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Acute Serotonin and Dopamine Depletion Improves Attentional Control: Findings from the Stroop Task

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Schizophrenia is associated with impairments of attentional control on classic experimental paradigms such as the Stroop task. However, at a basic level the neurochemical mechanisms that may be responsible for such impairments are poorly understood. In this study, we sought to investigate the influence of brain monoamine function on Stroop task performance in healthy participants using the established methods of acute dietary serotonin, dopamine, and combined monoamine depletion. The study was a double-blind placebo controlled design in which 12 healthy male participants completed the Stroop task under four acute treatment conditions: (a) balanced/placebo control, (b) acute tryptophan depletion, (c) acute tyrosine/phenylalanine depletion, and (d) acute tyrosine/phenylalanine/tryptophan depletion (combined monoamine depletion). Decreased Stroop interference indicating improved attentional control was observed after both tryptophan depletion and tyrosine/phenylalanine depletion, while there was no significant change in interference after combined monoamine depletion. Findings suggest that reduced tonic dopamine or serotonin activity within specific neural circuits (such as the striatum, anterior cingulate, or prefrontal cortex) may play a critical role in attentional control, possibly by improving gating of information via reducing noise in monoaminergic systems. These findings enhance our understanding of the neurochemical basis of attentional control and the possible cause of attentional control deficits in schizophrenia.
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INTRODUCTION

Schizophrenia is a severe, chronic and disabling psychiatric disorder prevalent in approximately 0.4–1% of the world's population (Bhugra, 2005; Jablensky, 2000). In addition to the positive and negative symptoms, patients also often experience severe disturbances in cognitive functioning (Abi-Dargham, 2004; Gold and Weinberger, 1995; Keefe *et al*, 2006). It has been suggested that cognitive deficits are the most 'functionally limiting' symptom dimension, and have the greatest impact on illness outcome in schizophrenia (Green, 1996; Green *et al*, 2000). With this recognition of cognitive impairment as a separable and unequivocal feature in schizophrenia, basic research into the neurochemical basis of this phenomena, and the development of treatments that more effectively target cognition in schizophrenia, has become an area of rapidly growing interest (Carter, 2005).

One of the most prominent cognitive deficits in schizophrenia involves patients impaired ability to flexibly adjust attention and to inhibit or control irrelevant or unwanted responses in cognitive tasks (Andreasen, 1994; Barch *et al*, 2004; Kuperberg and Heckers, 2000). Experimentally, this deficit is most evident when patients are administered paradigms such as the Stroop Color-Word Interference task (Barch and Carter, 2005; Barch *et al*, 1999a, b, 2004; Braver *et al*, 1999; Carter *et al*, 1992; Chen *et al*, 2001; Cohen *et al*, 1990; Henik and Salo, 2004). The Stroop task is a well-established measure of attentional control and response inhibition, in which participants are presented with words printed in different ink colors and are told to name the print color and to ignore the stimulus word. In the single-trial version of the Stroop task, stimuli are presented on a computer screen one at a time, and participants are

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required to name the ink color of the individual stimulus (Henik and Salo, 2004; MacLeod, 1991). When the word and the ink color conflict, that is they are incongruent, participants are slower to respond than when there is no (or less) conflict, such as on neutral or congruent trials (Stroop, 1935). This reaction time difference is referred to as Stroop interference and shows high reproducibility and robustness within and across individuals (MacLeod, 1991). In contrast, a less studied phenomenon, referred to as Stroop facilitation, occurs when participants are faster to respond to stimuli when the color is congruent with the semantic meaning of the word, than to neutral stimuli such as patches of color (Barch *et al*, 1999a). However, facilitation is considered to be a somewhat weaker phenomenon than interference (MacLeod, 1991). Research suggests that patients with schizophrenia exhibit increased facilitation, an increased number of errors and increased reaction times to all stimuli, but have no difference in interference, when compared to healthy control participants on the single-trial version of the Stroop task (Barch *et al*, 1999a, b, 2004; Carter *et al*, 1992; Chen *et al*, 2001; Henik *et al*, 2002; Nordahl *et al*, 2001; Perlstein *et al*, 1998; Taylor *et al*, 1996). These findings are thought to be as a result of a specific failure in contextual processing, whereby patients have difficulty implementing and/or maintaining a representation of the task goals over time (Barch and Carter, 2005; Barch *et al*, 1999a, b). It should be noted that the pattern of deficits observed in schizophrenic patients when using an alternate Stroop procedure (the blocked card version) is somewhat different to those observed when using the single-trial version, and this is suggested to be due to the slightly different processes inherent in these different tasks (see Henik and Salo (2004)).

While these deficits of attentional control in the Stroop task have been well characterized in schizophrenia, and have been theoretically linked to abnormalities in dopamine function (Braver *et al*, 1999; Cohen *et al*, 2002), the underlying relationships between neurochemistry and attentional control are yet to be fully determined. Although it has been extensively hypothesized that both dopamine (Abi-Dargham, 2004; Andreasen, 1994; Laurelle, 1998, 1999) and serotonin (Abi-Dargham *et al*, 1997; Kapur and Remington, 1996) play a critical role in the neurochemical underpinnings of schizophrenia and the action of antipsychotic drugs, the exact role of dopamine and serotonin on Stroop performance and attentional control is not fully characterized. It has been suggested that both dopamine and serotonin may be involved in the disturbances to attentional control observed in patients with schizophrenia (Cohen and Servan-Schreiber, 1992; Kivircik Akdede *et al*, 2005), although many of the findings are confounded by medication use and institutionalization of patients, making it difficult to know whether reported deficits are trait related, or are secondary to other factors. Therefore, in order to try and understand the role of dopamine and serotonin on attentional control, many researchers have attempted to modulate these neurotransmitter systems using pharmacological probes in healthy participants to then make inferences about the possible influence of these neurotransmitters on attentional control in schizophrenia.

