Neuropsychopharmacology (2005) 30, 1138–1147

www.neuropsychopharmacology.org

Serotonergic Modulation of Prefrontal Cortex during Negative Feedback in Probabilistic Reversal Learning

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This study used functional magnetic resonance imaging to examine the effects of acute tryptophan (TRP) depletion (ATD), a well-recognized method for inducing transient cerebral serotonin depletion, on brain activity during probabilistic reversal learning. Twelve healthy male volunteers received a TRP-depleting drink or a balanced amino-acid drink (placebo) in a double-blind crossover design. At 5 h after drink ingestion, subjects were scanned while performing a probabilistic reversal learning task and while viewing a flashing checkerboard. The probabilistic reversal learning task enabled the separate examination of the effects of ATD on behavioral reversal following negative feedback and negative feedback *per se* that was not followed by behavioral adaptation. Consistent with previous findings, behavioral reversal was accompanied by significant signal change in the right ventrolateral prefrontal cortex (PFC) and the dorsomedial prefrontal cortex. ATD enhanced reversal-related signal change in the dorsomedial PFC, but did not modulate the ventrolateral PFC response. The ATD-induced signal change in the dorsomedial PFC during behavioral reversal learning extended to trials where subjects received negative feedback but did not change their behavior. These data suggest that ATD affects reversal learning and the processing of aversive signals by modulation of the dorsomedial PFC.
Neuropsychopharmacology (2005) **30,** 1138–1147, advance online publication, 2 February 2005; doi:10.1038/sj.npp.1300663

Keywords: serotonin; reversal learning; functional imaging; punishment; inhibitory control; prefrontal cortex

INTRODUCTION

Serotonin (5-HT) has been extensively implicated in depressed mood and the processing of motivational signals (Graeff *et al*, 1986; Soubrie, 1986; Deakin, 1991; Wilkinson *et al*, 1995). Animal research has demonstrated that 5-HT-enhancing drugs attenuate the aversive effects of brain stimulation (Patkina and Lapin, 1976; Graeff *et al*, 1986; Smith and Kennedy, 2003), and, conversely, potentiate self-stimulation in so-called 'reward' centers and enhance the motivational properties of stimuli predictive of rewards (Redgrave and Horrell, 1976; Aronson *et al*, 1995; Sasaki-Adams and Kelley, 2001; Orosco *et al*, 2004). Reduced motivation (anhedonia, apathy) is a cardinal feature of depression, where neuropsychological studies have further emphasized the relevance of incentive motivation and the processing of reinforcement; depression has been associated with a 'catastrophic response to perceived failure' (Beats *et al*, 1996) or an oversensitivity to negative feedback (Elliott *et al*, 1997; Steffens *et al*, 2001; Murphy *et al*, 2003). Selective 5-HT reuptake inhibitors exert antidepressant effects and acute reduction of central 5-HT function through dietary depletion of tryptophan (TRP), a precursor of 5-HT, can induce temporary depressive relapse in remitted patients (Young *et al*, 1985; Smith *et al*, 1997).

5-HT neurotransmission has been implicated not only in the processing of reward and punishment signals, but also in the inhibitory control of behavior (Soubrie, 1986; Evenden, 1999), where impulsive pathology is typically associated with reductions in central 5-HT (Coccaro *et al*, 1989; Cherek and Lane, 2000). Findings from studies with clinical populations are corroborated by animal studies linking impulsive choice in delay-discounting paradigms and premature responding in choice reaction-time tasks with 5-HT dysregulation (Harrison *et al*, 1997; Puumala and Sirvio, 1998; Koskinen *et al*, 2000; Mobini *et al*, 2000; Dalley *et al*, 2002; Liu *et al*, 2004).

These studies with animals have implicated particularly the medial and orbital prefrontal cortex (PFC) in impulsive performance (Dalley *et al*, 2002; Chudasama and Robbins, 2003; Liu *et al*, 2004), and this concurs indirectly with findings that manipulation of the 5-HT system in humans affects tasks that implicate the ventral and medial aspects of PFC (Robbins, 2000). Thus, neuropsychological studies have shown that acute TRP depletion (ATD) impairs

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Received 21 June 2004; revised 19 November 2004; accepted 29 November 2004
Online publication: 1 December 2004 at http://www.acnp.org/citations/Npp120604040271/default.pdf

performance on tasks of reversal learning, response inhibition, and affective decision-making (Park *et al*, 1994; Murphy *et al*, 2002; Walderhaug *et al*, 2002; Rogers *et al*, 2003), which have all been associated with ventral and/or medial PFC circuitry (Iversen and Mishkin, 1970; Jones and Mishkin, 1972; Dias *et al*, 1996; Rogers *et al*, 1999; O'Doherty *et al*, 2001; Cools *et al*, 2002; Fellows and Farah, 2003; Kringelbach and Rolls, 2003; Hornak *et al*, 2004).

