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Mood state moderates the role of serotonin in cognitive biases

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Abstract

Reduction of the monoamine serotonin (5-HT) via the dietary manipulation of tryptophan (acute tryptophan depletion; ATD) has been shown to induce negative cognitive biases similar to those found in depression in healthy individuals. However, evidence also indicates that there can be positive effects of ATD on both mood and reinforcement processing. Here, we present two separate studies, with remarkably similar findings, which may help explain these discrepancies. In both experiments, we assessed cognitive biases following experimentally induced mood states under both a balanced amino acid drink (BAL) and ATD. A significant interaction between treatment, mood state and cognitive bias was observed in both experiments. In the first experiment, subjects undergoing positive mood induction demonstrated a positive cognitive bias on BAL, which was abolished by ATD. The same effect was observed in subjects undergoing neutral mood induction in the second experiment. These effects replicate findings in healthy individuals undergoing ATD. Subjects undergoing negative mood induction, by contrast, demonstrated the opposite pattern of results; in both experiments, they showed no bias under BAL but induction of a positive cognitive bias by ATD. These results mimic previous findings in currently depressed patients undergoing ATD. We therefore suggest that mood state moderates the effect of ATD on cognitive biases. This, in turn, has important implications for the understanding of the role of 5-HT in psychiatric disorders.

Key words

acute tryptophan depletion; bipolar disorder; cognition; depression; mania; mood; serotonin

Introduction

The monoamine serotonin (5-HT) has long been implicated in the modulation of affective processing (Cools, *et al*., 2008). In particular, reduced 5-HT has been consistently linked to depression (Deakin, *et al*., 1990; Owens and Nemeroff, 1994), anxiety (Deakin, 1998a; b) and negative mood (Van der Does, 2001; van der Veen, *et al*., 2007). One way to experimentally manipulate 5-HT is via the acute tryptophan depletion (ATD) procedure, which reduces central 5-HT by depriving the brain of tryptophan, the chemical precursor to 5-HT (Young, *et al*., 1985; Carpenter, *et al*., 1998). Perhaps surprisingly, however, several ATD studies have demonstrated that low serotonin does not lead directly to negative mood (Ruhe, *et al*., 2007; Robinson and Sahakian, 2008a). Although ATD can evoke negative mood in subjects who have recovered from an episode of depression, it has no consistent effects on the mood of subjects who have never suffered from depression or in patients with bipolar disorder (Van der Does, 2001; Booij, *et al*., 2003; Robinson and Sahakian, 2008b). Furthermore, ATD has actually been shown to provoke positive mood in subjects who are currently depressed (Delgado, *et al*., 1994; Price, *et al*., 1998). These apparently contradictory findings, alongside the observation of reduced 5-HT in both manic (Mendels, *et al*., 1972; Young, *et al*., 1994; Shiah and Yatham, 2000) and depressed (Owens and Nemeroff, 1994) patients, pose an intriguing challenge to the understanding of the precise role of 5-HT in mood regulation.

Neuropsychological studies using ATD reflect this diversity of findings. ATD has been shown to remove positive motivational biases (Cools, *et al*., 2005; Roiser, *et al*., 2006) to reduce reward sensitivity (Rogers, *et al*., 2003) and to enhance punishment

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prediction (Cools, *et al*., 2007) in healthy controls. But serotonin reduction has also been shown to induce positive motivational biases in individuals with the 'long' allele of the serotonin transporter (5HTTLPR) polymorphism (Roiser, *et al*., 2006) and increase reward-sensitivity in animals (Harrison, *et al*., 1997; Tran-Nguyen, *et al*., 2001).

In order to fully understand the role of 5-HT, it is therefore important to reconcile these discrepancies. One possibility is that the role of 5-HT in cognition depends on additional modulating factors (Baron and Kenny, 1986). In light of differential effects of ATD in healthy and depressed individuals, we hypothesised that mood state might be a factor that modulates the effect of 5-HT on cognition.

To test this hypothesis, we assessed the interaction between ATD and induced mood using two paradigms sensitive to cognitive biases in two separate experiments. In the cued-reinforcement reaction-time task (CRRT), a positive cognitive bias is expressed in a faster reaction time in response to anticipated reward. This bias is abolished by ATD (Cools, *et al*., 2005; Roiser, *et al*., 2006). In the self-referent encoding/retrieval task (SRET), a positive bias is demonstrated by increased recall of positive (relative to negative or neutral) self-referent words (Gilboa, *et al*., 1997; Timbremont and Braet, 2004; Ramel, *et al*., 2007; Hayden, *et al*., 2008). This task has not, to the best of our knowledge, been examined in healthy individuals undergoing ATD, but as subjects demonstrate a positive bias at baseline, we predicted that it would mimic the pattern seen on the CRRT.

In a double-blind placebo-controlled crossover design, all subjects received ATD and a balanced amino acid drink (BAL) on two separate visits. In the first experiment, one group of subjects underwent a negative mood induction procedure (MIP) on both visits, a second group underwent a neutral MIP on both visits and a third group underwent a positive MIP on both visits. In the second experiment, one group of subjects underwent negative MIP on both visits, whereas the other group underwent neutral MIP on both visits.

In both experiments, we predicted that 1) ATD would abolish positive biases present after BAL (in accordance with previous findings) and 2) mood state would moderate these effects.

Experiment (1): Reinforcement-related speeding

Procedures were approved by the Norfolk Research Ethical Committee (06/Q0101/5) and were in accord with the Helsinki Declaration of 1975.