A number of studies have used acute tryptophan depletion (ATD) to examine the role of serotonin on Stroop performance. This technique acutely depletes serotonin and its metabolites in humans (Carpenter *et al*, 1998; Nishizawa *et al*, 1997; Williams *et al*, 1999), and has been shown to consistently impair serotonin dependant processes such as memory consolidation and learning (Harrison *et al*, 2004; Riedel *et al*, 2002; Schmitt *et al*, 2000). To date, the studies examining the effects of ATD on Stroop task performance have been somewhat inconsistent. While some studies have reported improved performance (ie, decreased interference) after ATD (Evers *et al*, 2006; Rowley *et al*, 1997; Schmitt *et al*, 2000), other studies have found no effects (Gallagher *et al*, 2003; Horacek *et al*, 2005; Sobczak *et al*, 2002). Similarly, studies examining the role of dopamine on Stroop performance (using dopamine agonists) have also yielded inconsistent results. For example, stimulation of dopamine D_2 receptors using bromocriptine decreased Stroop interference (Roesch-Ely *et al*, 2005), while increasing synaptic dopamine with the indirect dopamine agonist, amphetamine, had no effect (Barch and Carter, 2005). Both studies, however, found no effects of dopaminergic modulation on Stroop facilitation, while the latter study also found improved reaction times to all stimuli (Barch and Carter, 2005). These conflicting findings may be due to factors such as the use of different pharmacological probes, as well as variability in the Stroop task methods used.

Therefore, to further explore the role of serotonin and dopamine on attentional control, we examined the effects of tryptophan (ie, serotonin) depletion and an analogous method of tyrosine/phenylalanine depletion (to deplete dopamine) on Stroop interference and facilitation. Acute tyrosine/phenylalanine depletion (ATPD) is a technique which has been shown to selectively decrease dopamine synthesis and release (Jaskiw and Bongiovanni, 2004; Leyton *et al*, 2004; McTavish *et al*, 1999a, 2001a, b; Mehta *et al*, 2005; Montgomery *et al*, 2003) and impair dopamine dependent cognitive processes such as working memory (Harmer *et al*, 2001; Harrison *et al*, 2004). To our knowledge, ATPD has never been used to investigate the effects of dopaminergic modulation on Stroop task performance. In addition, we also examined for the first time, the effects of simultaneous serotonin and dopamine depletion (ie, combined monoamine depletion) on Stroop interference and facilitation, using a method that was recently shown to simultaneously deplete tyrosine, phenylalanine, and tryptophan to levels that are expected to modulate central dopaminergic and serotonergic function (Nathan *et al*, 2004), and was subsequently shown to impair vigilance (Matrenza *et al*, 2004). Given the highly inconsistent findings concerning the direction of changes in Stroop performance associated with these depletion techniques, no specific predictions were made. However, in line with previous findings, it was predicted that both dopamine depletion and serotonin depletion would alter Stroop interference, but would have no effect on Stroop facilitation.

MATERIALS AND METHODS

Participants

The current study comprised 12 healthy nonsmoking male participants, aged 21–38 years (M = 25.83 years, SD = 4.67 years). Participants were recruited for the study through

university advertisements, and were considered for inclusion if they were not currently taking any medications, had no personal or family history of psychiatric disorders, had no history of substance abuse, and had no history of head injury. All participants underwent a medical examination by a registered physician prior to participation, in order to verify that they were physically and psychiatrically healthy and that they satisfied the inclusion criteria. Furthermore, all participants gave written informed consent for participation in the study, which was approved by the Swinburne University Human Research Ethics Committee.

Study Design

We used a double-blind, placebo controlled, repeated measures design in which each participant was tested under four acute treatment condition; (a) 100 g nutritionally balanced placebo control treatment (BAL), (b) acute tryptophan depletion treatment (ATD) (ie, serotonin depletion), (c) acute tyrosine and phenylalanine depletion treatment (ATPD) (ie, dopamine depletion), and (d) acute tyrosine, tryptophan, and phenylalanine depletion treatment (ie, combined monoamine depletion, CMD). Individual assignment to the order of completion of each treatment was randomized and counterbalanced using a computerized randomization program. Completion of each treatment was separated by a minimum 7-day washout period.

Amino-Acid Composition

The amino-acid composition for the depletion treatments were based on the 104 g balanced mixture developed by Young *et al* (1985) and Nathan *et al* (2004). In the current study, the balanced placebo control mixture consisted of 5.5 g of L-alanine, 3.2 g of glycine, 3.2 g of L-histidine, 8.0 g of L-isoleucine, 13.5 g of leucine, 11.0 g of L-lysine monohydrochloride, 5.7 g of L-phenylalanine, 12.2 g of L-proline, 6.9 g of L-serine, 6.5 g of L-threonine, 2.3 g of L-tryptophan, 6.9 g of L-tyrosine and 8.9 g of L-valine. L-Arginine (4.9 g), L-cysteine (2.7 g), and L-methionine (3.0 g) were encapsulated in 22 gelatin capsules and were administered separately due to their unpleasant taste. All treatment mixtures differed from the composition of the BAL mixture only in that, the mixture was deficient of L-tryptophan in the ATD treatment, the mixture was deficient of L-tyrosine and L-phenylalanine in the ATPD treatment, and in the CMD treatment the mixture was deficient of L-tryptophan, L-tyrosine, and L-phenylalanine.