While the ascending 5-HT projection has a widespread cortical distribution, receptor subtypes including the 5-HT_{2A} receptor show regional specificity to the frontal cortex and are overly expressed in medial and orbital regions in animal models of depression and anxiety (Poeggel *et al*, 2003; Preece *et al*, 2004). Structural and functional imaging studies in depressed patients also indicate reasonably selective abnormalities in the ventral and medial aspects of PFC (Drevets *et al*, 1997; Mayberg *et al*, 1999; Elliott *et al*, 2002; Ballmaier *et al*, 2004; Lacerda *et al*, 2004).

In the present study, we examined the effects of ATD on the blood oxygenation level-dependent (BOLD) response during probabilistic reversal learning, which requires the adaptation of behavior following changes in reward (and punishment) values as well as the maintenance of behavior in the face of misleading negative (probabilistic) feedback. ATD is a well-recognized research method for reducing central 5-HT in humans and studying the effects of low 5-HT on cognition. ATD produces a rapid decrease in the synthesis and release of brain 5-HT (Nishizawa *et al*, 1997; Carpenter *et al*, 1998; Williams *et al*, 1999). TRP is depleted by ingesting an amino-acid mixture that does not contain TRP but does include other large neutral amino acids (LNAA) (Young *et al*, 1985). ATD is achieved by increasing protein synthesis in the liver with subsequent decreases in plasma TRP stores. In addition, the amino-acid load results in competition for the active transport system that the amino acids share for entry across the blood–brain barrier, resulting in reduced availability of TRP in the brain. The probabilistic reversal learning task enables the relatively separate examination of behavioral adaptation following negative feedback (aversive signals) and the processing of negative feedback without subsequent behavioral adaptation. We used the same probabilistic reversal learning task that was previously employed by Cools *et al* (2002). This study revealed significant BOLD changes during probabilistic reversal learning in the ventrolateral prefrontal cortex (VLPFC) and the dorsomedial PFC. Based on the strong *a priori* association between depression, 5-HT, reversal learning, and orbital PFC, we decided not to restrict our regions of interest (ROIs) to the task-related brain areas but to extend these to the other orbital frontal regions not activated by the task. We predicted that ATD would modulate signal change in the ventral (including orbital) and medial PFC during the reception of negative feedback and the subsequent adaptation of behavior to the new contingencies.

MATERIALS AND METHODS

Participants

Twelve healthy right-handed male volunteers (18–28 years old; mean age of 23.8 ± 2.8) participated in this experiment. The study was approved by the Local Research Ethical Committee in Cambridge and carried out in accordance with the Declaration of Helsinki. Participants were recruited via local advertisements, and screened for psychiatric and neurological disorders and MRI contraindications by means of prescreening questionnaires and interview by EATE. All volunteers gave written informed consent, and were paid for participation. The exclusion criteria were any history of cardiac, hepatic, renal, pulmonary, neurological or gastrointestinal disorder, medication use, and a history of major depression or bipolar affective disorder.

One participant vomited after ingesting the amino-acid mixture and was replaced by a substitute. One participant was excluded from the analysis due to poor performance on the reversal learning task (final $n = 11$). His mean reaction time (RT) and the total number of trials on the reversal learning task were between 2.5 and 3.0 standard deviations higher than the group mean after the balanced drink.

Experimental Design

Participants attended two test sessions at least 1 week apart, and were administered either a TRP-depleted (TRP−) drink or a balanced (BAL) amino-acid drink in a double-blind crossover design (four participants received TRP− and seven received the BAL drink on the first session). Prior to a test session, volunteers fasted overnight and low-protein food was provided during the test days. Following a resting period of 5 h (4.5 h, SD = 35 min, in the TRP− condition and 5.0 h, SD = 40 min, in the balanced condition), to ensure stable and low TRP levels (Riedel *et al*, 1999), participants entered the functional magnetic resonance imaging (fMRI) scanner at the Wolfson Brain Imaging Centre (WBIC). They were scanned while performing three blocks of the probabilistic reversal learning task each for about 9 min (Cools *et al*, 2002) and the checkerboard task. Behavioral performance on the reversal learning task was assessed using button presses on a response box. Structural scans were obtained at the end of a test session or on a separate session.

Probabilistic Reversal Learning Task

The probabilistic reversal learning task was described in detail by Cools *et al* (2002). The task is a two-choice visual discrimination task where the same two abstract patterns were presented on each trial. Using trial-and-error feedback after each response (a green happy face or a red sad face), subjects learned to select the stimulus that was usually correct. This rule intermittently reversed so that the other stimulus was usually correct. Consequently, responding had to be adjusted in order to gain reward and avoid punishment. On a minority of trials (10–20%) false-negative feedback was provided to a correct response, the so-called 'probabilistic errors' (0–4 per reversal). Reversal of the stimulus-reward contingency occurred after 10–15 correct responses (including probabilistic errors). Participants performed three successive 9-min blocks of the task, each taking 140–160 trials (block length was determined by the number of errors made). Stimuli were presented for a 2000 ms response window (RTs > 2000 ms were followed by a 'too late' message). Feedback was presented immediately

after the response for 500 ms. After feedback, the stimuli were replaced by a fixation cross for a variable duration so that the overall interstimulus interval was 3215 ms, enabling precise desynchronization from the repetition time (TR) of 1600 ms.

