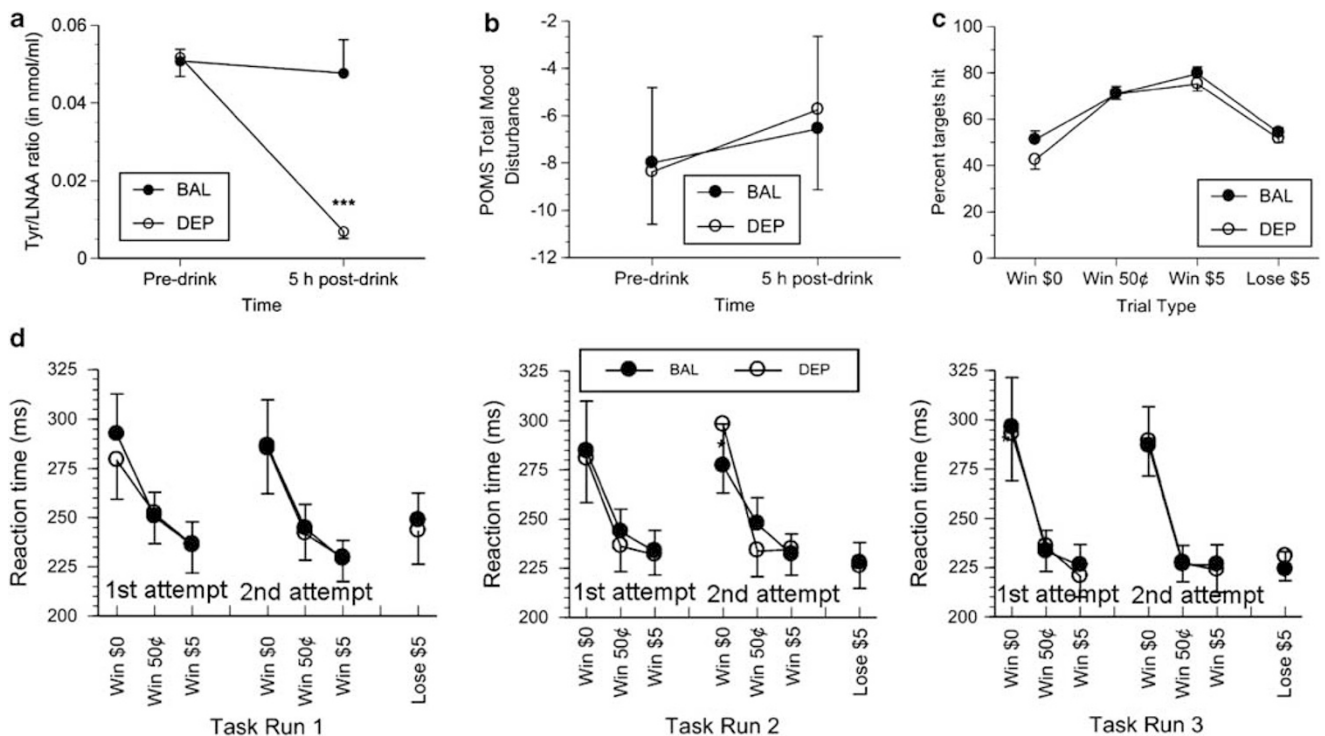


Figure 2 Effects of tyrosine-phenylalanine depletion (TPD) on free plasma amino-acid concentrations, mood, and task behavior. Acute TPD (tyr/phenyl-depleting beverage (DEP) beverage), but not the balanced-concentration (BAL) beverage resulted in a substantial reduction in the ratio of catecholamine precursor Tyr relative to competing large neutral amino acids (LNAA) in the plasma obtained 5 h after amino-acid beverage consumption (a). TPD had no effect, however, on Total Mood Disturbance as self-reported on the Profile of Mood States (POMS) (b). Target hit rates on the monetary incentive delay (MID) task (excluding second responses of double-response trials) varied as a function of the incentive at stake in the trial (c), where these differences in hit rates resulted from significant differences in reaction times to targets under different incentive conditions (d). *** denotes mixture \times time interaction effect at $P<0.0001$.