Subjects

Thirty-six subjects (21 females) were recruited. Three females were excluded due to participant drop-out and technical difficulties. The remaining 33 subjects were then divided into positive ($n = 9$; five females), negative ($n = 13$; five females) and neutral ($n = 11$; eight females) MIP groups. All subjects were screened for psychiatric and neurological disorders, gave written informed consent, and were compensated for participation. Exclusion criteria were cardiac, hepatic, renal, pulmonary, neurological, psychiatric or gastrointestinal disorders, medication/drug use and personal or family history of any depressive disorder. The mean age of subjects was [years] 25 (SD = 6).

Methods

Experimental procedure

Subjects were tested on two sessions, separated by at least 1 week. They were instructed to abstain from alcohol, caffeine, and food from midnight prior to each session. They arrived at the research centre between 08:30 and 10:30 and consumed the amino-acid mixture. Following this, they were allowed to consume water and were given a low protein snack (an apple) for lunch. After a resting period of approximately 5 h to ensure stable and low TRP levels (Carpenter, *et al*., 1998), the MIP was completed followed by the cued reinforcement reaction-time task.

Amino-acid mixtures

Central TRP was depleted by ingesting an amino-acid load that did not contain TRP but did include other large neutral amino acids (LNAAs). Amino-acid mixtures (prepared by SHS international; Liverpool UK) were as follows (Roiser, *et al*., 2006; Cools, *et al*., 2007; Robinson and Sahakian, 2008a):

L-alanine, 4.1 g; L-arginine, 3.7 g; L-cystine, 2.0 g; Glycine, 2.4 g; L-histidine, 2.4 g; L-isoleucine, 6 g; L-Leucine, 10.1 g; L-lysine, 6.7 g; L-Methionine, 2.3 g; L-proline, 9.2 g; L-phenylalanine, 4.3 g; L-serine, 5.2 g; L-threonine, 4.9 g; L-tyrosine, 5.2 g; L-valine, 6.7 g ± L-tryptophan, 3.0 g—total BAL: 78.2 / TRP-: 75.2 g

There was a 20% reduction in quantity for females to account for lower body-weight. The drinks were prepared by stirring the mixture into approximately 200 mL tap water. Lemon and lime or grapefruit flavouring was added to compensate for the unpleasant taste. Subjects were assigned in a double-blind approximately counterbalanced fashion to the 'ATD-first' ($N = 19$) or the 'BAL-first' group ($N = 14$).

Mood induction procedure

The MIP was programmed in Microsoft Visual Basic 6 (Microsoft Corporation, Redmond, WA, USA) and presented on a Paceblade tablet computer (11" monitor). A set of visual analogue scales (VAS) was administered at T_0 (admission), T_1 (5 h later pre-MIP) and T_2 (post-MIP) to determine self-reported mood.

Subjects were presented with 60 (positive, negative or neutral) Velten sentences (Velten, 1968) whilst music was played through Sennheiser HD 202 headphones. Each sentence was presented in

the centre of the screen for 12 s until a 'next' button appeared and subjects were able to move on to the next sentence by pressing the space bar. Subjects were instructed to 'relate the situation described by the sentence to situations in their own lives', to get 'as deeply as possible into any mood evoked' and to 'feel free to outwardly express any mood evoked'.

The negative version of the MIP contained light grey text on a dark blue background. The music played was either *Adagio for strings, Op. 11* by Samuel Barber or *Adagio in G Minor* by Tomaso Albinoni. Music was selected by asking the subjects (2 h prior to testing) which piece was the 'saddest'. The positive version featured peach text on a light yellow background. Either *Piano Concerto No. 4, Op. 58 in G Major: III. Rondo: Vivace* by Ludwig van Beethoven or *Serenade No. 13 KV 525 G Major: I. Serenade. Allegro* by Wolfgang Amadeus Mozart was played. The piece was selected by asking subjects which was the 'happiest'. The neutral version featured black text on a white background and *The Planets, Op. 32: VII. Neptune, the Mystic* by Gustav Holst was played.

Cued-reinforcement reaction-time task

The cued reinforcement reaction time task (CRRT) has been comprehensively described elsewhere (Cools, *et al*., 2005). It was programmed in Microsoft Visual Basic 6 and presented on a Paceblade tablet computer (11" monitor). Subjects were asked to select, as quickly as possible, the 'odd-one-out' from a selection of three horizontally adjacent circular patterns. Selection was made by pressing one of three buttons on a keyboard ('<', '>', or '?') representing the location of each pattern. Feedback was given on a proportion of trials. Three different colour-cues (red, blue and yellow) were associated with feedback on 10%, 50% and 90% of trials, respectively. On feedback trials, fast correct responses were rewarded with 100 points, a green smiley face and a flourish sound (500 ms); slow correct responses were rewarded with one point, a green smiley face and a short high frequency beep; incorrect responses were punished with zero points, a red unhappy face and a low tone. A cumulative score appeared at the bottom of the screen. Trials were separated by white fixation cross. On trials on which feedback was not available, the stimuli were followed immediately by this fixation cross. Subjects completed two blocks of 96 trials

At the start of testing, subjects completed two practice blocks of 20 trials in which no coloured boxes appeared and no feedback was presented. The mean and standard deviation of reaction times from the second of these practice blocks was used to determine the cut-off speed below which subjects were administered 100 points in the main task. After completing the task, subjects were asked to estimate feedback likelihood for each colour cue and to give confidence ratings of these subjective estimates.

Data analysis

Repeated measures analysis of variance (ANOVA) was used to examine the results of this three-group within-subjects experiment. All data were analysed using SPSS 10 (SPSS Inc, Chicago, Illinois, USA).