Procedure

On the day before each testing session, participants were required to adhere to a low-protein diet, with their total protein consumption to be <20 g (Young *et al*, 1985). In addition, participants were also required to fast from 1900 h that evening (with the exception of the consumption of water). This procedure has been employed in many previous studies as it has been suggested that it may enhance the effect of monoamine depletion and lessen the variability in baseline monoamine levels (Bel and Artigas, 1996; Bell *et al*, 2001; Harrison *et al*, 2004; Hood *et al*, 2005; Reilly *et al*, 1997). On arrival for testing, participants were required to complete the Visual Analog Mood Scales (VAMS) and then had a small sample of blood taken (12 ml) to establish baseline mood and amino-acid levels, respectively. Participants were then administered the amino-acid drink and capsules. The powdered amino acids were mixed with 180 ml of orange juice a few minutes prior to oral administration. Participants consumed the 22 capsules and then swallowed the amino-acid suspension immediately after. The participants were advised to drink this as quickly as possible given the unfamiliar and unpleasant taste. Upon completion of ingestion of the amino acids, participants were provided with sugar free chewing gum and a glass of water to cleanse the mouth. The process of amino-acid administration took approximately 10 min.

To enable the depletion effects to occur, participants rested for the following 4 h. During this time, they were allowed to consume water freely, but were restricted from any physical activity. At 2 h post-amino-acid administration, participants were provided with a low-protein snack of carrots and apples. At 4 h post-ingestion participants completed the Stroop task, which took approximately 10 min. This 4 h latency period for testing was chosen to coincide with the timing of maximal monoamine depletion determined in previous research detailing the time course of monoamine depletion in rats (Bel and Artigas, 1996; McTavish *et al*, 1999b), human plasma (McTavish *et al*, 1999b; Moja *et al*, 1996; Sheehan *et al*, 1996), and cerebrospinal fluid (Carpenter *et al*, 1998; Williams *et al*, 1999). Following completion of the Stroop task, participants were again administered the VAMS to examine mood changes following treatment, and were required to give another sample of blood (12 ml) in order to establish the levels of amino-acid depletion achieved. This was followed by further testing (electrophysiological recording) which will not be discussed in this study. Upon conclusion of the testing procedure, participants were provided with high-protein snacks in order to replenish their amino-acid levels. Participants resumed their normal diet between each of the testing sessions.

Stroop Task

Following the task used by Barch *et al* (1999a), we used a single trial version of the Stroop task consisting of 96 trials; with 24 (25%) congruent trials, 24 (25%) incongruent trials, and 48 (50%) neutral trials. Each trial consisted of a stimulus printed in one of four colors: red, green, blue, or purple. The congruent stimuli consisted of each of the four color names printed in its own color (eg, the word red printed in red ink). The incongruent stimuli comprised each of the four color words printed in one of the three remaining colors (eg, the word red printed in blue ink). The neutral stimuli consisted of four color unrelated words (dog, bear, tiger, and monkey) presented in one of the four colors. These neutral words were from a single semantic category in order to eliminate semantic confounds (MacLeod, 1991), and matched the response set color words in terms of number of letters and frequency of occurrence in the English language (Francis and Kucera, 1982). The use of these animal words as the neutral stimuli, rather than patches of color, was due to previous research suggesting

that these neutral stimuli are more likely to produce a Stroop facilitation effect in healthy participants (Barch and Carter, 2005; Barch *et al*, 1999a, 2004).

In each testing session, participants were seated 60 cm from a computer screen and told that they would view a series of stimuli one at a time, and that their task was to name the color in which the stimulus was printed as quickly and accurately as possible (Barch *et al*, 1999a). Each stimulus was presented on the screen for 2000 ms, and was then replaced by a fixation cross for 2000 ms. Regardless of the participant's reaction time to respond, a new trial began 4000 ms after the onset of the previous stimulus. This presentation was employed to ensure the task proceeded at a fixed pace for all participants (Barch *et al*, 1999a). Presentation of the stimuli was in a pseudo-random order. Participants were required to wear a headset with an attached microphone that recorded, and relayed to the computer, the reaction time for onset of the verbal responses. The verbal responses were monitored for accuracy (with the recording of errors in the naming of ink color) by the experimenter. To reduce any practice effects, each participant also completed a short practice version of the task (consisting of 25 stimuli) on two occasions before testing on the day of the first treatment session. Furthermore the order of treatment administration was randomized to further minimize practice effects, such that three participants received the order ABCD, three received the order BADC, three received the order CDAB, and three received the order DCBA.

Subjective Mood Assessment

Subjective mood ratings were obtained using the Visual Analog Mood Scales (Bond and Lader, 1974). The VAMS consists of 16 bipolar scales, anchored at each end of a 100 mm line. In factor analyses these scales reduce to three subscales: alertness (nine items), contentedness (five items), and calmness (two items).

Biochemical Analysis

The venous blood samples (12 ml) were separated by centrifugation for 20 min at 3000 r.p.m. within a few minutes of collection. Plasma was then yielded from the centrifuged samples and stored at -20°C. Concentrations of free amino acids tryptophan (TRYP), tyrosine (TYR), phenylalanine (PHE), valine (VAL), leucine (LEU), and isoleucine (ILE) in plasma were determined using precolumn derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by separation of the derivatives and quantification by reversed phase high-performance liquid chromatography (RP-HPLC) (Cohen, 2001). All amino acids except TRP were detected by fluorescence, while TRP required UV detection. VAL, LEU, and ILE levels were analyzed to calculate the ratio of plasma TRYP, TYR, or PHE, to other large neutral amino acids (LNAAs).