Four types of events were modeled: (i) a correct response followed by positive feedback, (ii) a correct response followed by negative feedback (probabilistic error), (iii) an incorrect response where the subject reversed on the subsequent trial (reversal switch error), and (iv) an incorrect response where the subject did not reverse (ie perseverated) on the subsequent trial (preceding error). Spontaneous discrimination errors (those which could not be categorized as reversal or probabilistic errors) were not included in the model.

Checkerboard Task

The checkerboard task was a passive visual task where the subject viewed two configurations of black and white squares in an 8×8 matrix that switched at a frequency of 8 Hz. Using a blocked ABAB design, 20 s checkerboard blocks alternated with 20 s crosshair fixation for six cycles, taking a total of 4 min.

Amino-Acid Mixture

The TRP-deficient amino-acid drink (TRP−) contained a total of 75 g of amino acids using the proportions described by Young *et al* (1985): 4.1 g L-alanine, 2.4 g glycine, 2.4 g L-histidine, 6.0 g L-isoleucine, 10.1 g L-leucine, 6.7 g L-lysine, 4.3 g L-phenylalanine, 9.2 g L-proline, 5.2 g L-serine, 4.3 g L-threonine, 5.2 g L-tyrosine, 6.7 g L-valine, 3.7 g L-arginine, 2.0 g L-cysteine, and 3.0 g L-methionine (SHS International Ltd, Liverpool, UK). The balanced mixture contained the same amino acids, plus 3.0 g TRP. The drinks were prepared with 200 ml tap water and fruit flavoring to compensate for the unpleasant taste.

Biochemical Measures

Blood samples (10 ml) were taken prior to ingestion of the amino-acid mixture and after the fMRI scan (about 6.5 h later), to determine the plasma TRP level and the TRP/ΣLNAA ratio. This ratio is important because the uptake of TRP in the brain is strongly associated with the amounts of other LNAA competing at the blood–brain barrier. Venous samples were taken in lithium heparin tubes, centrifuged, and stored at -20°C. Plasma TRP was determined by an isocratic high-performance liquid chromatography (HPLC) method of analysis. Plasma proteins were removed by precipitation with 3% trichloroacetic acid (TCA) and centrifugation at 3000 revs, 4° for 10 min, and then pipetted into heparin aliquots. An aliquot was then diluted in mobile phase before injection into the HPLC analysis column. Fluorescence end-point detection was used to identify TRP.

Paired-sample *t*-tests were used to compare the two baseline measurements of plasma TRP levels and TRP/ΣLNAA ratios, and to compare measurements of plasma TRP levels and the TRP/ΣLNAA ratio in the balanced and TRP− condition. A repeated-measures ANOVA was performed to look at the effect of ATD on plasma TRP levels and the TRP/ΣLNAA ratios.

Psychological Ratings

Visual Analogue Scales (VAS) containing the items drowsy, sad, happy, anxious and nauseous were administered five times during the test day (at roughly 90 min intervals). The Positive and Negative Affect Scale (PANAS; Watson *et al*, 1988) was completed prior to ingestion and after the scan. Repeated-measures ANOVA with drink treatment (TRP− and balanced) and time (two time points for the VAS, and two for the PANAS). Greenhouse–Geisser corrections were applied when the sphericity assumption was violated.

Behavioral Data Analysis

Dependent measures were the number of reversal contingencies during the task as a whole, the number of errors due to switching after a probabilistic error, mean RT, and a maintenance score that was calculated by dividing the number of errors made following five correct responses but prior to the next contingency reversal by the number of trials remaining prior to the next contingency reversal (adapted from Swainson *et al*, 2000) for each reversal block. Data were analyzed using repeated-measures ANOVA with block (1–3) and treatment (balanced and TRP−) as within-subjects factors and order of drug treatment (balanced first or TRP− first) as between-subjects factor. Greenhouse–Geisser corrections were applied when the sphericity assumption was violated. Simple effects of block and treatment were analyzed using *post hoc* tests with Bonferroni correction for multiple comparisons. Medians were used for analysis because RTs were not normally distributed. Measures that were not normally distributed were analyzed with the nonparametric Wilcoxon signed ranks test.

Image Acquisition

Participants were scanned in a 3 T Bruker Medspec scanner (S300; Bruker, Ettlingen, Germany), at the WBIC. T2*-weighted gradient echo planner images (EPI) (TE 27 ms) were acquired with blood oxygenation level-dependent (BOLD) contrasts. A whole-brain acquisition consisted of 21 slices (TR 1.6 s; voxel size before normalization $1.56 \times 1.56 \times 5\,\text{mm}^3$ and after normalization $3 \times 3 \times 3\,\text{mm}^3$; inter-slice gap 1 mm; matrix size 128×128; bandwidth 100 kHz; oblique orientation) and the total number of volumes acquired varied from run to run (from 142 to 166) depending on the participant's performance. In addition, high-resolution T1-weighted images for spatial normalization were acquired of each participant (voxel size $1 \times 1 \times 1\,\text{mm}^3$). We were unable to acquire reliable data from a section of ventromedial PFC because of susceptibility artifacts.