Amino-acid mixtures

Blood (venous) samples (10 mL) were taken immediately before ingestion of the amino-acid drink (T_0) and approximately 5 h after administration (T_1) to determine the TRP/ΣLNAA ratio. This ratio was calculated from the serum concentrations of total TRP divided by the sum of the large neutral amino acids (LNAAs; tyrosine, phenylalanine, valine, isoleucine, leucine). Venous samples were taken in lithium heparin tubes and stored at −20°C. Plasma TRP concentrations were determined by an isocratic high-performance liquid chromatography (HPLC). Plasma proteins were removed by precipitation with 3% trichloroacetic acid and centrifugation at 3000 revs, 4° C for 10 min, and then pipetted into heparin aliquots. An aliquot was diluted in mobile phase before injection onto the HPLC analytical column. Fluorescence end-point detection was used to identify TRP. Treatment (BAL/ATD) and time (T_0/T_1) were entered as within-subjects factors in analyses.

Mood induction procedure

Treatment (BAL/ATD) and time were entered as within-subjects factors; MIP and gender were entered as between subject factors in the analyses of self-reported mood state. Comparison between T_0 and T_1 was used to determine mood effects of the drink. Comparison between T_1 and T_2 was used to determine mood effects of the MIP.

Cued-reinforcement reaction-time task

For CRRT practice data, treatment (BAL/ATD) and block (1/2) were entered as within-subject factors, and MIP and gender were entered as between-subjects factors. For CRRT main-task data, treatment (BAL/ATD), block (1/2), half block (to determine any within-block learning effects: A/B) and feedback-probability (90%/50%/10%) were entered as within-subject factors. MIP group (positive, negative or neutral) was entered as the between-subjects factor in all analyses. Based on accumulating evidence for gender-dependent effects of ATD (Ellenbogen, *et al*., 1996; Booij, *et al*., 2003; Harmer, *et al*., 2003), gender was initially entered as a between-subjects factor before males were removed from the analysis and subsequent analysis was performed only in females. Treatment order (ATD-first/BAL-first) was entered as an additional between-subjects factor. However, if the main effect of order and interactions between order and treatment were found to be non-significant, then data were collapsed across order for subsequent analyses. Simple main effects were calculated from the estimated marginal means as is appropriate when

between-subject sample sizes are different and when there are missing data points (Searle, *et al*., 1980; SPSS, 2007).

Results

Amino-acid mixtures

Amino acid concentration data are presented in Table 1. Complete blood samples were not available for six subjects. A repeated-measures ANOVA revealed a significant two-way drink × time interaction for the critical TRP:ΣLNAA ratio (treatment × time: $F_{1,16} = 68$, $P < 0.001$). This was equivalent between the three mood groups (treatment × time × MIP: $F_{2,16} = 0.74$, $P = 0.49$) and between genders (treatment × time × gender: $F_{1,16} = 0.076$, $P = 0.79$) and there was no interaction with the order of administration (treatment × time × order: $F_{1,16} = 0.012$, $P = 0.91$). There was a similar interaction in the TYR/PHE: ΣLNAA ratio (treatment × time: $F_{1,16} = 13$, $P = 0.002$), which did not interact with order (treatment × time × order: $F_{1,16} = 0.23$, $P = 0.64$), gender (treatment × time × gender: $F_{1,16} = 0.68$, $P = 0.42$) or mood (treatment × time × MIP: $F_{2,16} = 0.62$, $P = 0.55$).

Simple effects analysis revealed that the significant drink × time interaction was due to a 84.6% decrease in the TRP:ΣLNAA ratio between T_0 and T_1 following ATD (simple effect of time: $T_{30} = 12.4$, $P < 0.001$) but a 19.1% increase in the TRP:ΣLNAA ratio between T_0 and T_1 following BAL (simple effect of time: $T_{28} = -2.9$, $P = 0.007$). By contrast, there was no change in the TYR/PHE: ΣLNAA ratio between T_0 and T_1 following ATD (no effect of time: $T_{30} = 0.40$, $P = 0.691$) but an 18.4% decrease following BAL (simple effect of time: $T_{28} = 4.7$, $P < 0.0001$).

Mood induction procedure

There were no treatment by time ($F_{1,29} = 1.4$, $P = 0.25$) or treatment by time by gender interactions ($F_{1,29} = 1.8$, $P = 0.19$) between T_0 and T_1 on the Happy–Sad VAS subscale. This indicates that the drink itself did not lower subjects' mood. However, there was a time by MIP interaction between T_1 and T_2 before and after the MIP ($F_{2,25} = 3.8$, $P = 0.035$). This was not influenced by treatment (no time × MIP × treatment interaction; $F_{2,25} = 1.4$, $P = 0.27$) or gender (no time × MIP × gender interaction; $F_{2,25} = 1.8$, $P = 0.18$).

Within-mood group comparison between T_1 and T_2 showed a significant shift towards the 'sad' end of the subscale following the negative MIP ($T_{25} = 2.9$, $P = 0.008$). These data indicate that the negative MIP was successful. There was a shift towards the positive end of the mood scale following the positive MIP which, although it was not significant ($T_{25} = 2.9$, $P = 0.35$), caused a significant difference between the T_2 ratings in the positive and negative groups ($T_{25} = 3.1$, $P = 0.004$), which was not present at T_1 ($T_{25} = 0.9$, $P = 0.4$) or between the negative and neutral groups at T_2 ($T_{25} = 3.2$, $P = 0.25$). There was no shift in ratings following neutral MIP ($T_{25} = 1.9$, $P = 0.24$).

Cued-reinforcement reaction-time task

Subjects were significantly faster on the second practice block than the first (main effect of block: $F_{1,22} = 57.0$, $P < 0.001$) but there was no effect of treatment (main effect of treatment: $F_{1,22} = 0.58$, $P = 0.45$), or treatment × MIP × gender interaction ($F_{2,22} = 0.22$, $P = 0.81$) on the mean practice RT. Therefore, any effects in the main task cannot be explained by a difference in terms of baseline reaction time (which was used to determine the cut-off below which large bonuses were assigned).