Prior to derivatization, the plasma samples (100 μl) were diluted 1 : 1 with internal standard solution and deproteinized by ultrafilration through a membrane with a 10 kDa nominal molecular weight cutoff (ultrafree MC with PL-10 membrane, Millipore, MA, USA). The filtrate (100 μl) was then subjected to AQC derivatization and HPLC analysis using the Waters AccTag amino-acid analysis system (Waters Corporation, MA, USA) (Cohen, 2001).

Statistical Analysis

Owing to the presence of a number of deviations from the normal distribution, and skewed variables within the biochemical data (which were unaltered with application of transformations), the biochemical data was analyzed with nonparametric Friedman's tests. Significant results were followed-up with the Wilcoxon Signed Ranks Test. To investigate whether the Stroop task used in the present study was able to elicit interference and facilitation effects, a one-way repeated measures ANOVA, with stimulus RT (neutral, congruent, and incongruent) as the within subjects variable, was conducted on the data from the balanced placebo control treatment only. Stroop interference (mean incongruent RT—mean neutral RT) and facilitation (mean neutral RT—mean congruent RT) were computed for each participant. Computed scores for interference and facilitation, as well as reaction times to the three types of stimuli, were all analyzed with separate one-way repeated measures ANOVA's, with treatment as the within subjects variable. As there was almost no variability concerning errors made during the task (ie, most participants committed no errors), analysis of the error data was excluded. In order to test for any relationship between level of amino-acid depletion and Stroop performance, Pearson *r* and Spearman *rho* correlational analyses were conducted. For each depletion treatment, percentage depletion ((post-level–pre-level)/pre-level)*100) of each amino acid (TRYP, TYR, and PHE) was correlated with interference and facilitation scores for that treatment. VAMS data was analyzed using a four (treatment: BAL, ATD, ATPD, CMD) by three (subscale: alertness, contentedness, calmness) by two (time: baseline, post-treatment) repeated measures ANOVA.

RESULTS

Amino-Acid Concentrations

Friedman's test showed a significant effect of treatment on the plasma levels of the amino acids (TRYP, TYR, PHE) ($\chi^2(23) = 148.41$, $p < 0.001$). Wilcoxon Signed Ranks tests (with significance set at $p < 0.05$) demonstrated that, when compared to baseline concentrations of amino acids, there were significant increases in TRYP, TYR and PHE after the BAL treatment, and significant decreases in these amino acids after the CMD depletion treatment. After the ATD treatment there was a significant decrease in concentrations of TRYP, and no significant change in concentrations of TYR, when compared to baseline levels. After the ATPD treatment there was a significant increase in TRYP levels, as well as significant decreases in TYR and PHE levels, when compared to baseline concentrations. For percentages see Table 1.

A separate Friedman's test also revealed a significant effect of treatment on the amino acid ratios ($\chi^2(23) = 138.06$, $p < 0.001$). Wilcoxon Signed Ranks Test (with significance set at $p < 0.05$) indicated that after the BAL treatment there was a significant decrease in the ratio of TYR:$\sum$LNAAs, but no significant change in the ratios of

Table 1 Plasma Concentrations of Amino Acids (μmol/l) (Mean (SD))

Amino acid	Treatment condition	Baseline	4 h post-treatment	Percent change
Plasma free TRYP	BAL	4.53 (1.88)	10.94 (3.42)	141.50**
	CMD	4.30 (1.68)	1.37 (0.52)	−68.14**
	ATD	5.10 (1.61)	0.80 (0.23)	−84.31**
	ATPD	4.23 (1.92)	15.07 (7.78)	256.26**
Plasma TYR	BAL	49.06 (11.35)	97.41 (27.26)	98.55**
	CMD	47.33 (10.83)	10.60 (3.33)	−77.60**
	ATD	65.49 (46.78)	96.74 (36.59)	47.72
	ATPD	46.73 (14.02)	12.93 (4.42)	−72.33**
Plasma PHE	BAL	44.96 (9.31)	101.74 (43.86)	126.29**
	CMD	43.90 (8.77)	8.04 (3.12)	−81.69**
	ATD	50.31 (14.51)	114.37 (52.31)	127.33*
	ATPD	41.76 (9.22)	7.77 (1.76)	−81.39**
TRYP/∑LNAAs	BAL	0.015 (0.006)	0.012 (0.003)	−20.00
	CMD	0.015 (0.005)	0.002 (0.001)	−86.67**
	ATD	0.015 (0.005)	0.001 (0.001)	−93.33**
	ATPD	0.014 (0.008)	0.016 (0.006)	14.29
TYR/∑LNAAs	BAL	0.162 (0.031)	0.109 (0.024)	−32.72**
	CMD	0.161 (0.033)	0.015 (0.005)	−90.68**
	ATD	0.162 (0.030)	0.131 (0.060)	−19.14
	ATPD	0.149 (0.059)	0.015 (0.005)	−89.93**
PHE/∑LNAAs	BAL	0.149 (0.022)	0.111 (0.033)	−25.50
	CMD	0.149 (0.023)	0.011 (0.004)	−92.62**
	ATD	0.142 (0.034)	0.140 (0.034)	−1.41
	ATPD	0.134 (0.027)	0.009 (0.003)	−93.28**

*$p<0.05$, **$p<0.02$ (Post treatment compared to baseline).
BAL, balanced condition; CMD, combined monoamine depletion condition; ATD, acute tryptophan depletion condition; ATPD, acute tyrosine/phenylalanine depletion condition.

