Mood-elevating effects of *d*-amphetamine and incentive salience: the effect of acute dopamine precursor depletion

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Objective: Midbrain dopamine transmission is thought to regulate responses to rewarding drugs and drug-paired stimuli; however, the exact contribution, particularly in humans, remains unclear. In the present study, we tested whether decreasing dopamine synthesis, as produced by acute phenylalanine/tyrosine depletion (APTD), would alter responses to the stimulant drug, *d*-amphetamine. **Methods:** On 3 separate days, 14 healthy men received *d*-amphetamine (0.3 mg/kg, given orally) plus a nutritionally balanced amino acid mixture, the phenylalanine/tyrosine-deficient mixture or the phenylalanine/tyrosine-deficient mixture followed by the immediate dopamine precursor, L-DOPA (Sinemet, 2 × 100 mg/25 mg). Responses to these treatments were assessed with visual analog scales, the Profile of Mood States, and a computerized Go/No-Go task. **Results:** *d*-Amphetamine elicited its prototypical subjective effects, but these were not altered by APTD. In comparison, APTD significantly increased commission errors on the Go/No-Go task and did so uniquely in conditions where subjects were rewarded for making correct responses; this effect of APTD was prevented by L-DOPA. **Conclusions:** Together these results support the hypothesis that, in healthy men, dopamine is not closely linked to euphorogenic effects of abused substances but does affect the salience of reward-related cues and the ability to respond to them preferentially.

Objectif : On croit que la transmission de la dopamine dans le mésencéphale assure la régulation des réponses aux drogues qui procurent une satisfaction et aux stimulis associés aux drogues, mais sa contribution exacte n'est toujours pas claire, en particulier chez les êtres humains. Dans cette étude, nous avons cherché à déterminer si une baisse de la synthèse de la dopamine causée par une déplétion aiguë de la phénylalanine-tyrosine (acute phenylalanine/tyrosine depletion ou APTD) modifierait les réponses à une drogue stimulante, la *d*-amphétamine. **Méthodes :** Quatorze hommes en santé ont reçu, pendant trois jours distincts, de la *d*-amphétamine (0,3 mg/kg, par voie orale) plus un mélange d'acides aminés carencé en phénylalanine-tyrosine ou un mélange d'acides aminés carencé en phénylalanine-tyrosine suivi d'un précurseur immédiat de la dopamine, le L-DOPA (Sinemet, 2 × 100 mg/25 mg). On a évalué les réponses à ces traitements au moyen d'échelles analogiques visuelles, du Profile of Mood States et d'une tâche informatisée oui ou non. **Résultats :** La *d*-amphétamine a produit ses effets subjectifs prototypes que l'APTD n'a toutefois pas modifiés. En guise de comparaison, l'APTD a augmenté de façon significative les erreurs commises au cours de l'exécution de la tâche oui ou non et l'a fait uniquement dans des conditions où les sujets ont été récompensés pour leur bonne réponse. Le L-DOPA a bloqué cet effet de l'APTD. **Conclusions :** Ces résultats appuient globalement l'hypothèse selon laquelle chez des hommes en bonne santé, il n'y a pas de lien étroit entre la dopamine et les effets euphorigènes de substances dont il est fait abus, mais elle a un effet sur la prégnance des indices reliés à la satisfaction et sur la capacité d'y réagir de façon préférentielle.

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Introduction

Stimulant drug-induced increases in mesocorticolimbic dopamine (DA) transmission are thought to affect responses to rewards. Still, controversy remains. Based on studies conducted in experimental animals, compelling arguments have been made that DA mediates the pleasure associated with reward,[1] the impetus to seek rewards,[2,3] the ability of reward-related cues to elicit and sustain interest,[4–8] the expectation of reward,[9] the judgement as to whether the reward was better or worse than expected,[9,10] the selection of attention to and behaviours directed toward the reward[11–13] and the learning of associations between rewards and their predictive cues.[14–18]

Recently, we have begun studies on the role of DA in drug reward in humans, using the acute phenylalanine/tyrosine depletion (APTD) method. In research animals, APTD decreases stimulated DA release[19–21] and cFos activation,[22] as well as striatal tissue concentrations of DA[23] and cerebrospinal fluid levels of the DA metabolite, homovanillic acid.[24] APTD might also decrease norepinephrine (NE) synthesis,[19,24] but this does not appear to be associated with diminished NE release.[19,22,25,26] In humans, tyrosine depletion decreases resting[27] and amphetamine-induced striatal DA release,[28] increases plasma levels of prolactin,[29–32] disrupts spatial working memory[29–31,33] (though see[32,34–36]), decreases alcohol self-administration,[37,38] and alters the ability to adjust betting behaviour in a gambling task.[36,39,40]