CRRT main task data are presented in Figure 1 and Tables 2 and 3. Analysis of median latency data revealed a treatment × feedback probability × MIP × gender interaction ($F_{2,23} = 4.6$, $P = 0.021$). Breakdown of this four-way interaction revealed a significant treatment × feedback probability × MIP interaction in females ($n = 18$; $F_{2,13} = 7.2$, $P = 0.008$) but not in males ($n = 15$; $F_{2,10} = 0.88$, $P = 0.44$).

Simple effects analyses of the 50% and 90% trials (which are most sensitive to manipulation by ATD (Cools, *et al*., 2005)) in females revealed a significant interaction between ATD and feedback probability ($F_{2,12} = 5.0$, $P = 0.026$) in the positive MIP group ($n = 5$) due to a simple main effect of feedback probability (i.e., feedback-related speeding) after BAL ($F_{1,13} = 6.1$, $P = 0.029$) but not after ATD ($F_{1,13} = 3.5$, $P = 0.084$).

Similarly, in the neutral MIP group ($n = 8$), a significant interaction between ATD and feedback probability ($F_{2,12} = 5.0$, $P = 0.027$) reflected ATD-induced reduction of feedback-related speeding, although the simple main effects of feedback probability were significant both after BAL ($F_{1,13} = 6.4$, $P = 0.026$) and ATD ($F_{1,13} = 5.8$, $P = 0.031$).

By contrast, in the negative MIP group ($n = 5$), feedback-related speeding was absent after BAL (simple main effect of

Table 1 Experiment 1: Cued-reinforcement reaction-time task (biochemical measure)

	BAL		ATD	
	T1	T2	T1	T2
TRP: ΣLNAA ($N = 34$)	0.11 (0.01)	0.14 (0.01)	0.12 (0.01)	0.02 (0.00)
TYR/PHE: ΣLNAA ($N = 34$)	0.23 (0.01)	0.19 (0.01)	0.22 (0.01)	0.21 (0.01)

BAL, balanced amino acid drink; ATD, acute tryptophan depletion.
Values represent mean (standard error of the mean).

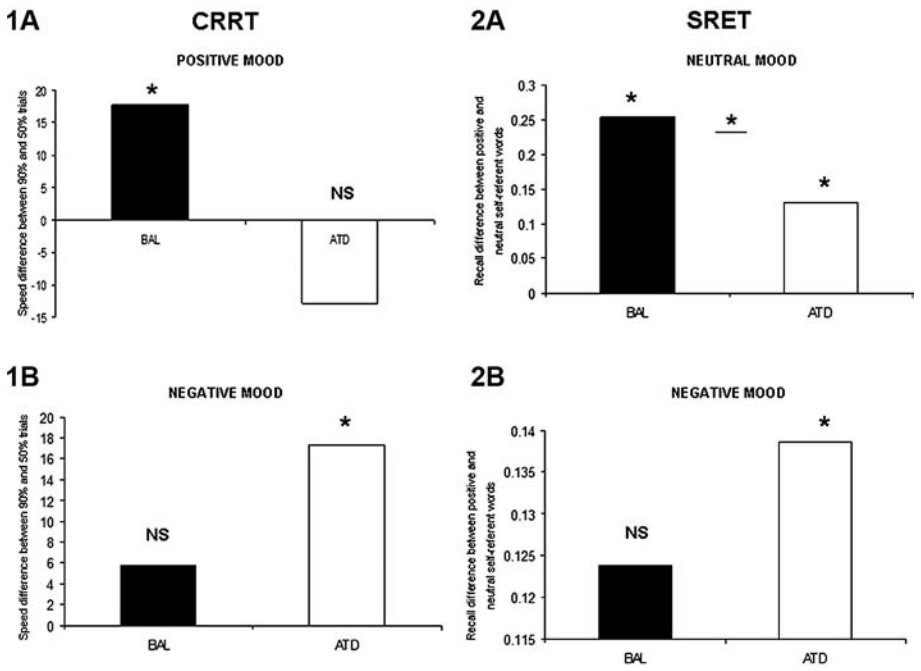

Figure 1 Mean difference scores–mood state moderates the effect of ATD on cognition in both the CRRT and SRET. In the CRRT task, females in a positive mood show a positive bias under BAL (as indicated by increased speed on 90%—relative to 50%—feedback likelihood) that is abolished by ATD (1A). The same effect is seen in all subjects in a neutral mood on the SRET (as indicated by increased recall of positive relative to neutral self-referent words), although the bias is significantly reduced rather than completely abolished (2A). By contrast, subjects in a negative mood show no positive bias under BAL but induction of a positive bias by ATD on both the CRRT (1B) and the SRET (2B). * = $P < 0.05$ (SED).

feedback probability: $F_{1,13} = 0.64$, $P = 0.44$) but present after ATD (simple main effect of feedback probability: $F_{1,13} = 6.4$, $P = 0.03$), although the simple interaction between ATD and feedback probability did not reach significance ($F_{2,12} = 3.0$, $P = 0.09$).

The significant differences driving these effects are presented in Table 3. A similar pattern was seen in the 10% and 90% trials and these contrasts are also presented in Table 3. No meaningful correlations were observed between biochemical and behavioural effects of ATD.

Analysis of the de-brief questionnaire revealed that all subjects were aware of the different colours and the feedback probabilities (main effect of cue: $F_{2,23} = 5.8$, $P = 0.01$) and that they were equally confident in their estimate for all cues (no effect of cue: $F_{2,23} = 1.3$, $P = 0.30$). There were no effects of treatment or MIP on these estimates.