TRYP:∑LNAAs or PHE:∑LNAAs, when compared to baseline amino acid ratios. There was a significant reduction from baselines in all amino acid ratios, after the CMD treatment. There were no significant decreases in the ratio of TYR:∑LNAAs or PHE:∑LNAAs after ATD; however, there was a significant reduction in the ratio of TRYP:∑LNAAs, when compared to baseline. Lastly, after the ATPD condition there were significant reductions in the ratio of TYR:∑LNAAs and PHE:∑LNAAs; however, there was no significant change in the ratio of TRYP:∑LNAAs, when compared to baseline. For percentages see Table 1.

In order to examine whether levels of amino-acid depletion achieved after the CMD treatment were as large as in the ATD and ATPD treatments, percentage change of the amino acid ratio ((post−pre/pre)*100) were compared across the treatment conditions. Wilcoxon Signed Ranks test (with significance set at $p<0.05$) revealed no significant difference in percentage change in the ratios of TYR:∑LNAAs or PHE:∑LNAAs between ATPD and CMD treatments. Similarly, there was no significant difference in the percentage change of the ratio of TRYP:∑LNAAs, between ATD and CMD treatments.

Stroop Performance

The one-way repeated measures ANOVA, with Greenhouse Geisser correction, of the stimulus reaction times in the balanced control treatment revealed a significant main effect of stimulus type ($F(1.34,17.39)=14.27$, $p=0.001$, *partial* $\eta^2=0.52$). Simple planned contrasts revealed that this significant main effect of stimulus was due to the presence of a significant difference between reaction times to incongruent stimuli when compared to neutral stimuli (ie, Stroop interference) ($F(1,13)=58.93$, $p<0.001$, *partial* $\eta^2=0.82$), but no significant difference between reaction times to congruent stimuli when compared to neutral stimuli (ie, Stroop facilitation) ($F(1,13)=0.47$, $p=0.51$, *observed power* $=0.10$) (ie, Table 2). Whereas a significant facilitation effect was not observed in the present study, and results are consistent with observed differences being attributable to expected sampling variation, the power was such that a real, but small, effect may have gone undetected.

The one-way repeated measures ANOVA of the reaction times revealed no significant main effect of treatment on reaction times to incongruent stimuli ($F(3,33)=1.18$, $p=0.33$, *observed power* $=0.29$), reaction times to congruent stimuli ($F(3,33)=1.16$, $p=0.34$, *observed power* $=0.28$) or reaction times to neutral stimuli ($F(3,33)=1.18$, $p=0.33$, *observed power* $=0.29$).

The one-way repeated measures ANOVA of the interference scores revealed a significant main effect of treatment ($F(3,33)=4.89$, $p=0.006$, *partial* $\eta^2=0.31$), with simple planned contrasts revealing a no significant difference in interference under the CMD treatment when compared to the BAL treatment ($F(1,11)=4.53$, $p=0.06$, *observed power* $=0.49$), and significant decreases in interference under both the ATD treatment ($F(1,11)=10.53$, $p=0.01$, *partial* $\eta^2=0.49$) and the ATPD treatment ($F(1,11)=7.39$, $p=0.02$, *partial* $\eta^2=0.40$), when compared to the BAL condition. In contrast, the one-way ANOVA of the facilitation scores revealed no significant main effect of treatment ($F(3,33)=0.31$, $p=0.82$, *observed power* $=0.10$) (Figure 1, Table 2).

Correlations between Stroop Performance and Depletion Levels

There were no significant correlations (Pearsons and Spearmans) between levels of depletion and Stroop performance under each treatment condition (all p's >0.05).

Subjective Mood

There were no significant changes in mood following any of the treatment conditions for VAMS subscales alertness, contentedness and calmness (all p's >0.05) (Table 3).

Table 2 Means and Standard Deviations of Stroop Task Measures Under Each Treatment Condition (Mean (SD))

	BAL condition	CMD condition	ATD condition	ATPD condition
Neutral RT (ms)	876.99 (171.82)	860.18 (157.02)	894.19 (194.54)	902.67 (184.96)
Incongruent RT (ms)	960.47 (161.16)	915.88 (164.78)	922.63 (179.73)	932.20 (171.03)
Congruent RT (ms)	866.31 (164.63)	835.17 (144.78)	865.10 (204.52)	889.32 (182.32)
Facilitation (neutral-congruent)	10.68 (24.61)	25.01 (14.07)	29.09 (19.63)	13.35 (18.51)
Interference (incongruent-neutral)	83.48 (9.97)	55.69 (7.32)	28.44 (14.71)*	29.53 (14.63)*

BAL, balanced control condition; CMD, combined monoamine depletion; ATD, acute tryptophan depletion; ATPD, acute tyrosine/phenylalanine depletion.
*Indicates significant differences between ATD or ATPD conditions in comparison to BAL condition ($p<0.05$).

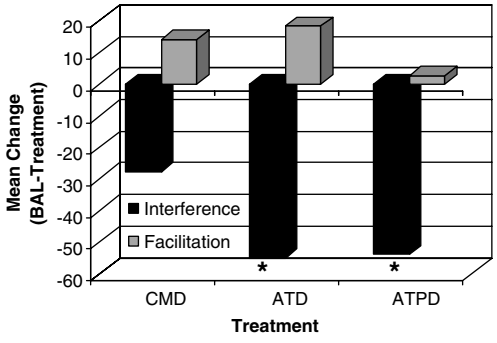

Figure 1 Mean change in interference and facilitation scores for each depletion treatment condition relative to the BAL treatment condition. *Indicates significant change in the ATD and ATPD treatments relative to BAL treatment; $p<0.05$. BAL = Balanced control condition, CMD = Combined monoamine depletion, ATD = Acute tryptophan depletion, ATPD = Acute tyrosine/phenylalanine depletion.