Image Analysis

Data analysis was performed using SPM99 and SPM2 (Statistical Parametric Mapping; Wellcome Department

of Cognitive Neurology, London, UK). Preprocessing procedures consisted of (linear) slice acquisition time correction, within-subject realignment (SPM2), geometric undistortion using fieldmaps (Cusack *et al*, 2003), spatial normalization using each individual subject's skull-stripped SPGR (using the Brain Extraction Tool; Smith, 2002), and the Montreal Neurological Institute (MNI) skull-stripped structural template (SPM2) and spatial smoothing using a Gaussian kernel (10 mm full-width at half-maximum).

A canonical hemodynamic response was used as a covariate in a general linear model and a parameter estimate was generated for each voxel for each event type. For each event, the hemodynamic response function was modeled to the onset of the response, which co-occurred with the presentation of the feedback for the reversal learning task.

For each subject, the following contrasts were computed: (i) Main task effect 1: Reversal switch errors *vs* baseline correct responses for the balanced condition only. (ii) Main task effect 2: Reversal nonswitch errors (which included probabilistic and preceding errors which were not followed by the subject switching responding) *vs* baseline correct responses for the balanced condition only. (iii) Main task effect 3: Reversal switch errors *vs* the other nonswitch errors for the balanced condition only. (iv) Treatment × task interaction 1, reflecting the effect of ATD on task effect 1. (v) Treatment × task interaction 2, reflecting the effect of ATD on task effect 2. (vi) Treatment × task interaction 3, reflecting the effect of ATD on task effect 3. Thus, treatment was modeled as a within-subject variable within each individual's general linear model. For the checkerboard task, an epoch (box-car) design contrasted checkerboard visual stimulation with crosshair fixation. Individual contrast images were taken to a second level analysis in which *t*-values were calculated for each voxel, treating inter-subject variability as a random effect.

The MarsBar tool (Brett *et al*, 2002) was used to average signal within independently defined ROIs at the group level. ROIs for the reversal task analysis were defined from the activation peaks found by Cools *et al* (2002); 10 mm spheres (corresponding to the smoothing filter) were built around the dorsomedial PFC ($x, y, z = 8, 32, 52$), right VLPFC ($x, y, z = 38, 24, -2$) and left VLPFC ($x, y, z = -32, 24, -4$). The random effects model was then reapplied to the average signal within these ROIs to test the statistical significance of the contrasts of interest (a one-sample *t*-test). Average signal change was extracted from each ROI and these are the values reported in Figure 2. In addition, we also performed whole-brain analyses. Both ROI and whole-brain analyses were thresholded at $P < 0.05$ (corrected for multiple comparisons). Given the *a priori* prediction concerning the modulation of the orbitofrontal cortex by ATD during reversal learning, we also examined the inferior, medial, and superior orbitofrontal cortex using ROIs from the Automated Anatomical Labelling (AAL) map based on the MNI average brain (Tzourio-Mazoyer *et al*, 2002), also thresholded at $P < 0.05$. Finally, the main effect of ATD was assessed by contrasting all task-related regressors from the TRP− condition with all task-related regressors from the balanced condition.

RESULTS

Functional Imaging Data

All significant task-related effects from the balanced condition are shown in Table 1. ATD significantly increased the BOLD response in the dorsomedial PFC during reversal switch errors relative to correct baseline responses. This effect reached significance in both the ROI (contrast iv; $T_{10} = 2.04$; $P = 0.03$; Figure 1) and whole-brain analyses (Talairach coordinates $x, y, z = 9, 39, 48$; $T_{10} = 11.95$; $P_{corrected} = 0.006$). However, the effect of ATD did not reach

Table 1 Significant Task Effects Revealed by Regions of Interest Analyses (From the Balanced Condition Only)

Increased activation	*T*-values	*P*
Reversal switch errors minus correct responses (contrast i)		
Dorsomedial PFC	4.22	<0.001
Right ventrolateral PFC	7.02	<0.001
Left ventrolateral PFC	3.43	<0.01
Other non-switch errors minus correct responses (contrast ii)		
Right ventrolateral PFC	4.83	<0.001
Left ventrolateral PFC	3.16	<0.01
Reversal switch errors minus other nonswitch errors (contrast iii)		
Dorsomedial PFC	5.15	<0.001
Right ventrolateral PFC	4.55	<0.001

