Selective effects of simultaneous monoamine depletion on mood and emotional responsiveness

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Abstract

Monoamines play a significant role in the regulation of mood and emotion. While the selective effects of serotonin and catecholamine depletion on mood have been described, the effects of simultaneous monoamine depletion on subjective measures of mood and emotional responsiveness are yet to be examined. The current study aimed to investigate the effects of simultaneous monoamine depletion on mood and emotional responsiveness in healthy participants. Twenty female participants completed a randomized, double-blind, placebo-controlled study, under a balanced control condition (B), and a combined monoamine depletion condition (CMD; via tryptophan, tyrosine and phenylalanine depletion). Mood ratings [Visual Analogue Mood Scale (VAMS) and Profile Of Mood States (POMS)] and measures of emotional responsiveness (Emotional Stroop and International Affective Picture System) were examined at baseline and 5 h post-depletion. Following CMD, participants rated themselves as feeling sadder, more antagonistic, and mentally slower on three VAMS subscales. There were no significant mood changes found on the POMS or measures of emotional responsiveness. These findings suggest that simultaneous depletion of all monoamines may have selective effects on mood. The findings provide evidence that the simultaneous monoamine depletion technique may be a useful experimental method to probe central monoamine function in humans.

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Introduction

The monoamine neurotransmitters, serotonin (5-HT), noradrenaline (NA), and dopamine (DA) have been widely implicated in the regulation of mood and emotion in humans (Bunney and Davis, 1965; Charney and Delgado, 1992; Kapur and Mann, 1992; Schatzberg and Schildkraut, 1995; Schildkraut, 1965; Young and Leyton, 2002), and have been implicated in the pathophysiology and treatment of depression (Coppen, 1967; Delgado, 2000; Hirshfeld, 2000; Prange, 1964). The hypothesis that lowered levels of monoamines may engender people to depressive symptoms has led to experimental studies of these neurotransmitters in healthy participants (Moore et al., 2000; Reilly et al., 1997; Van der Does, 2001).

Amino acid (AA) depletion provides a reliable tool for investigating the role of monoamine deficiencies in the regulation of mood (Bell et al., 2001; Moore et al., 2000; Reilly et al., 1997; Van der Does, 2001). As 5-HT synthesis is dependent on dietary levels of its precursor tryptophan (Trp), lowering the ratio of free Trp to other large neutral amino acids (LNAAs) can reduce 5-HT synthesis in the brain (acute tryptophan depletion; ATD). This is achieved through (1) the induction of protein synthesis, which incorporates Trp into protein and diminishes Trp supplies in the blood, and (2) competition with other LNAAs for transport across the blood–brain barrier (Fadda, 2000). In humans, ATD has been shown to decrease plasma Trp levels by as much as 90% over 5–6 h (see Reilly et al., 1997 for review), along with substantial declines in 5-HT metabolites (Carpenter et al., 1998) and brain 5-HT synthesis (Nishizawa et al., 1997). Similarly, acute
tyrosine/phenylalanine depletion (ATPD) is associated with reductions in plasma concentrations of the catecholamine precursors tyrosine (Tyr) and phenylalanine (Phe) (Moja et al., 1996), catecholamine metabolites (Palmour et al., 1998) and catecholamine synthesis in the brain (McTavish et al., 1999).

While both ATD and ATPD have been shown to reduce brain concentrations of either 5-HT or catecholamines, their effects on mood in healthy participants has not been consistent across studies. Some single depletion studies have reported a mild, transient lowering of mood with ATD (Ellenbogan et al., 1996; Leyton et al., 1999; Ravindran et al., 1999; Smith et al., 1987; Young et al., 1985) and ATPD (Grevet et al., 2002; Leyton et al., 2000), while other studies have found no mood effects following ATD (Benkelfat et al., 1994; Carpenter et al., 1998; Harrison et al., 2002; Knott et al., 1999) or ATPD (Harrison et al., 2002; McTavish et al., 2001).