Table 2 Experiment 1: Cued-reinforcement reaction-time task (reaction time in ms following 90%, 50% or 10% cues (SEM))

	BAL			ATD		
	10%	50%	90%	10%	50%	90%
Negative						
Male ($N = 8$)	472 (24.0)	474 (23.7)	450 (25.6)	504 (33.8)	511 (29.0)	492 (28.3)
Female ($N = 5$)	507 (40.5)	509 (40.4)	503 (41.7)	535 (37.7)	532 (38.0)	514 (38.0)
Positive						
Male ($N = 4$)	511 (29.4)	501 (29.1)	499 (31.4)	521 (41.5)	510 (35.5)	500 (34.7)
Female ($N = 5$)	492 (40.5)	489 (40.4)	471 (41.7)	488 (37.7)	481 (38.0)	494 (38.0)
Neutral						
Male ($N = 3$)	463 (34.0)	463 (33.6)	461 (36.2)	482 (47.9)	461 (41.0)	465 (40.0)
Female ($N = 8$)	502 (36.9)	498 (36.9)	482 (38.1)	519 (34.4)	514 (34.7)	499 (34.7)

BAL, balanced amino acid drink; ATD, acute tryptophan depletion.

Table 3 Experiment 1: Cued-reinforcement reaction-time task

	Contrast	BAL			ATD		
		Mean Difference (SEM)	T15	*P*	Mean Difference (SEM)	T15	*P*
Negative (*N* = 5)	90%–50%	−5.8 (7.21)	−0.80	0.437	−17.4 (6.89)	−2.52	0.026*
	90%–10%	−4.1 (7.72)	−0.53	0.608	−20.9 (10.18)	−2.05	0.061
Positive (*N* = 5)	90%–50%	−17.7 (7.21)	−2.46	0.029*	12.9 (6.89)	1.87	0.084
	90%–10%	−20.5 (7.72)	−2.65	0.020*	6.3 (10.18)	0.62	0.544
Neutral (*N* = 8)	90%–50%	−16.5 (6.58)	−2.51	0.026*	−15.2 (6.29)	−2.41	0.031*
	90%–10%	−20.3 (7.04)	−2.88	0.013*	−20.7 (9.30)	−2.23	0.044*

BAL, balanced amino acid drink; ATD, acute tryptophan depletion.
Mean difference in the female subjects (*N* = 18) reaction times following 90% versus 50% or 90% versus 10% cues (ms).
* = $P < 0.05$.

Experiment 2: Self-referent encoding/retrieval

Procedures were approved by the Norfolk Research Ethical Committee (06/Q0101/5) and were in accord with the Helsinki Declaration of 1975. Experimental procedure was the same as described above.

Subjects

Eighteen subjects (12 females) completed the self-referent encoding/retrieval task (SRET) on ATD and BAL and were divided into negative ($n = 9$; six females) or neutral ($n = 9$, five females) MIP groups. Subjects were screened as before. The mean age of subjects was [years] 25 (SD = 4).

Amino-acid mixtures

Subjects were assigned in a double-blind approximately counterbalanced fashion to the 'ATD-first' ($n = 8$) or the 'BAL-first' group ($n = 10$).

Mood induction procedure

Subjects completed the negative and neutral MIP as described above.

Self-referent encoding/retrieval task

The self-referent encoding/retrieval task (SRET) was programmed using the E-prime software (Psychological Software Tools, Inc., Learning 2002; Research and Development Center, University of Pittsburgh, Pennsylvania) and was based upon that used by Ramel, *et al*., (2007). Subjects were asked to indicate whether a word presented on a Paceblade tablet computer was self-referent. 36 positive, 36 negative, and 36 neutral words were selected from the Affective Norms for English Words (ANEW) (Bradley and Lang, 1999) and were presented in 18 blocks stratified by word valence. Each block consisted of six words.

Each word was presented until the subject made a response. Subjects completed six blocks of each valence (positive, negative, neutral). The blocks were randomly arranged but there were never two blocks of the same valence in a row. Words were randomly selected without replacement.

Before the task started, subjects were informed that they would be asked to recall as many words as possible. At the end of the task, participants were instructed to recall a minimum of three words and a maximum of 20 words within a 3-minute period. Recalled words were recorded by the researcher.

Data analysis

Amino-acid mixtures and MIPs were analysed as in Experiment 1.

Self-referent encoding/retrieval task

The variable of interest was the number of self-referent words of each valence (positive, negative or neutral) that were subsequently recalled, divided by the total number of self-referent words (all valences). This proportion of self-referent recall controlled for individual differences in endorsement (Symons and Johnson, 1997; Ramel, *et al*., 2007). Treatment (BAL/ATD) and word valence (positive/negative/neutral) were the within-subject factors and MIP was the between-subjects factor in main effects analysis. Simple contrasts were made between the estimated means (Searle, *et al*., 1980; SPSS, 2007) of the different word valences (positive-negative; positive-neutral; neutral-negative) with Bonferroni corrections applied to control for multiple comparisons.

Results

Amino-acid mixtures

Amino acid concentration data are presented in Table 4. Complete blood samples were not available for four subjects

Table 4 Experiment 2: Self-referent encoding/retrieval task (biochemical measures)

	BAL		ATD	
	T1	T2	T1	T2
TRP: ΣLNAA ($N = 18$)	0.14 (0.01)	0.18 (0.02)	0.16 (0.01)	0.02 (0.01)
TYR/PHE: ΣLNAA ($N = 18$)	0.26 (0.02)	0.19 (0.01)	0.24 (0.02)	0.21 (0.01)

BAL, balanced amino acid drink; ATD, acute tryptophan depletion.
Values represent mean (standard error of the mean).

due to difficulties with blood extraction. A repeated-measures ANOVA revealed a significant two-way drink × time interaction for the critical TRP: ΣLNAA ratio ($F_{1,7} = 47$, $P < 0.001$). This was equivalent between the two mood groups (treatment × time × MIP: $F_{1,7} = 2.4$, $P = 0.2$) and between genders (treatment × time × gender: $F_{1,7} = 0.19$, $P = 0.7$) and there was no interaction with the order of administration (treatment × time × order: $F_{1,7} = 0.14$, $P = 0.7$). There was no similar interaction in the TYR/PHE:ΣLNAA ratio (treatment × time: $F_{1,7} = 3.9$, $P = 0.09$).