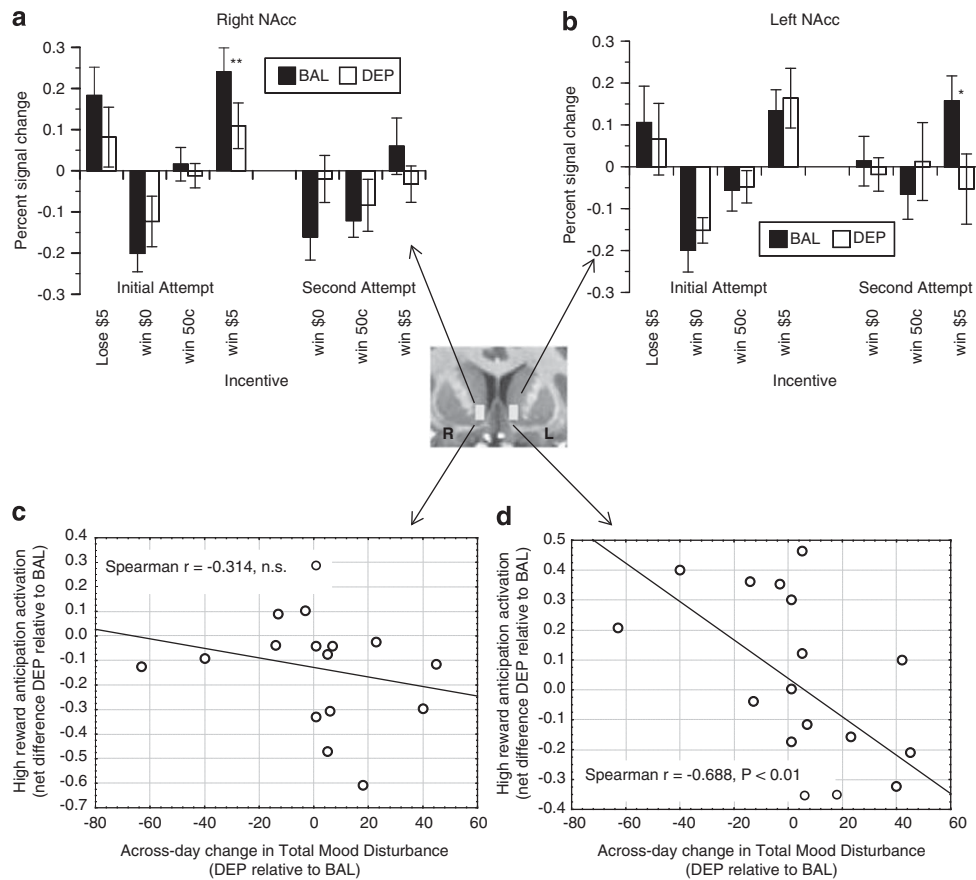


Figure 3 Anticipation cue-elicited signal change in the nucleus accumbens (NAcc) volume of interest (VOI). In a VOI drawn in the NAcc bilaterally (Talairach $\pm 10 + 10 - 4$; inset) modeled peak anticipatory blood oxygen level-dependent (BOLD) signal change reflected the prospective incentive value of hitting the target in both right (a) and left (b) NAcc. Notably, a significant attempt \times magnitude interaction effect indicated that this magnitude sensitivity of the NAcc responses was specific to initial/only attempt to hit the target, but not in second (repeat) responses to hit in the double-response trials. Across both sides of the NAcc, a significant mixture \times magnitude interaction effect suggested reduced reward-magnitude sensitivity under tyr/phe-depleting beverage (DEP) conditions compared with balanced (BAL) conditions. The net change (increase) in total mood disturbance across the day following tyrosine/phenylalanine depletion (TPD) relative to change under BAL conditions correlated with the net blunting of activation by high reward anticipation under DEP conditions relative to BAL conditions in left NAcc (d) but not right NAcc (c). * denotes mixture-wise $P < 0.10$, ** $P < 0.05$.