There is now abundant anatomical, physiological and neurochemical evidence for complex interactions between monoaminergic systems (Hirshfeld, 2000; Mongeau et al., 1997; Szabo et al., 1999, 2000). In addition, most antidepressant drugs affect both 5-HT and catecholamine systems either directly or indirectly (Blair and de Montigny, 1994; Leonard, 2000). It is therefore likely that mood and emotional processes may involve functional interactions between multiple neurotransmitter systems. The effects of simultaneous monoamine depletion on mood and emotion have not been examined in detail. In one study, Salomon et al. (1997) reported no mood effects with ATD when given in combination with \( \alpha \)-methyl-\( \beta \)-tyrosine (AMPT) (which reduces catecholamine synthesis). However, this study was limited by a very small sample size and the method involved 4 d of sequential testing, making it undesirable as an experimental probe of monoamine function in humans.

One of the limitations of previous depletion studies is the lack of investigation on a broader range of affective phenomena. To date, the majority of depletion studies have examined mood, while other aspects of emotion, such as emotional responsiveness, have been overlooked. Most theorists agree that moods are thought to be longer lasting and their cause often difficult to recognize or ambiguous, while emotions tend to be brief, in response to explicit stimuli, and are felt more intensely than moods (Davidson, 1994; Gainotti, 2001; Scherer and Peper, 2001). More specifically, emotional responsiveness may be considered the result of prior processing of emotional stimuli, involving cognitive, experiential, behavioural and physiological components.

The current study aimed to examine the effect of simultaneous monoamine depletion on mood and emotional responsiveness in healthy participants. Monoamine depletion was performed using a modified method of the method described by Young et al. (1985). In this method, depletion of monoamines was achieved by removal of Trp, Tyr and Phe from the balanced AA mixture. It was hypothesized that monoamine depletion would be associated with lowered mood, as assessed by the subjective mood scales, and changes to emotional responsiveness, as measured by the Emotional Stroop (ES) and International Affective Picture System (IAPS).

**Methods**

**Subjects**

Twenty healthy female participants were recruited through local and university advertisements, with ages ranging from 18 to 30 yr (mean, 22.2 yr; S.D., 3.56 yr). All participants were non-smokers, drug free and had no family or personal history of neurological or psychiatric disorders. Participants were screened using the Prime MD (APA, 1994) and a semi-structured clinical interview by a medical physician to satisfy the inclusion criteria. All participants gave written informed consent to take part in the study, which was approved by the Swinburne University Human Research Ethics Committee.

**Design**

The study was a randomized, double-blind, placebo-controlled, cross-over design in which all subjects were tested under two treatment conditions; the balanced control condition (B) and combined monoamine depletion condition (CMD). Treatment conditions were separated by a 5-d washout period.

**AA mixture**

The AA mixture used in the current study was modified, based on the 100 g balanced mixture developed by Young et al. (1985), which was adapted to an 86 g suspension and capsules to account for the lower body weight of women (Leyton et al., 1999). The B condition included the following AAs: L-alanine, 4.58 g; L-arginine, 4.08 g; L-cystine, 2.25 g; L-glycine, 2.97 g; L-histidine, 2.67 g; L-isoleucine, 6.67 g; L-leucine, 11.25 g; L-lysine monohydrochloride, 9.17 g; L-methionine, 2.50 g; L-proline, 10.17 g; L-valine, 7.42 g; L-serine, 5.75 g; L-threonine, 5.42 g; L-tryptophan, 1.92 g; L-tyrosine, 1.75 g; L-phenylalanine, 4.75 g; L-methionine, L-cystine, and L-arginine were given in capsules due to
their unpleasant taste. In the CMD condition all AAs were included except for Trp, Tyr, and Phe. Drinks were prepared by mixing the powdered AAs with 180 ml of orange juice within a few minutes of ingestion following administration of the capsules.

Procedure

To control for hormonal fluctuations, which may cause variations in mood, testing was conducted during the follicular phase of the menstrual cycle (days 1–13). On each testing day subjects arrived between 08:30 and 09:00 hours after following a low-protein diet, and fasting from 19:00 hours the day prior to testing. Upon arrival, a baseline blood sample and mood ratings were obtained, followed by the ingestion of the AA drink and capsules. Subjects then remained in the room and were allowed to do personal study/reading provided the content was emotionally neutral. Participants were given symptoms checklists at 1 and 3 h following AA ingestion. The mood ratings and blood sample were obtained again 5 h after ingestion of the AA load, together with measures of emotional responsiveness. The 5-h latency period was chosen as AA mixtures have been found to lower plasma-free Trp or Tyr/Phe levels by up to 90% in humans, with maximum depletion 5 h after ingestion (for review see Van der Does, 2001). Plasma was separated by centrifugation then stored for assay at −20 °C.