Simple effects analysis revealed that the significant drink × time interaction was due to a 86.1% decrease in the TRP: ΣLNAA ratio between T_0 and T_1 following ATD (simple effect of time: $T_{14} = 14$, $P < 0.001$) but no significant change in the TRP: ΣLNAA ratio between T_0 and T_1 following BAL (simple effect of time:$T_{17} = -1.7$, $P = 0.090$).

Mood induction procedure

There were no treatment × time ($F_{1,16} = 3.6$, $P = 0.08$) or treatment, time and gender interaction ($F_{1,16} = 1.9$, $P = 0.2$) between T_0 and T_1 on the Happy–Sad VAS subscale. This indicates that the drink itself did not lower subjects' mood. However, there was a time × MIP interaction between T_1 and T_2 before and after the MIP ($F_{1,13} = 8.1$, $P = 0.014$). This was not influenced by treatment (no time × MIP × treatment interaction; $F_{1,13} = 0.6$, $P = 0.44$) or by gender (no time × MIP × gender interaction; $F_{1,13} = 0.15$, $P = 0.7$).

Within-mood group comparison between T_1 and T_2 showed a significant shift towards the 'sad' end of the subscale following the negative MIP ($T_{13} = 3.5$, $P = 0.004$) but no shift in ratings following neutral MIP ($T_{13} = 0.5$, $P = 0.6$). These data indicate that the MIP was successful in both males and females.

Self-referent encoding/retrieval task

The proportion of self-referent recall for each valence is presented in Table 5. ATD had opposite effects on the recall of self-referent words in the two MIP groups, as confirmed by a significant treatment × word valence × MIP interaction ($F_{2,14} = 3.3$, $P = 0.05$). This effect was not present in the recall of non-self-referent words ($F_{2,14} = 0.2$, $P = 0.8$).

In the neutral MIP group ($n = 9$), ATD attenuated a positive bias (simple interaction between treatment and valence: $F_{4,12} = 5.8$, $P = 0.008$), although positive biases were present after both BAL (main effect of valence: $F_{2,14} = 11.0$, $P = 0.001$) and ATD (main effect of valence: $F_{2,14} = 6.6$, $P = 0.009$). ATD therefore significantly reduced the magnitude of the positive bias.

By contrast, in the negative MIP group ($n = 9$), ATD enhanced a positive bias (simple interaction between treatment and valence: $F_{4,12} = 4.0$, $P = 0.028$). Simple main effects of valence were significant after ATD ($F_{2,14} = 8.4$, $P = 0.004$) but not quite after BAL ($F_{2,14} = 3.0$, $P = 0.08$). The contrasts driving these results are presented in Table 6 and Figure 1. No meaningful correlations were observed between biochemical and behavioural effects of ATD.

Table 5 Experiment 2: Self-referent encoding/retrieval task (Proportion self-referent recall for each word valence (SEM))

	BAL			ATD		
	POS	NEG	NEUT	POS	NEG	NEUT
Neutral						
All ($N = 9$)	0.26 (0.05)	0.01 (0.01)	0.00 (0.01)	0.14 (0.03)	0.02 (0.01)	0.01 (0.01)
Female ($N = 5$)	0.30 (0.07)	0.00 (0.01)	0.00 (0.02)	0.11 (0.05)	0.02 (0.01)	0.00 (0.01)
Male ($N = 4$)	0.19 (0.06)	0.03 (0.02)	0.01 (0.01)	0.18 (0.02)	0.01 (0.02)	0.01 (0.02)
Negative						
All ($N = 9$)	0.14 (0.04)	0.02 (0.01)	0.02 (0.01)	0.16 (0.03)	0.04 (0.01)	0.02 (0.01)
Female ($N = 6$)	0.13 (0.06)	0.02 (0.01)	0.02 (0.01)	0.18 (0.04)	0.03 (0.01)	0.02 (0.01)
Male ($N = 3$)	0.17 (0.06)	0.01 (0.02)	0.00 (0.01)	0.13 (0.02)	0.05 (0.02)	0.03 (0.02)

BAL, balanced amino acid drink; ATD, acute tryptophan depletion; POS, positive words; NEG, negative words; NEUT, neutral words.

Table 6 Experiment 2: Self-referent encoding/retrieval task

	Contrast	BAL			ATD		
		Mean Difference (SEM)	T	P	Mean Difference (SEM)	T	P
All Subjects ($N = 18$)							
Neutral ($N = 9$)	Positive–Negative	0.246 (0.05)	4.81	0.001*	0.117 (0.04)	3.34	0.014*
	Positive–Neutral	0.254 (0.05)	4.93	0.001*	0.130 (0.03)	3.76	0.006*
Negative ($N = 9$)	Positive–Negative	0.120 (0.05)	2.49	0.074	0.125 (0.03)	3.79	0.005*
	Positive–Neutral	0.124 (0.05)	2.56	0.066	0.139 (0.03)	4.24	0.002*
Females ($N = 12$)							
Neutral ($N = 5$)	Positive–Negative	0.299 (0.07)	4.28	0.006*	0.087 (0.05)	1.81	0.310
	Positive–Neutral	0.299 (0.07)	4.08	0.008*	0.110 (0.05)	2.22	0.161
Negative ($N = 6$)	Positive–Negative	0.104 (0.06)	1.63	0.411	0.149 (0.04)	3.39	0.024*
	Positive–Neutral	0.103 (0.07)	1.54	0.473	0.158 (0.05)	3.47	0.021*

BAL, balanced amino acid drink; ATD, acute tryptophan depletion.
Mean difference in the proportion of self-referent recall of positive versus negative or positive versus neutral words.
* = $P < 0.05$.