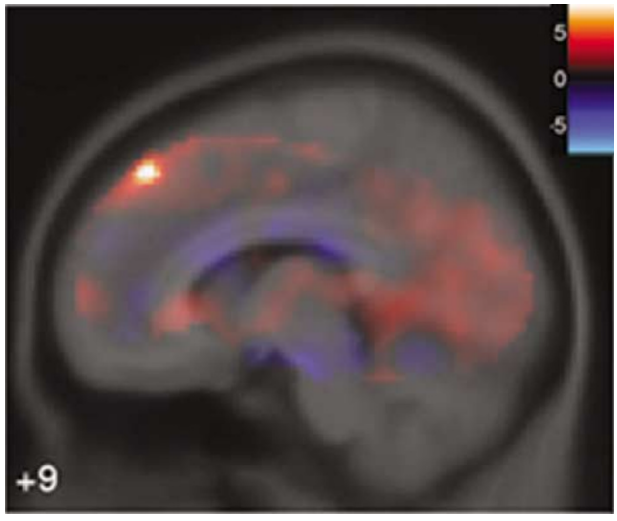

Figure 1 The continuous *t*-value image (statistical parametric map) (color-coding according to legend in top-right corner: light blue = extreme negative *t*-value and light red = extreme positive *t*-value) is shown as a sagittal section (MNI coördinate $y = 9$) superimposed upon the average MNI T1 template (average of 152 brains). The *t*-map represents functional activation changes following ATD relative to the balanced (placebo) condition during reversal switch errors relative to baseline correct responses (a treatment × task interaction effect). Whole-brain (and ROI) analyses revealed a significant signal increase following TRP depletion in the dorsomedial PFC, centered on MNI coördinates (x, y, z) = (9, 39, 48) ($T_{10} = 11.95$, $P_{corrected} = 0.006$).

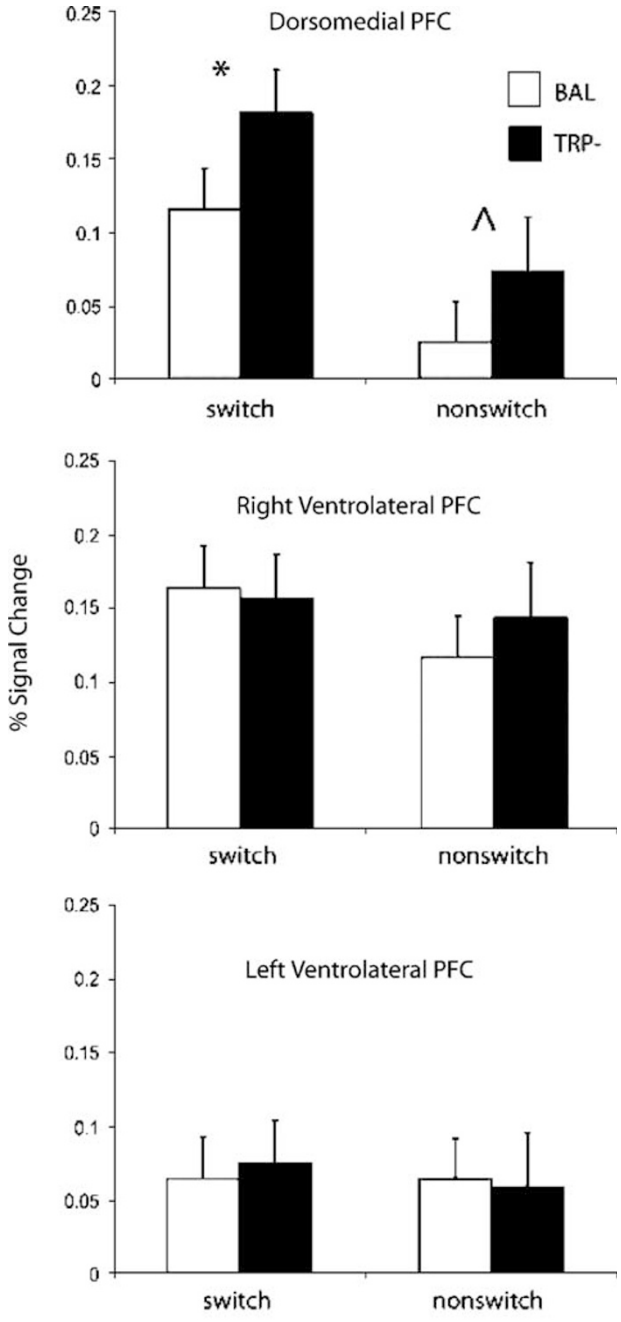

Figure 2 Percent signal change is shown for three ROI: the dorsomedial PFC, the right and the left ventrolateral PFC for reversal switch errors (relative to baseline correct responses), and nonswitch errors (including probabilistic and preceding errors relative to baseline correct responses) in the BAL and TRP− conditions. Errors bars represent SEMs. ROI (spheres with 10 mm radius) were defined from peak coordinates from Cools *et al* (2002) (see Materials and methods section for coordinates). $^{*}P<0.05$, $^{\wedge}P<0.1$.

significance when the reversal switch errors were compared with the other nonswitch errors (contrast vi; $T_{10}=1.33$; $P=0.1$). Furthermore, the increase in signal in the dorsomedial PFC tended towards significance when the reversal nonswitch errors were compared with baseline correct responses (contrast v; $T_{10}=1.44$; $P=0.09$) (Figure 2). Thus, ATD increased signal changes during all negative feedback, irrespective of whether the errors were followed by behavioral reversal. ATD did not affect the BOLD response in the left and right VLPFC ($T_{10}=-0.13$, $P=0.55$ and $T_{10}=-0.21$, $P=0.6$, respectively). Furthermore, ATD did not significantly affect global activation changes during the task, as revealed by both whole-brain and ROI analyses (ROI analyses: left VLPFC: $P=0.33$; right VLPFC: $P=0.32$; medial PFC: $P=0.23$).