cues deactivated the VS, low-reward cues had minimal effect, and cues for high rewards and for losses activated VS (Figure 3). There were no main effects of side (left vs right), attempt (first or second), or amino-acid mixture on anticipatory NAcc activation. There was a significant magnitude \times attempt interaction effect ($F(2,30) = 12.835$, $P < 0.0001$), however, where the effect of magnitude was specific to the *initial* cue presentation of double-response trials. Notably, a significant mixture \times magnitude interaction effect ($F(2,30) = 4.352$, $P < 0.05$) indicated that magnitude effects on anticipatory activation were more pronounced under BAL conditions, whereas under DEP conditions, peak signal change in NAcc was more similar across trial incentives (\$0, 50¢, \$5). *Post hoc t*-tests indicated a significant reduction in anticipation of responding for high rewards in right NAcc (first cue) and a trend for a reduction in left NAcc (second cue) under DEP conditions relative to BAL conditions. Activation by anticipation of potential losses indicated no main or interaction effects of mixture or side. There were no main effects of side, mixture, magnitude, or outcome (hit vs miss) on NAcc activation by trial notifications. There were no significant main or

interaction effects of side, mixture, or magnitude in hemodynamic responses to the 'Again!' demand to repeat a trial in double-response trials.

Correlation between TPD effects on brain activation by rewards and TPD effects on mood. We wished to determine whether blunted NAcc responses to high reward cues under DEP conditions were specific to subjects who reported worsening mood following TPD. To limit comparisons, we only analyzed anticipatory signal change elicited by initial/only high reward cues. We calculated the net difference in modeled peak BOLD signal response in NAcc elicited by high reward anticipation under DEP conditions minus the response under BAL conditions. Second, we calculated a POMS-TMD net change score as: $(TMD_{\text{post-DEP}} - TMD_{\text{pre-DEP}}) - (TMD_{\text{post-BAL}} - TMD_{\text{pre-BAL}})$. Across subjects, net worsening of mood following the DEP drink (relative to mood change following the BAL drink) correlated with lower NAcc reward-anticipatory activation under DEP conditions relative to BAL in left NAcc (Spearman's $r = -0.688$, $P < 0.01$) (Figure 3).