In concordance with the CRRT analysis, we also included gender as an additional between subjects factor. There was a significant treatment × word valence × MIP × gender interaction ($F_{2,12} = 5.17$, $P = 0.013$) on the recall of self-referent words on the SRET. When stratified by gender, there was a significant treatment × valence × MIP interaction in females ($F_{2,8} = 6.23$, $P = 0.006$) but not in males ($F_{2,3} = 6.23$, $P = 0.6$).

Results summary

Cued-reinforcement reaction-time task:

- ATD was associated with an 85% reduced tryptophan ratio, whereas BAL was associated with a 19% increased tryptophan ratio and an 18% decreased tyrosine/phenylalanine ratio.
- There was a significant interaction between mood state, treatment and cognitive bias in female ($n = 18$) but not male ($n = 15$) subjects.
- This female-specific effect reflected increased speed in anticipation of feedback (i.e., a positive bias) after BAL in the positive mood group ($n = 5$). This bias was abolished by ATD.
- The negative mood group ($n = 5$) showed the opposite pattern and demonstrated a positive bias after ATD but not after BAL.
- The neutral mood group ($n = 8$) showed a positive bias after both BAL and ATD.

Self-referent encoding/retrieval task:

- ATD was associated with an 86% reduced tryptophan ratio.
- A significant interaction between mood state, treatment and cognitive bias was seen in all subjects ($n = 18$).
- This effect reflected increased recall of positive- (over neutral- and negative-) self-referent words (i.e., a positive bias) after BAL in the neutral mood group ($n = 9$) (replicating previous findings; (Ramel, *et al*., 2007)). This bias was significantly reduced by ATD.
- The negative mood group ($n = 9$) demonstrated a positive bias after ATD but not after BAL.
- This constitutes a replication of the CRRT findings in a separate and larger group of subjects.

Discussion

The present study reveals that the effects of ATD on cognitive performance are moderated by mood. A significant interaction between treatment, mood induction and cognitive performance was observed across two independent paradigms measuring cognitive affective biases (see Figure 1). Subjects undergoing positive mood induction demonstrated a positive cognitive bias on the CRRT after BAL. This bias was abolished by ATD. Subjects undergoing neutral mood induction showed the same effect on the SRET. These findings replicate previously observed disruption of positive cognitive biases by tryptophan depletion (Rogers, *et al*., 2003; Cools, *et al*., 2005; Roiser, *et al*., 2006). Strikingly, however, subjects undergoing negative mood induction demonstrated the opposite pattern of results. In both paradigms, subjects showed no bias after BAL, but a positive cognitive bias after ATD; thereby mimicking the effect in depressed patients following ATD. This supports our hypothesis that mood state moderates the effects of ATD on cognition. These findings, which were consistent across two separate groups of subjects and on two different measures of cognitive performance, may explain some of the apparently contradictory findings pertaining to serotonin function.

Subjects completing the CRRT under a positive mood demonstrate motivational speeding under BAL that is removed by ATD. This disruption of a positive bias on the CRRT by ATD has been demonstrated before (Cools, *et al*., 2005; Roiser, *et al*., 2006). In addition, subjects completing the SRET in a neutral mood demonstrate increased recall of positive words under BAL, which is also reduced by ATD. This is consistent with the CRRT findings and extends previous findings demonstrating an ATD linked impairment in verbal recall (Sambeth, *et al*., 2007). Specifically, Sambeth, *et al*. (2007) have shown a valence-independent impairment in recall by ATD in healthy volunteers. Here, we reveal valence dependency as a function of mood state. At first sight, the effect appears inconsistent with another study, which failed to show an effect of ATD on a negative bias in self-referent recall. However, this finding was obtained in recovered depressed individuals (Hayward, *et al*., 2005) who are unlikely to respond to ATD in the same way as healthy individuals (Robinson and Sahakian, 2008b). Our present findings therefore concur with recent theorizing suggesting that 5-HT promotes an 'optimism bias' in healthy individuals, which is removed by ATD (Dayan and Huys, 2008). According to this theorizing, 5-HT promotes behavioural inhibition of states and thoughts with negative outcomes. This leads to a bias towards the sampling of positive over negative states/thoughts, which manifests as an 'optimism' bias. Removal of 5-HT, whether via natural fluctuations or ATD, then leads to disinhibition of these aversive states and thoughts and thus the removal of positive biases. Our finding in subjects in neutral and positive moods replicates previous findings in healthy individuals who have not been subjected to mood manipulation (McCabe and Gotlib, 1995; Erickson, *et al*., 2005). This is consistent with the hypothesis that healthy individuals possess a baseline ranging from neutral to positive mood, which confers resilience to affective disorders (Yehuda, *et al*., 2006). The presence of the effect in the neutral mood group on the SRET may be because the task is more sensitive to positive biases (which may be less pronounced under a neutral mood state), but future work comparing the modulation of SRET performance by ATD under both neutral and positive moods is necessary to clarify this.