Mood assessment

Subjective mood ratings were obtained using the Visual Analogue Mood Scale (VAMS; Bond and Lader, 1974) and the Profile of Mood States (POMS; McNair et al., 1988). The VAMS consists of five 100 mm horizontal lines each representing a bipolar dimensional mood state: Happy–Sad, Sociable–Withdrawn, Relaxed–Tense, Quick Witted–Mentally Slow, Amicable–Antagonistic. Participants place a mark on each line that best describes their current mood state. The POMS consists of 65 adjectives rated on a scale from 0 (not at all) to 4 (extremely), which are compiled into six mood dimensions including: Anger, Vigour, Anxiety, Fatigue, Depressed, and Confusion.

Assessment of emotional responsiveness

The ES (Gotlib and Cane, 1987) is a measure of emotional processing which requires subjects to name the colour of emotional and neutral words presented to them. Words were presented to participants in three blocks (40 pleasant, 40 neutral, 40 unpleasant) (Eide et al., 2002; Gotlib and Cane, 1987; Gotlib and McCann, 1984). The speed of colour naming emotionally salient words in the ES has been used to evaluate several emotional conditions and is believed to be an indicator of participants’ attentional biases (for review see Greco, 1993).

The IAPS (Lang et al., 1999) was used to measure any possible changes in the emotional responsiveness of participants. This involved randomly presenting 75 emotional pictures categorized as pleasant, neutral, or unpleasant (25 images per category) on a computer screen. Each picture was rated using bipolar valence (1 = unpleasant to 9 = pleasant) and arousal (1 = low arousal to 9 = high arousal) scales. The 75 images were selected according to the standardized arousal and valence ratings from the manual. Valence of the images was varied (unpleasant pictures, 1.8–3.47; neutral, 4.46–5.46; pleasant, 7.02–8.34), whilst arousal ratings remained relatively low (unpleasant, 3.52–5.5; neutral, 1.55–4.27; pleasant, 2.67–5.94). No differences were found between image categories for brightness and contrast (Kemp et al., 2002). The literature suggests that the presentation of photographic images can evoke a range of emotions similar to those experienced outside the laboratory (Lang et al., 1998).

AA measurement

Concentrations of free Trp, and plasma Tyr, Phe, valine (Val), leucine (Leu), and isoleucine (Iso) levels were analysed using high-pressure liquid chromatography (HPLC) with fluorometric detection (see Benkelfat et al., 1994). Plasma concentrations of Val, Leu and Iso levels were analysed to calculate the ratio of plasma-free Trp, Tyr, or Phe, to other LNAAs.

Statistical analysis

The mood and plasma data was analysed using repeated-measures ANOVAs with time [pre-treatment scores (0 h) vs. post-treatment scores (5 h)] and treatment (B or CMD) as the group factors. Plasma ratio data was analysed using a 3 (AA ratio; free Trp:ΣLNAAs, Tyr:ΣLNAAs, Phe:ΣLNAAs) × 2 (treatment) × 2 (time) repeated-measures ANOVA. In addition, absolute levels of free Trp, and plasma Tyr and Phe were analysed using a 2 (treatment) × 2 (time) repeated-measures ANOVA. Independent t tests were utilized to investigate whether mean baseline ratings on the POMS and VAMS subscales differed between the two testing days. Group factors for emotional responsiveness were category (pleasant, unpleasant, neutral) and treatment (B or CMD). In order to test for interference effects, new variables were created by subtracting the neutral scores from both the pleasant and unpleasant scores, in both treatments. Planned comparisons were
used to examine any differences between the pleasant, neutral, and unpleasant categories of the IAPS and ES.

Results

AA levels (µmol/l)

There was a significant 6 (AA: Trp, Tyr, Phe, Leu, Iso, Val) × 2 (time) (pre- and post-treatment administration) × 2 (treatment condition) (B, CMD) repeated-measures ANOVA interaction [F(1.49,11.93) = 12.86, p < 0.001] (Greenhouse–Geisser adjusted), indicating that treatment modified the effect of time on AA concentration levels. In order to further investigate this effect, we conducted two 6 (AA ratio) × 2 (time) repeated-measures ANOVAs. Results indicated a significant AA × time interaction [F(1.91,17.19) = 64.04, p < 0.001] (Greenhouse–Geisser adjusted) for the B condition, and a significant AA × time interaction [F(1.14,9.98) = 87.88, p < 0.001] (Greenhouse–Geisser adjusted) for the CMD condition. Following administration of the B condition, all concentrations of the 6 AAs increased significantly (all p < 0.001) compared to baseline. Following administration of the CMD condition, Trp, Tyr and Phe levels decreased significantly (all p < 0.001) compared to baseline, whilst remaining AA concentrations increased significantly (all p < 0.001) compared to baseline (see Table 1 for AA concentrations and percentage changes).