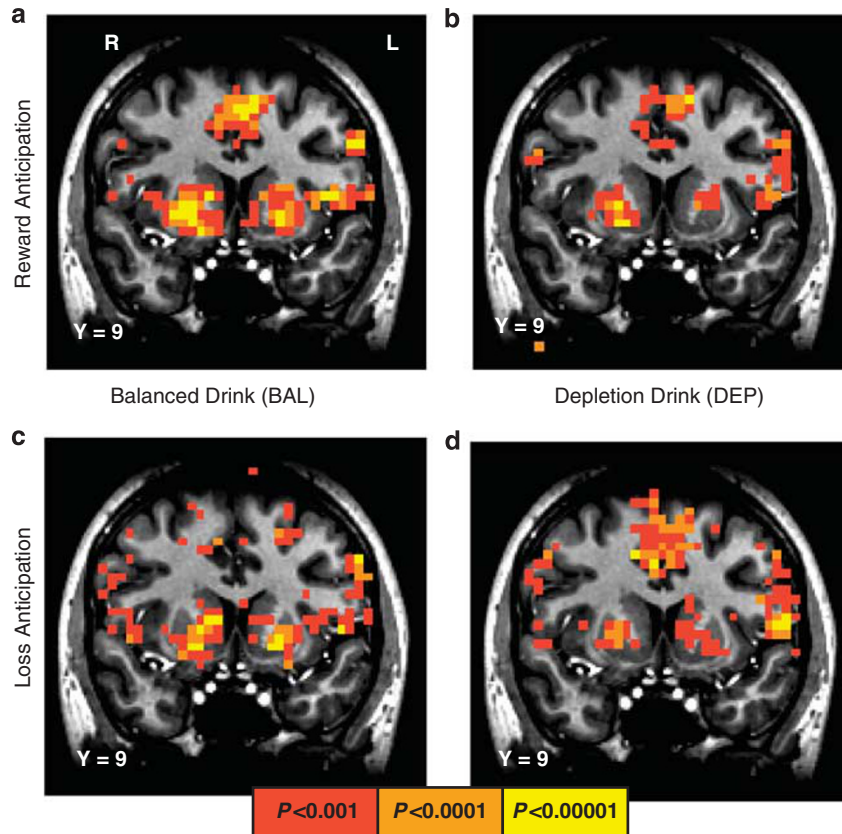


Figure 4 Statistical maps of activation by anticipation of potential rewards and losses. Anticipation of responding for rewards (50¢, \$5) contrasted with anticipation of responding for no incentive activated bilateral ventral striatum under balanced (BAL) (a) and tyr/phe-depleting beverage (DEP) (b) amino-acid conditions. Anticipation of responding to avoid losses (\$5) contrasted with anticipation of responding for no incentive also activated bilateral dorsal striatum in both BAL (c) and DEP (d) conditions. There were no voxel-wise amino-acid mixture differences in anticipatory activation for either potential rewards or losses. Statistical maps are right-left reversed per radiological convention, and anatomical underlays are derived from a T1-weighted scan of a representative subject at the planar Talairach coordinate shown. Color overlays depict uncorrected voxel-wise P statistics, where illuminated voxels or voxel clusters survive false discovery rate correction.

Task Contrast Activations in Brain-Wide Search

Reward vs non-incentive anticipation. Under BAL conditions, anticipation of responding for reward (50¢ and \$5 magnitudes combined) vs responding for no incentive activated a large bilateral cluster of voxels in the VS, with activation extending into anterior insula (Figure 4 and Supplementary Table 1). Additional activations included ventromesial frontal cortex (vmFC), posterior mesofrontal cortex, and several portions of occipital cortex. Under DEP conditions, these same regions were activated (with the exception of vmFC), with a reduced spatial extent of suprathreshold activations. There were no activation differences between mixtures, after correction for multiple comparisons.

Loss vs non-incentive anticipation. Under BAL conditions, anticipation of responding to avoid a \$5 loss vs responding for no incentive also activated a large bilateral cluster of voxels in the VS, with activation extending into anterior insula (Figure 4 and Supplementary Table 2). Additional activations by prospective losses also included vmFC, posterior mesofrontal cortex, and several portions of occipital cortex. Under DEP conditions, these same regions were activated (with the exception of vmFC), with a reduced spatial extent of suprathreshold activations. As with

rewards, there were no direct mixture differences in loss-anticipatory activation.

Gain vs nongain outcomes. This contrast did not yield activations that survived FDR correction in either the DEP or BAL conditions. Subthreshold (uncorrected voxelwise $P < 0.001$) activations in vmFC by gain notifications were present under both mixture conditions (Figure 5a and b and Supplementary Table 3), with an additional activation of right NAcc by gains under DEP condition only. A direct mixture-difference map revealed no contrast activation differences as a function of amino-acid manipulation.

Loss vs avoided-loss outcomes. This contrast did not yield any activations under BAL conditions. Under DEP conditions, however, loss notifications elicited activation in the thalamus, left anterior insula, subgenual anterior cingulate cortex, and posterior mesofrontal cortex (Figure 5c and d and Supplementary Table 3).

‘Again!’ vs non-reward outcomes in reward trials. ‘Again!’ demands activated right anterior insula, bilateral inferior frontal lobe, and bilateral inferior occipital lobe under both DEP and BAL conditions (Figure 5e and f and