While an ATD-induced increase in the orbitofrontal cortex during the reversal switch errors compared with correct responses did not reach significance in the ROI or whole-brain analysis according to our criterion (AAL's left middle orbital gyrus: $T_{10}=1.12$; $P=0.14$), for completion we report that whole-brain analysis revealed a nonsignificant effect at $x, y, z=-48, 42, -3$ ($T_{10}=5.72$; $Z=3.7$).

Supplementary analysis revealed that our findings are not confounded by the fact that four participants started with the TRP− condition and seven participants started with the balanced condition. This analysis of the individual parameter estimates, extracted from the dorsomedial PFC ROI (reversal switch errors minus baseline correct responses), revealed that the effect of ATD was not qualified by testing order (ATD × testing order interaction: $F_{1,9}=0.5$, $P=0.5$). No differences in signal change were observed between subjects who ingested the TRP− drink on the first occasion (mean signal change = 0.16) and subjects who ingested the TRP− drink on the second occasion (mean signal change = 0.19), and no differences were observed between subjects who ingested the balanced mixture on the first occasion (mean signal change = 0.11) and subjects who ingested the balanced mixture on the second occasion (mean signal change = 0.12).

Whole-brain analyses did not reveal any other significant effects.

Behavioral Effects of ATD on Reversal Learning

There was a nonsignificant tendency for ATD to slow overall RT ($F_{1,9}=4.72$, $P=0.06$); mean RT, TRP− = 554 ms, BAL = 522 ms). No other differences were found between the TRP− and balanced condition. Mean values are presented in Table 2.

Checkerboard Task

Visual stimulation was associated with a large and highly significant activation cluster ($T=13.63$, $P=0.002$) in the occipital cortex. No effects of ATD on visual stimulation in

Table 2 Behavioral Effects of ATD

	Balanced	TRP−	*P*-value
Total number of reversals	26.8 (0.1)	26.1 (0.5)	0.26
Total number of switches after probabilistic errors	10.3 (2.8)	10.3 (2.9)	0.55
Mean maintenance score	0.06 (0.0)	0.06 (0.1)	0.64
Mean overall RT	523 (19)	554 (31)[a]	0.06

Values represent means (SEMs).
[a]The effect of ATD on mean RT tended towards significance at $P=0.06$.

Table 3 Psychological Ratings of Mood at Baseline t0 and Post-Scanning t6.5

	BAL t0	BAL t6.5	TRP− t0	TRP− t6.5	*P*-value
VAS					
Drowsy	2.0 (2.1)	3.1 (2.3)	2.1 (1.8)	3.4 (3.1)	0.07
Sad	1.0 (0.7)	1.8 (1.6)	1.8 (1.6)	2.0 (1.6)	0.60
Happy	5.2 (2.0)	4.0 (2.3)	5.5 (1.5)	5.0 (1.4)	0.68
Anxious	2.6 (1.8)	1.2 (1.5)	1.9 (1.4)	1.4 (1.0)	0.37
Nausea	1.6 (2.0)	1.4 (1.1)	1.9 (2.0)	1.7 (1.3)	0.56
PANAS					
Positive	26.9 (1.9)	23.7 (1.6)	28.6 (0.9)	23.6 (1.8)	0.36
Negative	13.4 (0.8)	11.8 (0.5)	13.4 (0.7)	13.3 (0.8)	0.14

BAL = balanced condition; TRP− = tryptophan-depleted condition.

this region in occipital cortex were found, even at $P = 0.01$ uncorrected for whole-brain volume.

Biochemical Measures

The analysis of amino-acid levels revealed that the depletion was successful. No differences between conditions were found in terms of plasma TRP level (mean/SEM: BAL 12.2/2.3; TRP 12.3/3) and ratio TRP/ΣLNAA's (BAL 0.19/0.02; TRP− 0.16/0.02) at baseline. After 6.5 h, plasma TRP and the ratio TRP/ΣLNAA were significantly lower in the TRP− condition compared with the balanced condition (TRP levels: BAL 18.2/6.3; TRP− 4.4/1.8; $T_{10} = 8.3$, $P < 1$ and ratios: BAL 0.16/0.03; TRP− 0.04/0.01; $T_{10} = 4.0$, $P < 0.01$, respectively). Following the TRP− drink, plasma TRP was reduced by 64% and the ratio TRP/ΣLNAA's by 74% relative to baseline t0. Following the balanced drink, the plasma TRP was increased by 50% and the ratio TRP/ΣLNAA was reduced by 16%.

Psychological Ratings

No interaction effects of ATD by time on PANAS-positive and -negative effect scores were observed (positive effect: $F_{1,10} = 1.1$, $P = 0.3$; negative effect: $F_{1,10} = 1.0$, $P = 0.3$), while there were significant main effects of time across drink treatment on both scores (positive effect: $F_{1,10} = 6.7$, $P = 0.03$; negative effect: $F_{1,10} = 6.1$, $P = 0.03$) (Table 3). Similarly, while some main effects of time reached significance for VAS scores (Drowsiness increased over time: $F_{1,10} = 14.7$, $P = 0.003$; Happiness and anxiety decreased over time: $F_{1,10} = 011.3$, $P = 0.007$ and $F_{1,10} = 11.1$, $P = 0.008$, respectively), no significant interaction effects between time and drink treatment on VAS scores were observed (Drowsiness: $F_{1,10} = 0.04$, $P = 0.8$; Sadness: $F_{1,10} = 0.7$, $P = 0.4$; Happiness: $F_{1,10} = 1.0$, $P = 0.3$; Anxiety: $F_{1,10} = 1.6$, $P = 0.2$; Nausea: $F_{1,10} = 0.03$, $P = 0.9$).