Effects of APTD on subjective states have been more variable. Tyrosine depletion reduces manic symptoms in patients with a bipolar mood disorder,[41,42] craving responses to cocaine and cocaine cues[43] and psychostimulant effects of amphetamines[41,44]; however, although APTD has also been reported to elicit mild mood-lowering responses associated with boredom and apathy,[39,45] marked changes in mood have not been seen. APTD does not reinstate depressive symptoms in recovered patients with a history of major depression,[31,40] alter anxiogenic effects of stressors,[45,46] or diminish the mood-elevating effects of cocaine,[43] alcohol[37,38] or nicotine.[47] In the present study, we investigated whether APTD would decrease mood-elevating effects of *d*-amphetamine and behavioural responses to reward stimuli, using a computerized Go/No-Go task; effects of APTD, it was proposed, would be prevented by administrating the immediate DA precursor, L-DOPA.

Methods

Participants

We recruited 14 healthy men aged 24.1 years (mean, standard deviation [SD] 4.4 yr) from advertisements placed in local newspapers and on campus. All were healthy nonsmokers, as determined by a physical exam, standard laboratory tests, and an interview with the Structured Clinical Interview for DSM-IV,[48] axis I.[49] None had a first-degree relative history of axis I psychiatric disorders, as assessed by the Diagnostic Interview for Genetic Studies.[50] On each study day, all men tested negative on a urine drug screen sensitive to cocaine, opiates, phencyclidine, barbiturates, delta-9-tetrahydrocannabinol, benzodiazepines, and amphetamines (Triage Panel for Drugs of Abuse, Biosite Diagnostics, San Diego, Calif., US). The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of McGill University's Faculty of Medicine. All participants gave written informed consent. Go/No-Go data were missing for one participant.

Procedure

Administration of the amino acid (AA) mixtures was conducted double blind in a randomized, within subjects, counterbalanced design (Fig. 1). The day before each test session, participants ate a low-protein diet provided by the investigators and fasted from midnight. On the test day, participants arrived at 8:30 am and had blood samples drawn to measure plasma AA concentrations. They then ingested one of the AA mixtures. The APTD mixture's composition, preparation and administration are based on our 100 g nutritionally balanced mixture, with phenylalanine and tyrosine withheld.[45,51] After ingesting the mixture, participants remained awake in a room with neutral videos and reading material available to them.

One and 3 hours after AA mixture administration, participants received placebo or L-DOPA/carbidopa (Sinemet, 100 mg/25 mg, orally), the immediate DA precursor with a peripheral decarboxylase inhibitor. Four hours after AA mixture administration, subjects were given tablets of *d*-amphetamine (Dexedrine, 0.3 mg/kg, orally). PET/[^{11}C]raclopride studies indicate that this dose of *d*-amphetamine increases DA release in human limbic striatum[52] and that the effect is significantly diminished by APTD.[28]

Dependent measures

Subjective effects of *d*-amphetamine were measured with the bipolar Profile of Mood States (POMS)[53,54] and 10 visual analog scales (VASs).[55] The POMS comprises 6 scales (Elate-Depressed, Composed–Anxious, Agreeable–Hostile, Confident–Unsure, Energetic–Tired, and Clearheaded–Confused)

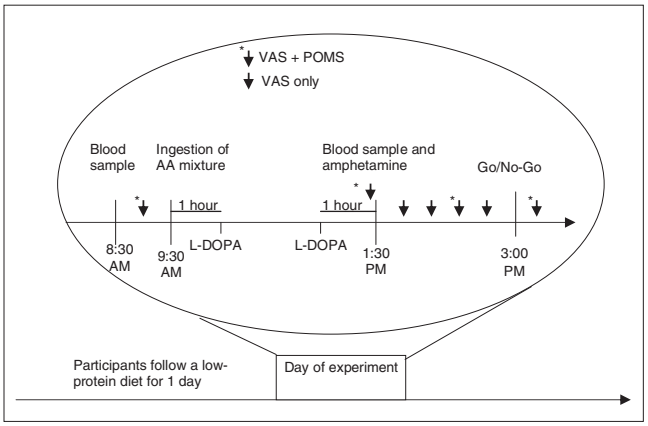

Fig. 1: Timeline of events on the 3 test sessions. VAS: visual analog scale; POMS: Profile of Mood States.

