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Article *in* Psychopharmacology · December 2005

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Psychopharmacology (2005) 182: 570–578
DOI 10.1007/s00213-005-0104-6

ORIGINAL INVESTIGATION

L. Clark · J. P. Roiser · R. Cools · D. C. Rubinsztein · B. J. Sahakian · T. W. Robbins

Stop signal response inhibition is not modulated by tryptophan depletion or the serotonin transporter polymorphism in healthy volunteers: implications for the 5-HT theory of impulsivity

Received: 27 April 2005 / Accepted: 17 June 2005 / Published online: 15 September 2005

Abstract *Rationale:* Reduced serotonin neurotransmission is implicated in disorders of impulse control, but the involvement of serotonin in inhibitory processes in healthy human subjects remains unclear. *Objectives:* To investigate the effects of an acute manipulation of serotonin and genotype at a functional polymorphism in a gene coding for the serotonin transporter (5-HTT) on an established measure of response inhibition. *Methods:* Serotonin function was reduced by the acute tryptophan depletion (ATD) procedure in a double-blind, crossover design in 42 healthy subjects. The Stop Signal Task (SST) was administered 5–7 h after drink administration. The influences of 5-HTT polymorphism, gender and trait impulsivity were investigated. *Results:* ATD was associated with significant depletion of plasma tryptophan levels but did not increase the stop signal reaction time in comparison to the balanced (placebo) amino acid mixture. Subjects possessing the short allele of the 5-HTT polymorphism were not more impulsive on the SST than subjects homozygous for the long allele under placebo conditions and were not disproportionately sensitive to the effects of ATD. There was no effect of gender or trait impulsivity on ATD-induced change. *Conclusions:* We find no support for the involvement of brain serotonin neurotransmission in this form of inhibitory control in healthy human subjects.

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Introduction

Central serotonin (5-HT) neurotransmission is implicated in several domains of psychological function, including threat processing, mood and memory. One influential account has posited an association between decreased 5-HT activity and impulsivity (Evenden 1999; Soubrié 1986). This model is supported by preclinical research using rodent and non-human primate models of impulsivity (e.g. Clarke et al. 2004; Mehlman et al. 1994; Mobini et al. 2000; Wogar et al. 1993). Evidence in humans is derived mainly from studies in clinical groups with impaired impulse control. For example, reduced levels of 5-HT metabolites in the cerebrospinal fluid have been reported in violent offenders (Linnoila et al. 1983; Virkkunen et al. 1994) and suicide victims (Asberg et al. 1976). Subsequent research using selective serotonergic manipulations showed an attenuation of impulsive responding by acute fenfluramine (a 5-HT releasing agent) and chronic paroxetine (a selective serotonin re-uptake inhibitor) in criminals with a history of conduct disorder (Cherek and Lane 2000; Cherek et al. 2002). Conversely, 5-HT depletion impaired impulse control in subjects at high-risk of developing alcoholism (Crean et al. 2002; LeMarquand et al. 1999).

The degree to which 5-HT is implicated in inhibitory control in healthy human subjects without impulsive or aggressive disorders is unclear. The acute tryptophan depletion (ATD) procedure has been commonly used to deplete 5-HT function in human experimental studies (Riedel 2004; Smith et al. 1997; Young et al. 1985). In this technique, subjects ingest an amino acid load specifically lack-

ing tryptophan, the precursor to 5-HT. 5-HT neurotransmission is reduced for several hours via increased competition for transport across the blood-brain barrier (Fernstrom 1981) and increased protein synthesis in the liver (Moja et al. 1991). ATD studies in healthy volunteers have found mixed effects on inhibitory function, with previous studies reporting impairment (Walderhaug et al. 2002), improvement (Crean et al. 2002) or no effect (LeMarquand et al. 1999; Murphy et al. 2002; Rubinsztein et al. 2001).