There was a significant interaction between 3 (AA ratio) (Trp:ΣLNAAs, Tyr:ΣLNAAs, Phe:ΣLNAAs) × 2 (time) × 2 (treatment condition) repeated-measures ANOVA interaction [F(1.16,10.45) = 18.99, p = 0.001] (Greenhouse–Geisser adjusted), indicating that treatment modified the effect of time on AA ratios. In order to further investigate this effect, we conducted two 3 (AA ratio) × 2 (time) repeated-measures ANOVAs on treatment condition (B, CMD). Results indicated a significant AA × time interaction [F(1.12,10.11) = 7.66, p = 0.018] (Greenhouse–Geisser adjusted) for the B condition, and a significant AA × time interaction [F(1.23,11.04) = 72.76, p < 0.001] (Greenhouse–Geisser adjusted) for the CMD condition. The ratio of Trp:ΣLNAAs, Tyr:ΣLNAAs and Phe:ΣLNAAs was significantly reduced in the B condition, however a much greater reduction in the same ratios was observed in the CMD condition (see Table 1).

Effects of monoamine depletion vs. balanced control condition on mood

Significant interaction effects were found on three of the VAMS subscales: Happy–Sad [F(1,19) = 4.88, p < 0.05], Amicable–Antagonistic [F(1,19) = 4.50, p < 0.05], and Quick Witted–Mentally Slow subscales [F(1,19) = 5.38, p < 0.05], indicating that treatment modified the main effects for time. Following CMD, participants rated themselves as feeling sadder, more antagonistic, and mentally slower than baseline, compared to the B condition in which ratings did not differ from baseline to post-treatment (see Table 2).

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**Table 1. Plasma concentrations of amino acids (µmol/l) (mean ± S.E.M.) (n=10)**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Group</th>
<th>Baseline</th>
<th>5 h</th>
<th>Per cent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma free Trp</td>
<td>B</td>
<td>3.47 (0.19)</td>
<td>8.89 (1.19)</td>
<td>+156.20**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>3.19 (0.17)</td>
<td>1.73 (0.13)</td>
<td>-45.77**</td>
</tr>
<tr>
<td>Plasma Tyr</td>
<td>B</td>
<td>39.43 (2.15)</td>
<td>112.15 (14.31)</td>
<td>+184.43**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>42.64 (4.15)</td>
<td>11.19 (0.83)</td>
<td>-73.75**</td>
</tr>
<tr>
<td>Plasma Phe</td>
<td>B</td>
<td>37.88 (0.87)</td>
<td>107.64 (13.46)</td>
<td>+184.16**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>37.84 (1.88)</td>
<td>8.28 (0.84)</td>
<td>-78.12**</td>
</tr>
<tr>
<td>Plasma Val</td>
<td>B</td>
<td>150.25 (5.02)</td>
<td>622.55 (44.86)</td>
<td>+314.34**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>150.20 (6.78)</td>
<td>685.14 (48.98)</td>
<td>+356.15**</td>
</tr>
<tr>
<td>Plasma Iso</td>
<td>B</td>
<td>67.49 (4.32)</td>
<td>264.96 (17.65)</td>
<td>+292.59**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>62.88 (4.06)</td>
<td>316.69 (26.92)</td>
<td>+403.64**</td>
</tr>
<tr>
<td>Plasma Leu</td>
<td>B</td>
<td>83.57 (2.95)</td>
<td>368.47 (37.86)</td>
<td>+340.91**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>83.85 (3.33)</td>
<td>504.73 (44.61)</td>
<td>+501.94**</td>
</tr>
<tr>
<td>Free Trp/ΣLNAAs</td>
<td>B</td>
<td>0.0092 (0.001)</td>
<td>0.0060 (0.001)</td>
<td>34.78**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>0.0088 (0.001)</td>
<td>0.0012 (0.000)</td>
<td>-86.36***</td>
</tr>
<tr>
<td>Tyr/ΣLNAAs</td>
<td>B</td>
<td>0.1168 (0.009)</td>
<td>0.0839 (0.011)</td>
<td>-28.17**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>0.1263 (0.008)</td>
<td>0.0078 (0.001)</td>
<td>-93.82***</td>
</tr>
<tr>
<td>Phe/ΣLNAAs</td>
<td>B</td>
<td>0.1109 (0.004)</td>
<td>0.0767 (0.006)</td>
<td>-30.84**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>0.1109 (0.004)</td>
<td>0.0061 (0.001)</td>
<td>-94.50***</td>
</tr>
</tbody>
</table>