and is highly sensitive to nonclinical changes in mood states. It was administered 4 times: at baseline before ingesting the AA mixture, immediately before *d*-amphetamine administration (4 hours after AA mixture ingestion) and 60 and 120 minutes postdrug. The VASs were labelled "Rush," "High," "Euphoria," "Excited," "Anxious," "Energetic," "Like drug," "Mind racing," "Alert" and "Would like drug again." They were administered 7 times, at baseline before ingesting the AA mixture, immediately before amphetamine administration and every 20 minutes after amphetamine administration for 2 hours.

A computerized Go/No-Go task measured responses to rewards and punishments in 4 conditions.[56] In each condition, participants are presented with a set of 2-digit numbers. The first time that subjects do the task, each condition has 8 numbers. The second and third times, 10 numbers. This modification is thought to compensate for the potential effect of repeated testing.[57] By trial and error, subjects learn that one-half of the numbers signal that a button should be pressed, and one-half indicate that the button should not be pressed. In 2 of the 4 conditions (reward–reward and reward–punishment), pressing a button in response to the correct stimulus leads to a reward (win 10 cents); in the other 2 conditions (punishment–punishment and punishment–reward), pressing correctly avoids a punishment (lose 10 cents). Subjects are provided with 12 training trials (15 on test days 2 and 3) before starting the task, and performance during this phase does not count toward the final score. After this initial phase, each number is briefly shown on the screen, 10 times each. The task was administered 5 and a half hours after the AA mixture was given and takes approximately 30 minutes to complete. Thus, participants were taking the Go/No-Go task between 90 and 120 minutes after *d*-amphetamine administration, which corresponds to the period when plasma drug levels peak.[58]

Blood samples were drawn at morning baseline before AA mixture ingestion and 6 hours later. Phenylalanine and tyrosine were measured with precolumn derivatization with o-phthalaldehyde and gradient reverse phase HPLC with aminoadipic acid as an internal standard and fluorometric detection. Plasma concentrations of amphetamine were analyzed with electron-capture gas chromatography after extraction and derivatization of amphetamine with pentafluorobenzenesulfonyl chloride.[59] In 3 participants, plasma samples could not be drawn at all time points.

Statistical methods

We analyzed the plasma, subjective state, and Go/No-Go data by separate repeated-measures, univariate analyses of variance (ANOVA). AA mixture and time were within-subject factors. For the plasma data, time had 2 levels (immediately before and 6 hours after AA administration). For the subjective state data, the time factor was defined as change in mood from pre-AA mixture to 4 hours postmixture and maximum change from preamphetamine to postamphetamine. For the Go/No-Go analyses, AA mixture and Go/No-Go condition were also within-subject factors. Significant results revealed by these procedures were further examined by post hoc Least Significant Difference tests. All tests were 2-tailed.

Results

Plasma amino acids

APTD lowered plasma concentrations of phenylalanine and tyrosine, as reflected by significant AA mixture × time interactions (tyrosine: $F_{2,16} = 23.5$, $p \leq 0.002$; phenylalanine: $F_{2,16} = 38.4$, $p \leq 0.001$). Compared with morning baseline, phenylalanine and tyrosine levels were reduced by 74.4% and 74.6% and 80.1% and 83.1% by the APTD and APTD + L-DOPA treatments, respectively ($p < 0.05$). The effect of the 2 APTD conditions did not differ ($p \geq 0.80$). The nutritionally balanced (BAL) mixture increased plasma phenylalanine and tyrosine by 38.2% and 192.6% ($p \leq 0.005$) (Table 1).

Plasma amphetamine

Amphetamine was not present in any of the morning baseline samples and was present at a concentration of 26.0 (SD 5.7) ng/mL 120 minutes after drug administration. The AA mixtures did not alter amphetamine bioavailability (BAL: 26.9 [SD 5.8], APTD: 27.5 [SD 6.0], APTD + L-DOPA: 24.1 [SD] 6.3). In line with this, the AA mixture × time ANOVA yielded a significant main effect of time ($F_{1,17} = 211.8$, $p < 0.001$), but not AA mixture ($F_{2,14} = 1.85$, $p \geq 0.20$) or a mixture × time interaction ($F_{2,14} = 1.45$, $p \geq 0.27$).