DISCUSSION

The present study examined the effects of acute serotonin and dopamine depletion on performance of the Stroop task in healthy participants. In addition this study examined the effects of simultaneous serotonin and dopamine depletion on Stroop task performance. Improvements in Stroop performance, as measured by decreased interference, were observed after independent dopamine and serotonin depletion. Simultaneous depletion of both dopamine and serotonin produced a level of interference that was not significantly different from the balanced control, and was intermediate between the balanced control and the levels produced by individual depletions. These effects were observed independent of any effects on mood, consistent with growing evidence for lack of mood changes following amino-acid depletion methods in healthy subjects (see Harrison *et al*, 2002). Thus, our findings suggest an important role for dopamine and serotonin in the neurochemical basis of attentional control.

Acute tryptophan depletion resulted in a 93% decrease in the ratio of tryptophan to other LNAA's (TRYP/LNAA), which is thought to be a more accurate indicator of brain serotonin concentrations than absolute plasma levels of tryptophan (Oldendorf and Szabo, 1976; Fernstrom *et al*, 1979). This decrease in TRYP/LNAA ratio is comparable to, and in fact exceeds that obtained in many studies (Schmitt *et al*, 2000; Leyton *et al*, 1999, 2000). Decreases of tryptophan of comparable magnitude have been shown to significantly affect central serotonin function, as evidenced by significant decreases in CSF concentration of serotonin metabolites (Carpenter *et al*, 1998; Williams *et al*, 1999), moderate decreases in brain serotonin synthesis (Moja *et al*, 1989; Nishizawa *et al*, 1997), and modulation of serotonin-dependent cognitive processes such as declarative episodic memory (Schmitt *et al*, 2000; Harrison *et al*, 2004). Following tryptophan depletion, we observed a significant reduction of Stroop interference, but no effects on Stroop facilitation or overall reaction times. The decrease in Stroop interference following tryptophan depletion indicates improved Stroop task performance, presumably through mechanisms of enhanced attentional control. Importantly, these effects were independent of speed of processing, as tryptophan depletion did not modulate overall reaction time performance. These findings are consistent with a number of studies which have also found decreased Stroop interference following tryptophan depletion (Evers *et al*, 2006; Schmitt *et al*, 2000). A relationship between serotonin and attentional control is also supported by studies in rats using the 5 choice serial reaction time task (5-CSRTT) in which enhanced premature responses (a measure of failure of behavioral inhibition) have been noted under conditions of increased serotonin neurotransmission (Dalley *et al*, 2002; Koskinen *et al*, 2003; Koskinen and Sirvio, 2001), particularly in the infralimbic/prelimbic regions of the medial prefrontal cortex (Dalley *et al*, 2002). Similarly, it has been found that increased serotonergic function can increase premature responses in a behavioral inhibition task in healthy humans (Del-Ben *et al*, 2005). Together these findings suggest that the serotonergic system within specific areas of the cortex may play an important role in attentional control, with reduced serotonergic transmission associated with greater attentional control or behavioral inhibition.

Interestingly, in a recent fMRI study (Evers *et al*, 2006), the effect of tryptophan depletion on Stroop interference was also associated with concurrent increased activation of the anterior cingulate and lateral prefrontal cortices, two brain regions thought to be critically involved in the performance of this task (eg, see Botvinick *et al* (2004)). It has been previously suggested that this improved performance may be due to the removal of the inhibitory actions of serotonin over cortical arousal (Schmitt *et al*, 2000). To this end, because serotonin appears to act to promote cortical de-arousal systems (Robbins, 1997), decreasing serotonergic function would reduce this inhibitory function thereby improving arousal and attention (Robbins, 1997).

Table 3 Means and SD of VAMS Scores, Pre and Post Each Treatment Condition (Mean (SD))

Subscale	Time of measurement	BAL condition	CMD condition	ATD condition	ATPD condition
Alertness	Pre-treatment	34.22 (21.75)	32.49 (18.96)	37.69 (21.27)	34.96 (17.43)
	Post-treatment	38.83 (20.35)	45.09 (19.06)	39.85 (23.03)	42.39 (22.06)
Contentedness	Pre-treatment	27.71 (18.86)	27.83 (17.00)	28.19 (14.59)	28.66 (15.30)
	Post-treatment	27.44 (13.02)	30.81 (14.27)	31.05 (13.14)	33.06 (15.31)
Calmness	Pre-treatment	23.46 (14.93)	23.79 (14.62)	28.82 (19.43)	28.29 (18.72)
	Post-treatment	25.18 (14.22)	26.14 (14.64)	32.50 (19.50)	27.79 (20.33)

BAL, balanced control treatment; CMD, combined monoamine depletion; ATD; acute tryptophan depletion; ATPD, acute tyrosine/phenylalanine depletion.

Furthermore, it has been shown that a close interaction exists between the serotonergic and noradrenergic systems, such that destruction or inhibition of serotonergic neurons results in an activation of noradrenergic neurons (McRae-Degueurce *et al*, 1985; Tian *et al*, 1993). Therefore, it is possible that acute tryptophan and serotonin depletion in the current study also resulted in indirect activation of noradrenergic function, leading to greater attentional control and improved Stroop task performance. In support, increasing noradrenergic function (via noradrenergic reuptake inhibition) has been shown to improve response inhibition in a stop signal task (Chamberlain *et al*, 2006).