DISCUSSION

The present findings reveal that acute depletion of central 5-HT in young healthy male volunteers significantly increased the task-related BOLD response in the dorsomedial PFC during probabilistic reversal learning. By contrast, ATD did not modulate task-related signal change in the ventrolateral PFC. These effects contrast with those observed following manipulation of the central dopamine system in young healthy volunteers, which reduced the BOLD signal in the left ventrolateral PFC during reversal learning but did not affect signal in the dorsomedial PFC (Clark *et al*, 2004), and suggest that changes in the 5-HT system affect probabilistic reversal learning via action in the dorsomedial PFC.

The observed reduction in plasma TRP was comparable to that seen in previous studies (Young *et al*, 1985; Murphy *et al*, 2002) and data from both animal and human studies indicate that acute precursor depletion induces significant reductions in the synthesis and release of brain 5-HT (Biggio *et al*, 1974; Nishizawa *et al*, 1997; Carpenter *et al*, 1998). The BOLD changes following ATD are unlikely to reflect indirect effects of the dietary manipulation on mood or arousal, because no effects of ATD on any of the subjective ratings were observed. As predicted, ATD did not affect depressive symptomatology in this sample of young volunteers without a history of psychiatric disorder (Benkelfat *et al*, 1994). Although there was a tendency towards an overall slowing of response latencies following depletion of central 5-HT, the BOLD changes were not accompanied by significant behavioral changes and are thus unlikely to reflect increased task difficulty. The observed tendency towards a behavioral effect concurs with previous observations that ATD induces significantly slower latencies in a neuropsychological version of the probabilistic reversal learning task (Murphy *et al*, 2002), which involved only a single reversal of contingencies. Murphy *et al* (2002) showed that ATD slowed responding only in a first test session, in which the task was novel to the subjects. In light of these previous observations, we suggest that the present behavioral measure of repeated, well-practiced and serial reversal learning was not sufficiently sensitive to reveal significant behavioral effects of ATD. Furthermore, we propose that the physiological BOLD response provided a more sensitive measure of the effects of ATD, hypothetically accompanying behavioral changes that surface only in more sensitive, single reversal learning paradigms.

The increased response in the dorsomedial PFC after 5-HT depletion was significant when the reversal switch errors were compared with correct responses, but did not reach significance when the reversal switch errors were compared with the reversal nonswitch errors. Moreover, ATD increased (although only marginally significantly) the BOLD signal during the reversal nonswitch errors relative to correct responses. The ATD effect was therefore not restricted to errors that were followed by a behavioral switch, but extended to the other error types where negative feedback was presented without subsequent reversal. The present task thus implicates 5-HT in processes other than reversal switching, which may include the detection/monitoring of errors and/or conflict. This is consistent with cognitive models of medial frontal cortex function, which emphasize the region's involvement in error detection and conflict monitoring (Paus *et al*, 1993; Carter *et al*, 1998; Gehring and Fencsik, 2001; Kerns *et al*, 2004; MacDonald *et al*, 2000; Garavan *et al*, 2003).

Based on evidence for a role of 5-HT in the processing of aversive signals, as well as models of depression as an oversensitivity to negative feedback (Elliott *et al*, 1997; Steffens *et al*, 2001; Murphy *et al*, 2003; Graeff *et al*, 1986; Smith and Kennedy, 2003), we hypothesize that the increased BOLD signal in the dorsomedial PFC reflects enhanced processing of aversive signals (error feedback) leading to enhanced response conflict on both switch and nonswitch trials. Clearly, the task does not fully dissociate negative feedback, conflict and behavioral switching: negative feedback initiates conflict and subsequent reversal, and a probabilistic task places demands on inhibitory control to maintain responding after misleading feedback. This is illustrated by the findings of Murphy *et al* (2003), showing that depressed people exhibit impaired maintenance of responding in the probabilistic reversal learning task, specifically following misleading negative feedback. Serotonergic modulation of medial frontal function is also consistent with the selective abnormalities seen in this region in structural imaging and post-mortem investigations in mood disorders (Drevets *et al*, 1997; Rubinsztein *et al*, 2001; Merali *et al*, 2004).