Subjective effects

Repeated-measures analyses of the POMS data indicated that there were no main effects of AA mixture ($p \geq 0.35$) or AA mixture × time interactions ($p \geq 0.46$). In comparison, significant effects of time were seen for the POMS scales Elated–Depressed ($F_{1,13} = 12.89$, $p \leq 0.01$), Energetic–Tired ($F_{1,13} = 10.26$, $p \leq 0.01$), Confident–Unsure ($F_{1,13} = 8.93$, $p \leq 0.01$), Agreeable–Hostile ($F_{1,13} = 10.67$, $p \leq 0.01$) and Clearheaded–Confused ($F_{1,13} = 6.01$, $p \leq 0.03$), whereas scores on the Com-

Table 1: Plasma concentrations of phenylalanine and tyrosine before and 6 hours after ingesting the AA mixture

Amino acid	Morning baseline; mean (and SD) μmol/L	Postmixture; mean (and SD) μmol/L
Phenylalanine balanced	47.7 (4.6)	65.9 (23.8)†
Phenylalanine APTD	49.6 (9.2)	12.7 (5.8)‡
Phenylalanine APTD + L-DOPA	46.2 (5.5)	11.7 (4.7)‡
Tyrosine balanced	52.9 (7.3)	154.8 (87.7)‡
Tyrosine APTD	54.3 (9.2)	10.8 (3.2)*
Tyrosine APTD + L-DOPA	52.1 (8.1)	8.8 (2.7)*

AA = amino acid; APTD = acute phenylalanine/tyrosine depletion; L-DOPA = L-dihydroxyphenylalanine; SD = standard deviation.
Planned comparisons:
*$p < 0.05$
†$p < 0.01$
‡$p < 0.001$

posed–Anxious scale remained unchanged ($F_{1,13}$ = 1.85, $p \geq 0.20$) (Fig. 2).

Inspection of the data indicated that the effects of time reflected mood-elevating effects of *d*-amphetamine. Collapsed across test days, and compared with POMS scores obtained immediately before *d*-amphetamine administration, the stimulant drug significantly increased Elated–Depressed ($F_{1,13}$ = 16.46, $p \leq 0.001$), Agreeable–Hostile ($F_{1,13}$ = 9.83, $p \leq 0.008$), Energetic–Tired ($F_{1,13}$ = 12.79, $p \leq 0.003$), Confident–Unsure ($F_{1,13}$ = 9.47, $p \leq 0.009$), and Clearheaded–Confused scores ($F_{1,13}$ = 6.87, $p \leq 0.02$), whereas POMS scores on the Composed–Anxious scale remained unchanged ($F_{1,13}$ = 0.14, $p \geq 0.72$) (Fig. 2).

For each of the VAS items except for "Anxious," repeated-measures analyses also indicated statistically significant main effects of time ($p \leq 0.02$) (Table 1) but not effects of AA mixture or AA mixture × time interactions. VAS items that significantly increased after *d*-amphetamine, compared with preamphetamine, were "Rush" ($F_{1,13}$ = 18.70, $p \leq 0.001$), "High" ($F_{1,13}$ = 16.81, $p \leq 0.001$), "Euphoria" ($F_{1,13}$ = 11.40, $p \leq 0.005$), "Excited" ($F_{1,13}$ = 25.98, $p \leq 0.001$), "Energetic" ($F_{1,13}$ = 19.72, $p \leq 0.001$), "Like drug" ($F_{1,13}$ = 11.06, $p \leq 0.005$), "Mind-racing" ($F_{1,13}$ = 22.87, $p \leq 0.001$), "Alert" ($F_{1,13}$ = 16.05, $p \leq 0.001$), and "Would like drug again" ($F_{1,13}$ = 8.41, $p \leq 0.01$) but not "Anxious" ($F_{1,13}$ = 1.34, $p \geq 0.27$).

Go/No-Go commission errors

An AA mixture × Go/No-Go condition ANOVA of Go/No-Go commission errors during the initial learning phase yielded a near significant effect of AA mixture ($F_{2,24}$ = 3.19, $p \leq 0.059$) but not an AA mixture × Go/No-Go condition interaction ($F_{6,72}$ = 1.62, $p \geq 0.15$). Data inspection suggested that there was a tendency for commission errors to be higher during the test session with L-DOPA (mean ± SEM, 13.3 ± 1.5), compared with the sessions with BAL (10.8 ± 1.3, $p \leq 0.045$) and APTD (11.0 ± 1.3, $p \leq 0.035$).