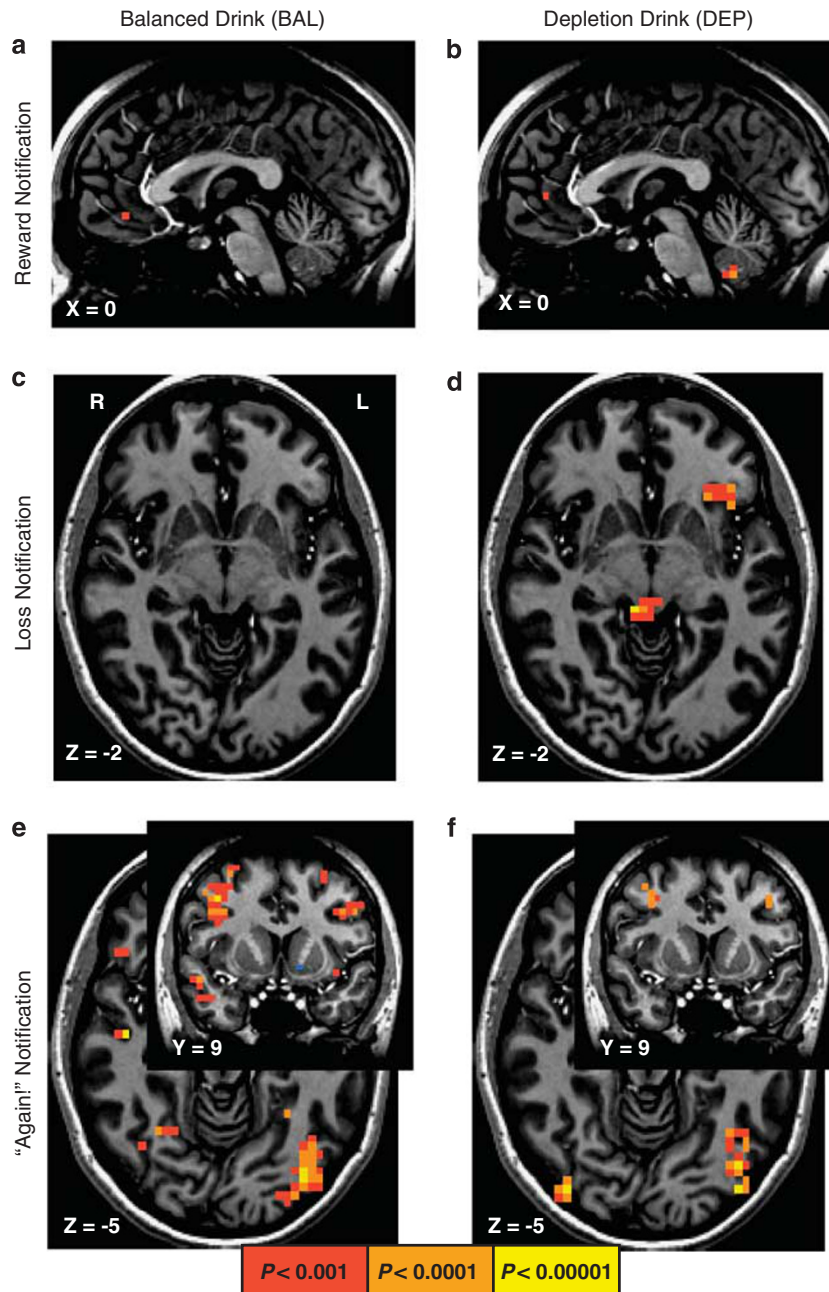


Figure 5 Statistical maps of activation by notification of rewards, by demands to repeat trial, and by notification of losses. Notification of rewards (50¢, \$5) contrasted with notification of failure to win reward activated a focal region of vmFC under both balanced (BAL) (a) and tyr/phe-depleting beverage (DEP) (b) conditions. Notification of losses (\$5) contrasted with notification of avoided losses did not activate any region above threshold under BAL conditions (c), but activated left anterior insula in under DEP conditions (d). Replacement of outcome notification with the demand to repeat the trial effort ('Again!') in reward trials (50¢, \$5) contrasted with notification of non-wins in single-response reward trials activated inferior occipital and middle frontal cortex under both BAL (e) and DEP (f) conditions, with an additional suprathreshold deactivation of left ventral striatum (VS) under BAL conditions. vmFC, ventromesial frontal cortex.