** p < 0.001, *** p < 0.0001 indicates significant change from baseline to 5-h post-treatment.
There were no significant interaction effects on any of the POMS subscales (all \(p > 0.05\)) indicating that treatment did not modify the main effects of time. In addition, there were no main effects of treatment or time on POMS subscales, except for the Vigour subscale for which ratings decreased significantly 5 h after AA ingestion regardless of condition \(F(1,19) = 8.22, p < 0.05\). Mean baseline ratings on the POMS and VAMS subscales did not differ significantly between the two testing days, indicating that our subjects did not differ on these scales for the two testing sessions (all \(p > 0.05\)) (see Table 2).

### Table 2. Mood and emotional regulation measures (mean±s.e.m.) (\(n = 20\))

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>CMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 5 h</td>
<td>Baseline 5 h</td>
</tr>
<tr>
<td><strong>POMS ratings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anger</td>
<td>4.25 (0.97)</td>
<td>4.55 (1.11)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5.55 (0.80)</td>
<td>6.70 (1.21)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>5.95 (0.72)</td>
<td>6.50 (1.01)</td>
</tr>
<tr>
<td>Depressed</td>
<td>3.60 (0.92)</td>
<td>4.60 (1.37)</td>
</tr>
<tr>
<td>Confusion</td>
<td>5.95 (0.61)</td>
<td>6.40 (0.75)</td>
</tr>
<tr>
<td>Vigour</td>
<td>15.05 (1.45)</td>
<td>14.40 (1.52)</td>
</tr>
<tr>
<td><strong>VAMS ratings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sad</td>
<td>28.00 (2.93)</td>
<td>27.63 (4.43)</td>
</tr>
<tr>
<td>Antagonistic</td>
<td>25.65 (3.99)</td>
<td>24.45 (3.98)</td>
</tr>
<tr>
<td>Mentally slow</td>
<td>40.98 (4.14)</td>
<td>41.08 (4.89)</td>
</tr>
<tr>
<td>Tense</td>
<td>25.90 (3.16)</td>
<td>32.80 (5.30)</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>30.90 (3.79)</td>
<td>33.28 (5.36)</td>
</tr>
<tr>
<td><strong>ES reaction times (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleasant</td>
<td>650.30 (25.93)</td>
<td>651.21 (26.19)</td>
</tr>
<tr>
<td>Unpleasant</td>
<td>649.46 (21.09)</td>
<td>656.46 (21.35)</td>
</tr>
<tr>
<td>Neutral</td>
<td>621.30 (21.30)</td>
<td>638.87 (24.17)</td>
</tr>
<tr>
<td><strong>IAPS valence ratings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleasant</td>
<td>6.43 (0.15)</td>
<td>6.33 (0.15)</td>
</tr>
<tr>
<td>Unpleasant</td>
<td>2.91 (0.16)</td>
<td>2.97 (0.13)</td>
</tr>
<tr>
<td>Neutral</td>
<td>5.00 (0.07)</td>
<td>5.01 (0.09)</td>
</tr>
<tr>
<td><strong>IAPS arousal ratings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleasant</td>
<td>3.70 (0.35)</td>
<td>3.54 (0.36)</td>
</tr>
<tr>
<td>Unpleasant</td>
<td>4.01 (0.27)</td>
<td>4.06 (0.30)</td>
</tr>
<tr>
<td>Neutral</td>
<td>1.87 (0.14)</td>
<td>1.81 (0.16)</td>
</tr>
</tbody>
</table>

For mood dimensions on the POMS and VAMS, larger scores represent greater intensity of the mood state. For the IAPS valence scale, higher ratings represent more pleasant feelings associated with images.