Analyses of performance after the learning phase yielded a significant AA mixture × Go/No-Go condition interaction ($F_{6,72}$ = 2.32, $p \leq 0.05$). Data inspection indicated that APTD's effects were restricted to the 2 conditions where correct responses lead to a reward (win 10 cents; reward–punishment and reward–reward); in the 2 other conditions (punishment-

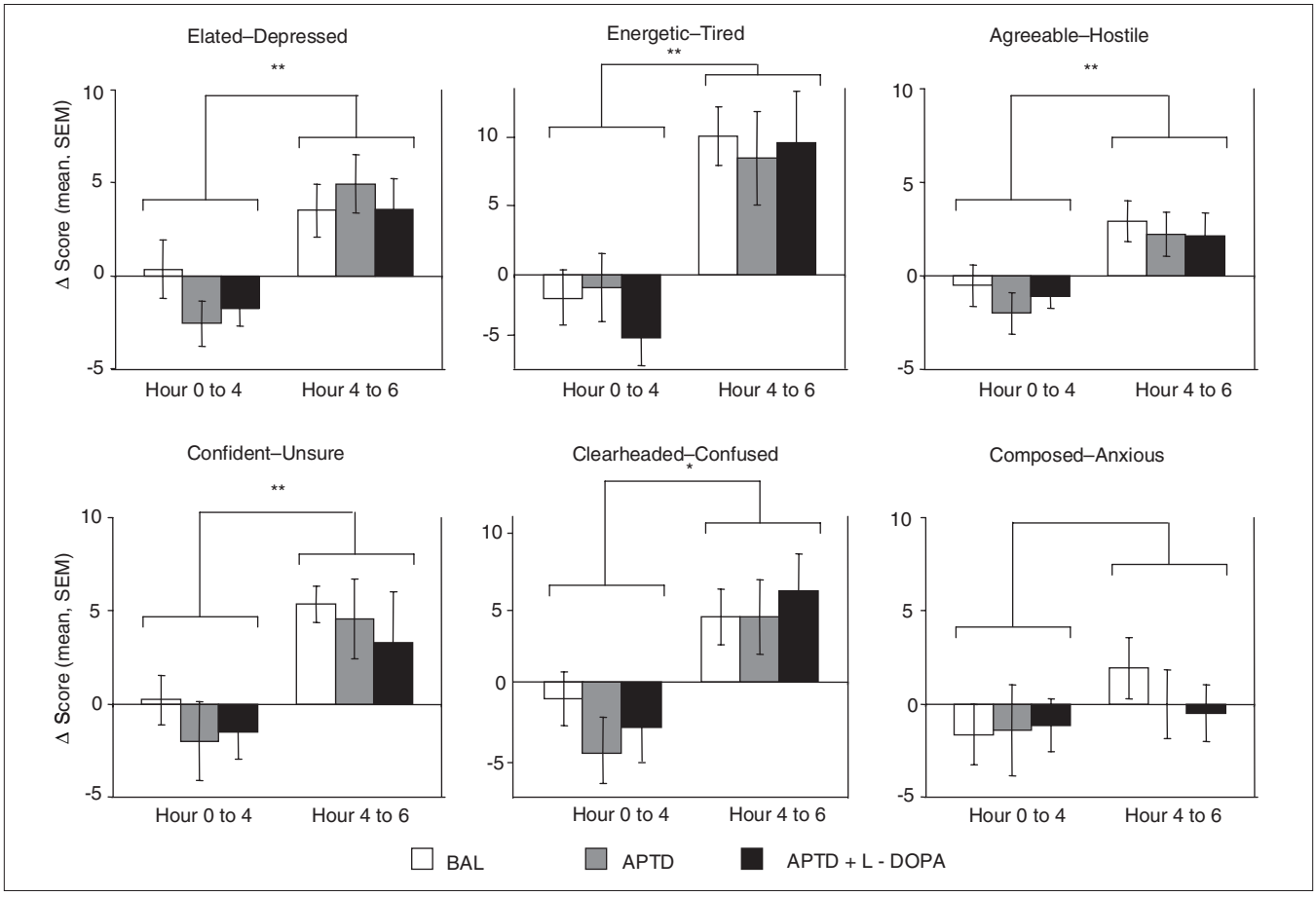

Fig. 2: Effect of acute phenylalanine/tyrosine depletion (APTD) and *d*-amphetamine on mood, as measured by the bipolar Profile of Mood States (POMS). The graphs depict mean change scores ± SEM for each of the POMS subscales from hour 0 to hour 4 (post-AA minus pre-AA) and from hour 4 to hour 6 (postamphetamine minus preamphetamine), as a function of type of amino acid mixture (APTD, BAL, APTD + L-DOPA). A higher score denotes more positive mood. Postamphetamine scores are defined as the maximum value after amphetamine administration. *$p \leq 0.02$; **$p \leq 0.01$.