Several factors may contribute to this disparity. Primarily, impulsivity is unlikely to represent a unitary construct (Evenden 1999). Tasks measuring discrete processes such as response inhibition, perseveration and delay of gratification may draw on distinct neural mechanisms and correlate only weakly with one another (de Wit et al. 2002; Swann et al. 2002; Winstanley et al. 2004). Secondly, some measures of impulsivity may lack sensitivity due to ceiling levels of performance in healthy subjects. Specifically, the Go–No Go procedure may fail to detect impulsivity following pharmacological challenge because healthy subjects typically make very few commission errors over the course of the task (e.g. LeMarquand et al. 1999; Rubia et al. 2005). Thirdly, individual differences may mediate susceptibility to ATD. The cognitive effects of 5-HT challenge may vary as a function of personality, such as self-report ratings of impulsivity (Cools et al. 2005) or aggression (Dougherty et al. 1999). Gender may also be an important factor: female subjects show reduced rates of 5-HT synthesis (Nishizawa et al. 1997) and greater cognitive effects following ATD (Harmer et al. 2003) compared to male subjects. In addition, many sources of genetic variation in 5-HT neurotransmission exist (Hariri and Weinberger 2003). The 5-HT transporter (5-HTT) linked polymorphic region (5-HTTLPR) is a functional polymorphism associated with two common alleles, termed short (s) and long (l) (Lesch et al. 1996). Subjects possessing one or two copies of the s allele display reduced 5-HTT expression (Lesch et al. 1996), with concomitant effects on 5-HT neurotransmission (David et al. 2005; Whale et al. 2000; Williams et al. 2001). These individuals are at increased risk of mood and anxiety disorders (Caspi et al. 2003; Hoefgen et al. 2005; Lesch et al. 1996) and display a slowed response to antidepressant treatment (Durham et al. 2004; Pollock et al. 2000; Smeraldi et al. 1998). Healthy subjects carrying the short allele of this polymorphism also displayed increased fear conditioning (Garpenstrand et al. 2001), heightened amygdala reactivity to fearful faces (Hariri et al. 2002) and increased negative affect in response to ATD (Neumeister et al. 2002). However, there has been no systematic investigation of this polymorphism in relation to laboratory measures of impulsivity.

The present study focussed specifically on the construct of response inhibition, as measured by the Stop Signal Task (SST). In this task, subjects must suppress a prepotent motor ('Go') response on a minority of trials when an auditory stop signal is presented. The stop signal occurs at a short delay (the stop signal delay, SSD) after the presentation of the Go stimulus. Consequently, the response must be curtailed after some level of motor initiation. This feature increases the sensitivity of the stop signal procedure relative to the Go–No Go procedure. The key measure of inhibitory function on the SST, the stop signal reaction time (SSRT), can be estimated based on the assumptions of the race model (Logan et al. 1984), by assuming that Go- and Stop-related processes compete for behavioural output.

In our previous experiment (Cools et al. 2005; Evers et al. 2005), the ATD procedure was administered to 12 healthy volunteers in a crossover design. We measured performance on a novel task that assessed the speeding of reaction time by cues that were predictive of high reinforcement certainty. ATD was shown to abolish 'reinforcement-related speeding' in highly impulsive participants (Cools et al. 2005). We reported no influence of ATD on the SST (Cools et al. 2005). However, this observation remained inconclusive due to the relatively small group size and the inclusion of male volunteers only. The present report supplements that group of subjects with an additional 30 volunteers prescreened for the 5-HTT polymorphism. We predicted that the effect of the ATD procedure would be more pronounced in s carriers, who display reduced 5-HT neurotransmission under baseline conditions (Neumeister et al. 2002; Whale et al. 2000). We also examined the influence of gender and trait impulsivity [assessed using the Barratt Impulsivity Scale version 11 (BIS 11)] on ATD-induced responses.