Like ATD, acute tyrosine/phenylalanine depletion resulted in an 83% decrease in the ratio of tyrosine to other LNAA's (TYR/LNAA) and a 93% decrease in the ratio of phenylalanine to other LNAA's (PHE/LNAA). These depletion levels were similar in magnitude to those observed in previous studies using the TYR/PHE depletion method (plasma TYR/PHE: 53–76% depletion; TYR/PHE to LNAA ratio: 99% depletion) (Leyton *et al*, 1999, 2000; Harmer *et al*, 2001). Previous studies in animals with TYR depletion between 30 and 40%, have shown significant reductions in catecholamine metabolites (Palmour *et al*, 1998) and catecholamine synthesis in the brain (Jaskiw and Bongiovanni, 2004; McTavish *et al*, 1999a). In humans, depletion of TYR/PHE to this magnitude has been shown to cause significant increases in plasma prolactin levels (an indirect measure of dopamine function) (Harmer *et al*, 2001; Harrison *et al*, 2004) and to impair dopamine dependent cognitive processes such as spatial working memory function (Harmer *et al*, 2001) due to reductions in 'tonic' dopamine. Depletion of dopamine with tyrosine/phenylalanine depletion in the present study also resulted in a decrease in Stroop interference, with no changes in Stroop facilitation or overall reaction times. The improved Stroop performance following dopamine depletion (like serotonin depletion) suggests greater attentional control, as these effects were observed independent of effects on general speed of information processing (ie, overall reaction time). These findings are somewhat contrary to the findings of Roesch-Ely *et al* (2005) who found decreased Stroop interference with stimulation of D_2 receptors. These findings are also contrary to molecular imaging studies (ie, PET Imaging with [^{18}F]FDOPA) in which a negative association between regional [^{18}F]FDOPA uptake (a measure of dopamine synthesis) and Stroop interference has been found in normal control subjects and patients with Parkinson's disease and schizophrenia (Bruck *et al*, 2005, 2001; McGowan *et al*, 2004).

The inconsistency in the findings may be related to differences in the potency and specificity of the pharmacological probes used to manipulate dopamine function and consideration of the impact of global *vs* regional changes in dopamine function on Stroop performance and attentional control. For instance, tyrosine/phenylalanine depletion is thought to affect global brain dopamine function via modulation of the synthesis and transport processes (Fadda, 2000; Oldendorf and Szabo, 1976; Reilly *et al*, 1997), while the method used by Roesch-Ely *et al* (2005), that is the administration of a dopamine D_2 receptor agonist, is thought to affect dopaminergic function through stimulation of D_2 receptors specifically, at either cortical or striatal areas. Further, given that D_2 receptors are found both pre- and post-synaptically on dopamine neurons in different agonist affinity states (Wolf and Roth, 1987), the resultant effect on dopamine neurotransmission may vary depending on the site of action and the dosage. Consistent with this proposal, and the findings of the current study, are observations from a number of studies in marmosets indicating differential effects of frontal *vs* striatal dopamine depletion on cognitive processing (Crofts *et al*, 2001; Roberts *et al*, 1994). Whilst 6-OHDA lesions to the prefrontal cortex caused increased distractibility and impairment in maintenance of an attentional set (Crofts *et al*, 2001; Roberts *et al*, 1994), lesions of the striatum induced greater focusing on the relevant perceptual dimension during maintenance of an attentional set (Crofts *et al*, 2001). That is, marmosets with decreased striatal dopamine function were significantly more task engaged than control marmosets (Crofts *et al*, 2001). In addition, findings from the 5-CSRTT indicate that premature responding (or failure of behavioral inhibition) can be decreased in rats by the dopamine D_2 receptor agonist quinpirole, which is thought to decrease dopaminergic neurotransmission by stimulating presynaptic dopamine D_2 receptors (Passetti *et al*, 2003); while increasing dopamine neurotransmission with amphetamine and cocaine (Cole and Robbins, 1987; Harrison *et al*, 1997; van Gaalen *et al*, 2006) and with dopamine reuptake inhibitors (van Gaalen *et al*, 2006) increases premature responding, an effect that is suppressed with dopamine D_2

receptor antagonism (van Gaalen *et al*, 2006) and dopamine depletion (Cole and Robbins, 1987). Together, these findings suggest that the findings by Roesch-Ely *et al* (2005) of decreased Stroop interference (ie, improved attentional control) with stimulation of D_2 receptors may reflect a presynaptic mechanism leading to decreased dopamine neurotransmission. This is also supported by the evidence that haloperidol (a D_2 antagonist which inhibits post-synaptic dopamine transmission) has been shown to similarly reduce Stroop interference in healthy participants (Williams *et al*, 1996).