Supplementary Table 4). Under BAL conditions (only), this contrast resulted in additional activations of precentral gyrus and left inferior parietal lobule, as well as a deactivation of left NAcc.

DISCUSSION

As we hypothesized, the administration of a large bolus of amino acids devoid of catecholamine precursors blunted brain activation by reward-predictive cues in the NAcc, a

structure extensively implicated in the energizing of instrumental behavior (Haber and Knutson, 2010), presumably by reducing DA available for release. Notification of losses activated anterior insula under DEP conditions, but not under BAL conditions. Although there were no significant effects of TPD on task activation after brain-wide correction for multiple comparisons, we note that the spatial extent of most task activations tended to be reduced under DEP conditions in the striatum and other regions with mesolimbic dopaminergic innervation.

As we suspected, relatively reduced NAcc recruitment by reward anticipation under DEP conditions was specific to subjects who reported worsened mood following administration of the DEP beverage relative to the BAL beverage. Previously, blunted VS responses (Stoy *et al*, 2012) to reward cues and slower RTs (Hasler *et al*, 2009) to MID task targets have been found in clinically depressed subjects, with no activation differences in remitted (Dichter *et al*, 2012) or medicated (Stoy *et al*, 2012) depressed subjects. Of interest for future experiments would be collecting brain signal following TPD in subjects with histories of depression, anhedonia, or other affective disorder.

Our secondary hypothesis, that TPD would blunt VS deactivation by expected reward omission, was mildly supported. The contrast between 'Again!' notifications *vs* notification of failures to win in single-response trials elicited a significant focal left NAcc deactivation under BAL conditions, but not under DEP conditions. However, there were no direct significant mixture differences on VS deactivation by 'Again!' notifications. Although subject success on the large majority (>70%) of rewarded trials likely created some expectation of wins, we did not collect information on subject expectancies.

Notification of gains activated vmFC under both mixture conditions, and also mildly activated right NAcc in the DEP condition. Mesolimbic DA signaling has been elicited by both learned reward-predictive signals as well as by receipt of unexpected rewards (Schultz, 2007). As cue-reward associations are MID task are already trained before the scan, and because reward outcomes are highly probable (here ~75%), we suspect that phasic striatal DA activity elicited by the MID task is most robust (and by extension vulnerable to TPD effects) during reward anticipation.

Notification of losses activated left anterior insula and subgenual anterior cingulate cortex under DEP (but not BAL) conditions. Taken together with the presence of VS activation by gain notification under DEP (but not BAL) conditions, it is tempting to speculate that TPD may have increased limbic sensitivity to acute valenced instrumental outcomes through some other mechanism, to essentially mimic the responses of groups (eg, alcoholics) ostensibly characterized by impaired monoaminergic functioning. In the previous experiment with this MID task (Bjork *et al*, 2008), both left anterior insula activation by losses and VS activation by gains were greater in alcoholics. As we did not probe affective responses to gain and loss *notifications*, this question will require additional studies, where mood could be probed during the task.

RTs were essentially identical between the two mixture conditions. These behavioral data reflect previous findings in healthy controls, where TPD did not markedly affect RT in a simple signal-detection task (Nagano-Saito *et al*, 2012), and suggest that brain signal effects of TPD did not arise from sedation. Similar RT coupled with titration of target durations fostered consistent win rates across subjects and between mixtures. Therefore, NAcc activation differences between mixtures did not arise from differential *framing* of gains or losses against accumulation of winnings. Rather, mixture differences represented an absolute change in processing of the expected values of the specific trial magnitudes and outcomes.

Subjects generally reported positive mood, and TPD exerted no main effects on mood or on affective reactions to the anticipatory cues. The lack of a main effect of TPD on POMS-TMD ratings or MID task cue affective ratings also reflects negative findings in the literature when TPD is administered to normal controls (eg, Lythe *et al*, 2005; Coupland *et al*, 2001; Nagano-Saito *et al*, 2012) and even in subjects with histories of depression who were in remission at the time of testing (McTavish *et al*, 2004, 2005). There were large individual differences in absolute TMD ratings on each study day, however, and in net difference scores in POMS-TMD between mixture conditions. This also reflects previous findings of large individual differences in effects of TPD on mood (eg, Harmer *et al*, 2001).