punishment and punishment–reward), correct responding avoids a loss (lose 10 cents). Analyses of the reward versus no reward conditions confirmed this distinction; the ANOVA again yielded a significant AA mixture × Go/No-Go condition interaction ($F_{2,24} = 3.60$, $p \leq 0.04$), and relative to the BAL test session, APTD disturbed responses to stimuli in the reward ($p \leq 0.005$) but not the comparison condition ($p \geq 0.60$) (Fig. 3). L-DOPA prevented this effect of APTD ($p \leq 0.001$), improving performance and restoring it to a level not significantly different from that seen on the BAL test day ($p \geq 0.55$). Only the number of incorrect responses (commission errors) was affected; APTD did not alter omission errors ($p > 0.05$).

Discussion

The primary finding in the present study was that APTD did not alter mood-elevating effects of *d*-amphetamine, a result similar to previous APTD studies with cocaine,[43] alcohol[37,38] and nicotine.[47] In comparison, APTD significantly decreased the ability to preferentially respond to stimuli that predicted reward; this deficit was prevented by the immediate DA precursor, L-DOPA.

Although functional neuroimaging studies in humans have suggested that stimulant drug-induced striatal DA release correlates with both euphoria[60–65] and drug craving,[52,65] mood elevating effects of stimulant drugs are not consistently decreased by treatments that diminish DA transmission.[43,66–71] In comparison, there is a small but more consistent literature suggesting that decreased DA transmission diminishes

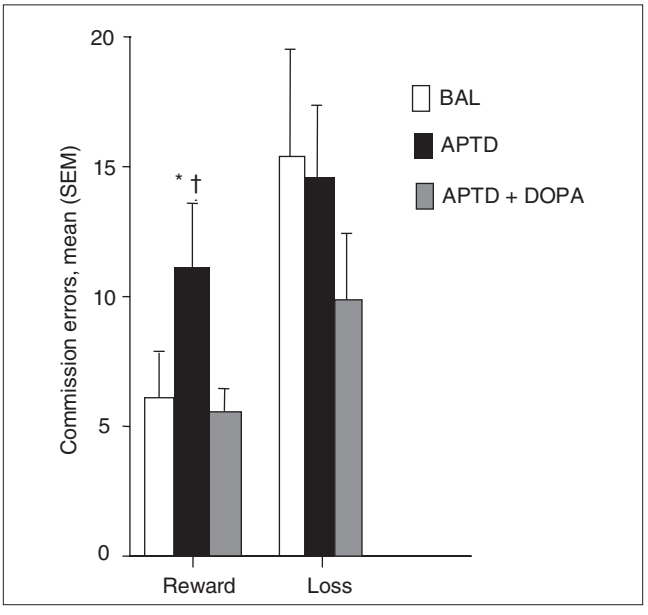

Fig. 3: Go/No-Go task commission errors (CEs) after the learning phase. Compared with the test session with the nutritionally balanced mixture (BAL), acute phenylalanine/tyrosine depletion (APTD) increased CE in the reward condition (reward–reward and reward–punishment), and performance was restored by L-DOPA. These effects of APTD and L-DOPA were not seen in the control condition. *$p \leq 0.005$, compared with the BAL test day; † $p \leq 0.001$, compared with the APTD + L-DOPA test day.

cocaine- and cocaine cue-induced craving[43,72] and the sustained motivation to obtain alcohol.[38] However, the finding that APTD does not alter the mood-elevating effects of *d*-amphetamine does not rule out a relation between DA and all aspects of emotion.[73–75] Since mood and motivational processes affect each other,[76] mesolimbic DA transmission might influence mood by moderating the anticipatory and appetitive components of positive affect rather than providing a sufficient substrate of mood elevation, per se.[77,78] Other contributors to drug-induced mood elevation might include NE,[79] serotonin[80] and endogenous opioids.[81]

The ability of APTD to disrupt preferential responding to reward-paired stimuli resembles the finding that tyrosine depletion alters the ability to adjust betting behaviour in a gambling task.[36,39,40] Similarly, the ability of L-DOPA to restore the APTD-induced performance deficit supports a recent report that L-DOPA administration improves responding to stimuli predictive of reward but not loss.[82] Together these findings add to the evidence that midbrain DA transmission is not closely linked to drug-induced mood-elevation but, across a range of tasks, enhances the ability to identify reward-related cues and respond to them preferentially.