* \(p < 0.05\) indicates significant change from baseline to 5-h post-treatment in the CMD condition relative to the B condition.

There were no significant interaction effects on any of the POMS subscales (all \(p > 0.05\)) indicating that treatment did not modify the main effects of time. In addition, there were no main effects of treatment or time on POMS subscales, except for the Vigour subscale for which ratings decreased significantly 5 h after AA ingestion regardless of condition \(F(1,19) = 8.22, p < 0.05\). Mean baseline ratings on the POMS and VAMS subscales did not differ significantly between the two testing days, indicating that our subjects did not differ on these scales for the two testing sessions (all \(p > 0.05\)) (see Table 2).

### Effects of monoamine depletion vs. balanced control condition on emotional responsiveness

**ES**

A repeated-measures ANOVA revealed a significant main effect for category for the ES \(F(2,38) = 4.81, p < 0.05\) and planned comparisons revealed that reaction times were significantly different between pleasant and neutral categories \(F(1,19) = 4.83, p < 0.05\) as well as unpleasant and neutral categories \(F(1,19) = 14.705, p < 0.01\), but not between pleasant and unpleasant categories \(F(1,19) = 0.07, p > 0.05\). No significant interaction or main effect for treatment was found for the ES. In addition, no significant interaction or main effects were found for the interference data (see Table 2).

**IAPS**

Repeated-measures ANOVAs revealed significant category effects for both valence ratings \(F(1,19) = 195.68, p < 0.001\) and arousal ratings \(F(1,19) = 30.37, p < 0.001\). Planned comparisons for the valence rating scale showed significant differences between the neutral and unpleasant categories \(F(1,19) = 265.43, p < 0.001\).
Treatment interaction effects were found for valence not between the pleasant and unpleasant categories \( F(1,19) = 79.32, p < 0.001 \). For the arousal rating scale, planned comparisons revealed significant differences between the neutral and unpleasant categories \( F(1,19) = 194.33, p < 0.001 \), and the neutral and pleasant categories \( F(1,19) = 38.05, p < 0.001 \), but not between the pleasant and unpleasant categories \( F(1,19) = 0.17, p > 0.05 \). No significant category x treatment interaction effects were found for valence \( F(1,19) = 1.03, p > 0.05 \) or arousal \( F(1,19) = 0.69, p > 0.05 \) (see Table 2).

**Adverse effects**

Of the 20 subjects, one experienced nausea and vomited after CMD. However, there were no significant adverse effects between the B and CMD as assessed via a symptoms checklist that included headache, cold, hot, dizzy, sweating more than usual, blurred vision, nauseous, racing heart, dry mouth, and stomach pains.

**Discussion**

The current study was designed to explore the effects of simultaneous monoamine depletion on mood and emotional regulation in healthy participants. Simultaneous depletion of all monoamine precursors was associated with selective effects on mood. Mood changes were observed on three of the VAMS subscales but not on any of the POMS subscales, ES response times, interference effects or IAPS valence and arousal ratings.

In the current investigation, the POMS subscales revealed that participants felt less vigorous following each testing day, but did not show any depletion-specific mood changes. This is consistent with the findings of Salomon et al. (1997) who did not find any subjective mood changes with the POMS following combined ATD and AMPT depletion. However, in contrast to Salomon et al. (1997), our findings suggested that participants felt sadder, more antagonistic, and mentally slower following CMD as rated on the VAMS Happy–Sad, Amicable–Antagonistic, and Quick Witted–Mentally Slow subscales respectively. Previous studies using single depletion methods (i.e. ATD or ATPD) have also found variable effects on subjective mood measures. While mood effects have been reported on the POMS in some studies (Ellenbogan et al., 1994; Knott et al., 1999; Leyton et al., 2000; Smith et al., 1997) and/or VAMS (Leyton et al., 2000; Smith et al., 1997), others have found no mood changes using the POMS (Bennkelfat et al., 1994; Bhatti et al., 1998; Harrison et al., 2002; Oldman et al., 1994) and/or VAMS (Ellenbogan et al., 1996; Harrison et al., 2002; Oldman et al., 1994). It is likely that specific effects on subjective mood ratings are dependent on methodological differences between studies, such as AA load administered, gender-related differences, and the use/non-use of pre-treatment low-protein diet.