Our findings may also be consistent with the growing evidence that tasks which are associated with time constraints, or are dependent on processing within short durations, and those that rely on suppressing attention to competing information (such as the Stroop task), may be linked to dopaminergic modulation of the striatum (and basal ganglia) or fronto-striato-thalamic circuitry (for a review see Cropley *et al*, 2006). Therefore, it is possible that, consistent with McTavish *et al* (1999a), our findings relate to a predominant effect of tyrosine/phenylalanine depletion on dopamine function within the striatum. Indeed, recently Mehta *et al* (2005) demonstrated that tyrosine/phenylalanine depletion predominantly depleted dopamine in the striatum (indicated by an increase in D_2 receptor binding measured with PET imaging using [^{11}C]raclopride) and this was directly correlated with changes in cognitive function. Performance of the Stroop task appears to engage a widely distributed network of regions (Harrison *et al*, 2005), with studies consistently reporting activation of medial (ie, anterior cingulate cortex) and lateral prefrontal cortices (for a review see Neumann *et al*, 2005). Therefore, it is likely that changes in dopaminergic neurotransmission within specific neural circuits linked to attentional control (such as the striatum, anterior cingulate, or prefrontal cortex) may be more specifically linked to improved performance. The role of dopamine in attentional control is also consistent with the model of dopamine function and cognitive control suggested by Braver *et al* (1999). In this model, dopamine acts as a gating mechanism to prefrontal cortex attentional control, such that increased noise level in mesocortical dopamine induces deficits in maintenance and updating of context (which is critical for cognitive control). Such a disturbance was linked to both increased tonic and decreased phasic dopamine activity. Our findings of improved cognitive control following decreases in tonic dopamine, fits with such a model. Future studies investigating the relationship between Stroop performance, pharmacological probes for tonic (ie, dopamine depletion methods) and phasic dopamine (ie, amphetamine challenge) and PET imaging studies of dopamine function (ie, [^{18}F]FDOPA uptake in the striatum, cingulate, and prefrontal cortex) would further enhance our understanding of the relationship between dopamine function and neuroanatomical substrates of cognitive control.

Combined monoamine depletion with acute tyrosine/phenylalanine/tryptophan depletion resulted in a 86% decrease in the ratio of tryptophan to other LNAA's, as well a 90% decrease in the ratio of tyrosine to other LNAA's, and a 92% decrease in the ratio of phenylalanine to other LNAA's. These decreases were not statistically different to those achieved with tryptophan depletion or tyrosine/phenylalanine depletion alone, suggesting that all three monoamine precursors were simultaneously depleted to levels that are expected to affect central dopaminergic and serotonergic function (Nathan *et al*, 2004). However, simultaneous depletion of serotonin and dopamine did not result in significant changes in Stroop interference, Stroop facilitation or reaction times. An apparent non-significant improvement in Stroop interference was observed ($p = 0.057$), however, the power was low in this comparison, most likely due to the small effect size. It is possible that this attenuated effect may have been due to a complex interaction between the serotonin and dopamine systems. Indeed, both systems have been shown to interact at a neuronal level, although the functional relevance of this interaction has not been fully determined (Costall *et al*, 1976; Jones *et al*, 1981; Parsons and Justice, 1993; Soubrie *et al*, 1984). It is possible that tryptophan depletion may result in the removal of serotonin's inhibitory influence on dopamine and noradrenaline neurotransmission (Gately *et al*, 1985; Lucki and Harvey, 1976; McRae-Degueurce *et al*, 1985; Segal, 1976; Tian *et al*, 1993), and this disinhibition is counteracted by simultaneous tyrosine depletion (impacting upon dopamine neurotransmission), thus resulting behaviorally in an attenuated effect on cognitive control.

In the current study, there was insufficient power to detect a small but real facilitation effect in the healthy participants, if it were there. Obviously this would have hindered our ability to detect any changes in Stroop facilitation under the different treatment conditions, although previous studies have also not found any changes in facilitation following dopaminergic or serotonergic modulation (Roesch-Ely *et al*, 2005; Schmitt *et al*, 2000). Despite this, future research may consider using faster stimulus presentation, or a larger sample size, in order to reproduce this phenomenon of facilitation (Barch *et al*, 2004), and to extend our observations. In addition, we also found no significant correlations between the level of tryptophan or tyrosine/phenylalanine depletion in plasma and Stroop performance. However, the extent to which changes in plasma precursor levels reflect corresponding changes in neurotransmitter concentrations in the brain remains unresolved. There is some evidence that peripheral precursor levels are poor correlates of central neurotransmitter levels and associated cognitive changes (Mehta *et al*, 2005), thus it is not all that surprising that they did not correlate with Stroop performance in the current study.

Given that the amino-acid depletion techniques are thought to alter global brain neurotransmitter function (Fadda, 2000; Oldendorf and Szabo, 1976); the current study was limited in that it could not directly test the hypotheses regarding the effects of region specific neurotransmitter disturbances in schizophrenia. Thus, such acute depletion techniques are not appropriate models of the hypothesized neurotransmitter disturbances in schizophrenia (such as cortical *vs* striatal dopamine dysfunction). However, this study has aided our understanding of the effects of serotonin and dopamine modulation on Stroop performance, which in turn can be used to make future inferences about the possible causes of disturbances to Stroop task performance in schizophrenia. The findings of the current study suggest that decreases of overall dopamine or serotonin can improve attentional control, and thus treatments

causing similar changes in dopamine and serotonin neurotransmission may be of use in ameliorating the disturbances to attentional control in schizophrenia. Furthermore, given that global depletion of serotonin and dopamine in the current study did not replicate the deficits observed in schizophrenia using the single-trial Stroop task, and the administration of amphetamine (which similarly causes global changes in dopamine neurotransmission) to patients with schizophrenia also did not abolish these specific deficits (Barch and Carter, 2005), it suggests that Stroop abnormalities in schizophrenia may be due to dopamine and serotonin dysfunction in specific pathways (ie, increased striatal dopamine). Furthermore, the findings suggest that the function of both neurotransmitters should be considered in future models of the neurochemical basis of Stroop task disturbances in schizophrenia.

In conclusion, the findings of the current study suggest that reduced tonic serotonin or dopamine, possibly in the frontal cortex and striatum respectively, may lead to improved attentional control, through greater focusing on the relevant perceptual dimension (ie, color naming). These findings go towards increasing out understanding of the neurochemical basis of attentional control, and highlight the necessity for basic neurochemical studies in healthy participants in order to improve our understanding of the nature of cognitive deficits in schizophrenia.

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