Noting the above observations, the results should be interpreted in light of the following considerations. First, the administered dose of *d*-amphetamine (0.3 mg/kg, orally) elicited modest subjective effects, and the inability of APTD to diminish these effects might reflect the low dose. However, tyrosine depletion also failed to decrease the mood-elevating effects of a 5-fold range of cocaine doses,[43] and it left unaltered the mood-elevating effects of 0.15 mg/kg of intravenously injected methamphetamine, although it moderately diminished 2 effects related to accelerated thoughts: "Mind-Racing" and the subjective sense of "buzz."[41]

Second, participants received *d*-amphetamine on all 3 test sessions, precluding an assessment of whether the stimulant drug itself affected responses to reward cues. However, a study conducted elsewhere indicates that *d*-amphetamine (10 and 20 mg, given orally), compared with placebo, improves responding to reward stimuli in the Go/No-Go task.[83]

Third, we have interpreted the change in Go/No-Go performance to reflect a change in the ability to identify and preferentially respond to reward-related cues. Alternative explanations include changes in motor function, attention or memory. However, a motor hypothesis would predict the opposite of what we observed. Decreased DA transmission would be expected to decrease, not increase, behavioural activation. Moreover, motor, attentional and memory disturbances would be expected to alter both omission and commission errors and to do so in all 4 Go/No-Go subconditions. This was not seen.

Fourth, although L-DOPA was able to prevent the effect of APTD on responses to reward-predictive stimuli, in 2 previous studies it was unable to prevent APTD's effects on cocaine craving[43] or alcohol self-administration progressive ratio breakpoints[38]; in this study, it tended to worsen performance during the learning phase. This differential efficacy was unexpected. However, the ability of L-DOPA to

increase DA synthesis and release[84–86] but transiently reduce DA cell firing under conditions of diminished DA function[87,88] raises the possibility that phasic DA cell firing versus tonic DA release mediate different aspects of reward-related behaviour.[89,90] DA cell burst firing has been found to tightly covary with the expectation of reward[9,91] and to facilitate set shifting[92] and the acquisition of reward-related behaviours.[93] In comparison, slow tonic increases in DA release appear to have neuromodulatory effects,[94] sustaining interest in motivationally relevant events,[4,90,95–98] facilitating the learned association between rewards and reward-paired cues[14–17,92] and eliciting approach.[2,13] Acute L-DOPA administration, therefore, might restore behaviours that require elevated tonic DA levels but not phasic cell bursts. Craving for drug reward, in comparison, might emerge from increases in both phasic DA cell firing and tonic DA release.[38,43,90,91,95,99]

Fifth, APTD might affect NE synthesis[19,24]; however, accumulating evidence suggests that, to the extent that effects on NE synthesis occur, they are insufficient to alter NE release.[19,22,25,26] Finally, all the participants were men. Sex differences in response to stimulant drugs have been reported,[100,101] and it would be of interest to repeat this study in women.

In conclusion, the present study suggests that stimulant drug-induced changes in mood are not closely related to changes in DA transmission. In comparison, APTD perturbed the ability to preferentially respond to reward-paired stimuli, an effect that was prevented by the immediate DA precursor, L-DOPA. These and findings from related studies support the hypothesis that drug-induced increases in DA transmission enhance the perceived value of reward stimuli, increasing the tendency to focus on and approach drug-paired cues and facilitating the production of motivational and craving states.

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Competing interests: None declared.

Contributors: Drs. Leyton and Young designed the study. Dr. Leyton, Ms. aan het Rot and Drs. Baker and Benkelfat acquired the data, which Drs. Leyton, Booij, Baker and Benkelfat analyzed. All authors gave approval for the final version of the article to be published. Drs. Leyton and Booij wrote the article, and Ms. aan het Rot and Drs. Booij, Baker, Young and Benkelfat critically reviewed it.

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