An interesting observation in the current study was that monoamine depletion affected some of the basic emotions, such as happy and sad. The basic emotions of happiness and sadness, fear and anger, surprise and disgust, can be thought of as the building blocks of emotion, according to the structural models of emotions (Gainotti, 2001). This is supported by a recent observation of impaired recognition of fearful facial expressions following Trp depletion (Harmer et al., 2003a) and enhanced recognition of fearful as well as happy facial expressions following serotonergic enhancement with the selective serotonin reuptake inhibitor (SSRI) citalopram (Harmer et al., 2003b).

The current study found no changes on the ES, a measure of emotional processing, following monoamine depletion. This suggests that acute manipulation of neurotransmitters may not alter mood state to the extent that might also alter emotional biases, that is likely to occur with chronic abnormalities of monoamine function, such as in depression. In support of this, studies have found that depressed patients took longer to name the colour of negative words compared to naming the colour of positive and neutral words than healthy controls (Gallardo et al., 1999; Gotlib and Cane, 1987) as well as participants with dysthymia or sad mood state (Gallardo et al., 1999). Likewise, no effects were found in the current study on the IAPS, a task designed to measure emotional responsiveness (Lang et al., 1999). It was found that participants’ ratings did not differ between treatments on this task. It might be possible that changes to emotional responsiveness did occur but were not accurately reported by participants at a conscious level. It has been suggested by Peper (2000) that the effects of emotional stimulation on subcortical activities and autonomic reactions may evade conscious awareness and verbal description. This theory is supported by our own electrophysiological study showing that augmentation of 5-HT with the SSRI citalopram does not affect subjective ratings of valence and arousal on the IAPS, but suppressed the processing of unpleasant affective stimuli in the frontal cortex and enhanced the processing of pleasant affective stimuli in the parietal–occipital cortices (Kemp et al., In Press). Similarly, Harmer et al. (2003b) showed that citalopram facilitated recognition of fear and happy facial expressions as...
evidenced by greater accuracy and reduced response times without subjective changes in mood. This difference in subconscious aspects of emotional experience and the expression of emotion has been supported by investigators who have noted that self-reports seem to diverge from the actual physiological response. Davidson (1995) suggests that information about emotion is communicated using primary neural controls, which are likely to be different from the principal neural controls employed in the experience of actual emotion. There may be a difference between the actual experience and expression of emotion, a view supported by others (Leventhal and Tomarken, 1986). Peper (2000) stated that the difference in physiological responses and self-report could be due to a restricted capacity to suitably monitor intrinsic reactions.

It is likely that in this study, significant reductions in free Trp, and plasma Tyr and Phe lead to significantly lowered 5-HT and catecholamine synthesis in the brain. Plasma-free Trp/LNAA ratio was depleted by approx. 86%, which was comparable to that achieved using the ATD method (Moore et al., 2000; Reilly et al., 1997). Similarly, plasma Tyr and Phe concentrations and Tyr/LNAA and Phe/LNAA ratios were depleted by 73–94% and the magnitude of these depletions was similar to that observed in previous studies using the ATPD method (Harmer et al., 2001; Harrison et al., 2002, In Press; Leyton et al., 1999). The ratio of precursors to other LNAAs has been suggested to be a more accurate indicator of central precursor depletion than absolute plasma precursor levels, as the levels of other LNAAs are also pertinent due to the competition for brain entry via the same AA transporter (Fernstrom et al., 1979; Moore et al., 2000; Oldendorf and Szabo, 1976). Given the large reduction in the ratio of monoamine precursors to other LNAAs, it is unlikely that the magnitude of depletion contributed to the lack of effects observed on the POMS, ES, and IAPS.

In summary, the current findings suggest that simultaneous depletion of monoamines using a monoamine precursor depletion method produces selective effects on subjective measures of mood. Additionally, these findings provide evidence that the simultaneous monoamine depletion technique may provide a useful probe to examine monoamine function in relation to mood and emotional responsiveness in humans. Previous studies in healthy, never-depressed subjects with genetic, familial and gender-related vulnerability to depression has shown evidence for greater mood-lowering effects following Trp depletion (Moore et al., 2000). It is possible that this group may also show a biological diathesis to the mood-lowering effects of simultaneous monoamine depletion.

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Statement of Interest

None.

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