Altered 5-HT neurotransmission in the PFC has also been associated with failures of inhibitory control (Leyton *et al*, 2001; Clarke *et al*, 2004; Liu *et al*, 2004). A recent study has demonstrated increased perseverative responding in marmosets following prefrontal 5-HT depletion, using a simple (nonprobabilistic) reversal learning task (Clarke *et al*, 2004). There are significant methodological differences between the tasks used in that study and the current fMRI task. Primarily, the probabilistic nature of the fMRI task increases the incidence of error processing and is likely to enhance the salience of punishment during the task. This may render the task particularly sensitive to changes in punishment sensitivity. The nature of punishment is also different in the two tasks. The fMRI task employs a specific error signal (a red sad face), whereas reversal is indicated to the marmoset by the omission of reward for the previously relevant stimulus, co-occurring with unexpected reward for the newly relevant stimulus. The hypothesized increase in sensitivity to negative feedback induced by ATD in the current study may coexist with a deficit in the inhibitory control of behavior. In probabilistic reversal learning, this may diminish the ability to maintain responding to the relevant stimulus following misleading negative feedback, as shown in depressed patients by Murphy *et al* (2003). However, in a nonprobabilistic reversal learning task that de-emphasizes the processing of punishment cues, behavioral changes may surface as impaired inhibitory control, in the form of perseverative responding. Of course, it also remains to be established at the present time whether a global depletion of 5-HT precursor in humans is functionally equivalent to a prefrontal lesion of 5-HT fibres in marmosets.

The present pattern contrasts with effects of the (dopamine) DA D2 receptor antagonist as well as the indirect catecholamine agonist methylphenidate, which resulted in significant reductions in the BOLD signal in the ventrolateral PFC that were specific to behavioral switching and cannot have been solely induced by the reception of negative feedback (Clark *et al*, 2004). This dissociation concurs with the observation that the indoleamine 5-HT modulates in particular a medial and orbital prefrontal neural system associated with associative learning and affective decision making, while catecholamines (ie DA and norepinephrine) affect predominantly a system connecting to the lateral PFC associated with set shifting, response inhibition, and working memory (Robbins, 2000; Aron *et al*, 2004).

The PFC is densely innervated with large ascending serotonergic projections from the raphé nuclei and there are several mechanisms via which 5-HT may modulate PFC neuronal responses. For example, evidence from *in vitro* studies show that activation of 5-HT_{2A} receptors induces a rapid increase in excitatory postsynaptic potentials/currents in virtually all layer V pyramidal cells of neocortex, but most prominently in medial PFC and other frontal regions where 5-HT_{2A} receptors are enriched (Marek and Aghajanian, 1998). Activation of postsynaptic 5-HT_{1A} receptors in the PFC, on the other hand, have been found to inhibit pyramidal cell activity (Puig *et al*, 2004). It is unlikely that our findings reflect vasoconstrictive or vasodilative effects of ATD leading to indirect (non-neuronal) changes in the hemodynamic response, because the increase was regionally selective to dorsomedial PFC and did not extend to other task-related brain areas, which were modulated by dopaminergic drugs. Vascular effects would be predicted to affect task-related signal globally. Moreover, ATD did not affect significantly task-related signal during the presentation of a checkerboard task that elicited a robust response in visual striate cortex.

The present study only used male volunteers. Previous studies showed that females are more sensitive to mood effects after ATD than males (Ellenbogen *et al*, 1996) and thus male volunteers were selected for the present study to avoid the potentially confounding effects of mood on task performance and task-related BOLD signal change. However, the cognitive and biological effects of 5-HT depletion may be markedly greater in female subjects (Nishizawa *et al*, 1997; Harmer *et al*, 2003). For example, ATD reduced the rate of 5-HT synthesis by 40 times in females compared to only 10 times in males (Nishizawa *et al*, 1997). Our results might be representative for a male population only, and need to be extended to female subjects in subsequent research. In a further feature of the experimental design, we employed a 75 g TRP− mixture rather than the more typical 100 g mixture, in order to minimize the incidence of nausea and vomiting during the scanning procedure. The biochemical analyses confirmed a successful depletion with this reduced volume, whereby plasma TRP was reduced by 64% on average, and the ratio of TRP to other LNAA was reduced by 74%. The ratio of TRP/ΣLNAA was virtually unaffected by the balanced mixture (only a 16% decrease relative to baseline), and this ratio is associated most closely with reduced 5-HT synthesis.

In conclusion, acute depletion of brain 5-HT levels in healthy male volunteers increased the BOLD signal in dorsomedial PFC during performance of a task that involves feedback processing and the adaptation of behavior. The effects of 5-HT were related to the processing of negative feedback more than behavioral adaptation *per se*, and this provides one of the first direct demonstrations of

serotonergic modulation of medial frontal processes in human subjects. During reversal learning, the dorsomedial PFC response is part of an extended network of frontal activity, and these data highlight the potential of pharmacological fMRI as a tool for dissecting the neurochemical contributions to cognitive control mechanisms.

ACKNOWLEDGEMENTS

We thank Wim Riedel and Ed Bullmore for helpful discussion, Matthew Brett for analysis support, Ruth Bisbrown-Chippendale and Clair Sleator from the Wolfson Brain Imaging Centre (Cambridge, UK) for radiographic assistance, and nursing staff of the Wellcome Trust Clinical Research Facility at Addenbrooke's hospital. This study was funded by a Wellcome Trust programme grant to TW Robbins, BJ Everitt, AC Roberts, and BJ Sahakian (LC), and completed within the MRC Centre for Behavioural and Clinical Neuroscience. EATE is funded by the ZonMW grant 912-02-050 from the dutch Organization for Scientific Research (NWO). RC holds a Royal Society Dorothy Hodgkin Fellowship and a Junior Research Fellowship from St John's College, Cambridge, UK.

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