Most intriguingly, however, we find that on both tasks, ATD has the opposite effect on performance when subjects are under a negative mood. Following BAL, subjects respond equally to different levels of reinforcement on the CRRT and recall positive, negative and neutral self-referent words equally on the SRET. However, following ATD, the positive biases seen under BAL in neutral and positive moods are reinstated. The influence of ATD under a negative mood is therefore diametrically opposite to its influence under positive/neutral moods. This apparently paradoxical finding suggests that serotonin has dramatically different functions under different mood states. It has a precedent in ATD-induced positive mood in currently depressed patients (Delgado, *et al*., 1994; Price, *et al*., 1998), which is seen on the day after consumption of the drink. This effect has previously been attributed to a 'rebound' increase in serotonin function 24 h after depletion but the present data highlight the possibility that it may be due to mood state. To reconcile our findings with the current model (Dayan and Huys, 2008), therefore, it may be that instead of solely inhibiting negative states, serotonin inhibits all states that are inconsistent with the prevailing mood. Under a positive or neutral mood state, serotonin might inhibit negative thoughts, while actually inhibiting positive thoughts under a negative mood state. In other words, ATD might induce a positive cognitive bias by disinhibiting positive thoughts under negative mood induction. The contrary manipulation, elevation of serotonin via antidepressant medication, would therefore have the opposite effect (i.e. elevate the inhibition of positive states). This is consistent with the observation of increased anxiety or suicidal behaviour in certain depressed individuals at the start of antidepressant treatment (Kent, *et al*., 1998; Burghardt, *et al*., 2004; Jick, *et al*., 2004; Harmer, 2008), However, antidepressant medication clearly improves mood symptoms in the long-term so it may be that these early effects may be restricted to acute, rather than chronic, serotonin manipulation (Burghardt, *et al*., 2004; Harmer, 2008; Robinson and Sahakian, 2008b). It has, nevertheless, been suggested that the early effect of antidepressant medication is to reduce 5HT levels via presynaptic action so our hypothesis is therefore highly speculative and requires explicit testing in future studies.

It should be noted that an alternative explanation for our findings is that the interaction between ATD and mood reflects a modulation of mood state by ATD, rather than a modulation of the effects of ATD by mood state. Although we cannot exclude this possibility, we consider it unlikely because we find no interaction with ATD in the self-reported response to mood induction; subjects within each mood group report the same mood following the MIP following both BAL and ATD.

Another point to consider is that in the first study, BAL was associated with a 19% increased tryptophan ratio and an 18% decreased tyrosine/phenylalanine ratio. Although this effect did not interact with mood state and was considerably smaller than the reduction of tryptophan seen in the ATD session, without baseline measures or additional biomarkers (such as prolactin response), it is difficult to say that the effects under BAL represent 'normal' processing (Badawy, 2005). The magnitude of our SRET effects under BAL and neutral mood are somewhat larger than previous findings (Ramel, *et al*., 2007), so this may be attributable to such BAL associated changes. An important avenue of future research, therefore, is to compare the present findings with a control baseline condition.

One final point to consider is the gender specificity of the findings. This finding is consistent with accumulating evidence suggesting that females are more sensitive to ATD (Ellenbogen, *et al*., 1996; Booij, *et al*., 2003; Harmer, *et al*., 2003; Sambeth, *et al*., 2007; Robinson and Sahakian, 2008a). Females may also be more susceptible to mood manipulation and hence the modulation of 5-HT function by mood. This sensitivity, in turn, may pertain to the increased risk of depression in females. As this finding was not anticipated, the second experiment did not include enough male subjects to appropriately examine these gender

effects. Future work should therefore include a balanced number of males and females in order to examine these findings more closely. Furthermore, although the findings are consistent with previous literature and the replication of the effect in two separate samples is promising, these data should be treated with caution until they are replicated in a larger sample of subjects. Nonetheless, the present data suggest that there are a number of apparent discrepancies in the role of 5-HT in cognition and that these may be a result of modulating factors, one of which may be mood.

Conclusion

These findings demonstrate that ATD can either disrupt or induce positive cognitive biases depending on mood state. This effect is seen in both a small preliminary sample and a larger second sample across two different aspects of cognition. In order to explain these findings, we propose that mood states may promote inhibition of affective states that are inconsistent with the mood and that 5-HT fuels this inhibition. The reduction of 5-HT, via ATD or natural fluctuations, releases the affective states opposite to the previously prevailing state and promotes cognitive biases that are in opposition to this prior state. This is consistent with the proposed role of 5-HT in inhibition (Soubrié, 1986; Dayan and Huys, 2008) and would explain both the promotion of negative biases by ATD in subjects under healthy (neutral or positive) moods (Rogers, *et al*., 2003; Cools, *et al*., 2005; Roiser, *et al*., 2006; Cools, *et al*., 2007) and the promotion of positive biases by ATD in subjects in negative moods (Delgado, *et al*., 1994; Price, *et al*., 1998). This would also help to explain the role of serotonin in both depression and mania.

However, the findings warrant replication. The overall message, therefore, is that there are a number of apparent discrepancies in the role of serotonin in cognition and that these may be explained by the presence of modulating factors. Mood state may be one such modulator, but it is quite possible that there are others. Given that affective disorders are a considerable social, emotional and financial burden upon both the individual and society (Robinson and Sahakian, 2008b); this is clearly an avenue of research worth pursuing.

Disclosure/Conflict of Interest

Professor Barbara Sahakian consults for Cambridge Cognition. She holds shares in CeNeS. She has consulted for Novartis, Shire, GlaxoSmithKline and Lilly. She has also received honoraria for Grand Rounds in Psychiatry at Massachusetts General Hospital (CME credits) (Boston, 27 April 2007) and for speaking at the International Conference on Cognitive Dysfunction in Schizophrenia and Mood Disorders: clinical aspects, mechanisms and therapy (Brescia, 17–19 January 2007). She is on the Medical Research Council Neurosciences and Mental Health Board and on the Science Co-ordination Team for the Foresight Project on Mental Capital and Wellbeing, (Office of Science, The Department of Innovation, Universities and Skills). As an Associate Editor, she also receives an honorarium from the journal Psychological Medicine. OJR, RC and MJC report no biomedical financial interests or potential conflicts of interest.

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