In fMRI studies where brain activation differences are found in the absence of behavioral differences, activations are unconfounded by groupwise differences in success or frustration. Yet, a critical interpretive question arises: What is the functional meaning of the brain changes and the perturbation (or individual differences) that caused them? We contend that detection of covert markers (only) of brain alteration is meaningful (Wilkinson and Halligan, 2004). First, although controls showed a resistance to behavioral and mood effects of TPD, vulnerable populations might not. The literature is replete with examples of brain differences in response to a cognitive task in clinical populations, where the pathophysiological process had not yet advanced to the point of a behavioral decrement (eg, Ernst *et al*, 2009). Therefore, differences in brain signal (only) could provide an advanced signal of compromised processing. Second, that behavior and affect regarding the incentive cues remained intact despite the fact that some blunting of mesolimbic metabolic activity reveals a resistance of at least some elements of catecholaminergic neurocircuitry to acute precursor restriction. This indicates that synthetic pathways feature significant buffering capacity to maintain motivated behavior in the face of temporary changes in dietary resources. Finally, self-report measures of mood are subjective or insensitive, and can be prone to a variety of biases or other individual differences in self-awareness. In contrast, brain markers of incentive processing might represent an unbiased signal of valuation (Knutson *et al*, 2009).

These findings should be interpreted with some caveats. First, our sample size, while respectable for a repeated-measures pharmacological fMRI experiment, precluded examination of potential laterality differences in striatal activation, sex differences in mixture effects, as well as correlations between magnitude of plasma amino-acid level alterations and beverage effects on task activations or mood. Second, acute TPD is an imprecise intervention. The conventional BAL beverage was not a true 'placebo' and may have appreciably affected other transmitter systems. Second, although scans were scheduled at the nadir of plasma TyrPhe/LNAA ratio, when plasma prolactin concentrations (as a peripheral marker of reduced central DA function) previously elicited by this mixture were at their peak (Harmer *et al*, 2001), it is possible that the nadir of restricted *central* catecholamine synthesis occurred later (Palmour *et al*, 1998). Moreover, TPD decreases norepinephrine (NE) synthesis as well as DA synthesis (eg, Palmour *et al*, 1998). Therefore, activation changes cannot be definitively attributed to changes in DA synthesis. Other

evidence, however, suggests that TPD disproportionately affects the DA system. McTavish *et al* (2001) demonstrated that TPD blunted locomotor responses to amphetamine, with disproportionate reductions in amphetamine-induced DA release in the striatum (McTavish *et al*, 1999b). In contrast, they reported a more modest effect of TPD on amphetamine-induced (McTavish *et al*, 1999b) and idazoxan-induced (McTavish *et al*, 1999a) NE metabolite concentrations.

In addition, both amino-acid mixtures depleted L-histidine (His). His competes with other LNAAs for transit into the brain, where it is transformed into histamine, such that His restriction holds the potential for an antihistamine-like sedative effect. In a degraded-stimulus signal-detection task, however, His depletion only mildly affected some task-elicited EEG signatures, but did not significantly affect RTs or accuracy of signal detection (van Ruitenbeek *et al*, 2009). Similarly, RTs in this experiment were substantially similar to those of controls in the previous study with this MID variant (Bjork *et al*, 2008) that featured no pharmacological manipulation. However, it cannot be ruled out that some effects may have resulted from reduced histamine signaling (on both mixture days).

In conclusion, in healthy adults, TPD mildly reduced the overall dynamic response of the mesolimbic DA system to incentives, where this temporary disruption of catecholamine synthesis was not sufficient to cause a *functional* change in the incentive-driven behavior. We interpret this as evidence of the resilience of the brain's catecholaminergic pathways to dietary alterations. Future experiments could examine extended effects of DA precursor restriction, or could apply acute TPD to at-risk populations.

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