Methods

Participants

Forty-two healthy volunteers attended two sessions at the Wellcome Trust Clinical Research Facility in Cambridge. Participants were recruited via local advertisement and consisted of 29 males and 13 females, with a mean age of 25.9 years [range 18–48, standard deviation (sd) 6.8]. The study was approved by the Local Research Ethics Committee, and all participants provided written informed consent after complete description of the study. Exclusion criteria were a history of psychiatric or neurological disorder, significant visual or auditory impairment and current use of medication, assessed by questionnaire and interview with a postgraduate psychologist. Twelve participants received a functional magnetic resonance imaging scan prior to cognitive testing on each session (Evers et al. 2005). These 12 subjects were genotyped for the 5-HTT polymorphism from a blood sample taken on the first test session. Eleven subjects carried one (n=7) or two (n=4) copies of the short allele, none were long–long homozygotes, and genotype could not be determined for one subject due to insufficient DNA material. The other 30 participants were preselected on the basis of homozygosity of the 5-HTT polymorphism, i.e. 15 long–long (eight males, seven females) and 15 short–short (nine males, six females), assessed from a blood sample taken during a screening session from 150 volunteers.

Procedure

Subjects were administered the ATD procedure in a randomised, placebo-controlled, double-blind crossover design. The ATD procedure entails the ingestion of an amino acid load that specifically lacks tryptophan. The placebo condition involved the ingestion of an amino acid mixture with a balanced (BAL) proportion of tryptophan. The two test sessions were separated by at least 1 week. Participants abstained from alcohol for 24 h prior to each test session and followed a low-protein diet during test days with a light snack at +2.5 h. On arrival at the test facility, a baseline blood sample was taken, and the amino acid mixture (ATD or BAL mixture) was ingested. Session order was approximately counter-balanced (23 subjects, ATD-BAL; 19 subjects, BAL-ATD). After a resting period of 5-7 h to allow depletion of central serotonin neurotransmission (Carpenter et al. 1998), a second blood sample was taken, and subjects performed the SST as part of a cognitive assessment taking 1.5-2 h (see Cools et al. 2005; Roiser et al. unpublished observations). Subjects also completed the BIS 11 (Patton et al. 1995) during the post-ingestion rest period as a measure of self-reported trait impulsivity.

Amino acid mixtures

The ATD mixture was a 75-g load of amino acids following 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, 3.0 g L-methionine. The BAL drink contained an additional 3.0 g tryptophan but was identical in all other respects. Female participants were administered a 20% reduced amino acid load to take into account lower bodyweight and the greater effect of ATD on serotonin metabolism in women (Nishizawa et al. 1997). The drinks were prepared blind by suspending the amino acid powder in 200 ml tap water with fruit flavouring added.

Stop signal task

Subjects were administered the SST on an Advantech PC with a 10.5-in. monitor (Aron et al. 2003a,b). The task was programmed using Experimental Run Time Software (ERTS; Berisoft, Germany) with a two-choice response box. Subjects responded using the index and middle fingers of their dominant hand. The task involves rapid two-choice 'Go' responding to a left- or right-facing arrow (i.e. a left button press to a left facing arrow and vice versa). On a minority (25%) of trials, an auditory stop signal (a 100-ms, 300-Hz tone) was presented at a short delay after the Go signal had appeared. Subjects were instructed to attempt to suppress their Go response when the Stop signal was presented. Subjects performed a practice block of 16 Go trials, before commencing 5 blocks of 64 trials, with 16 stop trials per block (hence, 80 stop trials in total). On each trial, a 500-ms fixation cue (a circle) preceded the Go stimulus. The Go stimulus remained on the screen until the subject responded, with a minimum inter-trial interval of 1,500 ms. Discrimination errors (e.g. a right-button press to a left-facing arrow) yielded the message 'Wrong!' for 200 ms. No feedback was provided in relation to correct or failed stopping on any trial. To maintain rapid Go responding across the task, mean reaction time data were appended to a line graph presented after each block: subjects were encouraged to maintain a constant speed of responding, whilst trying their best to inhibit whenever possible.

The difficulty of stopping was manipulated across trials by varying the delay between the onset of the Go signal and the Stop signals (the stop signal delay, SSD). The SSRT can be estimated by titrating the SSD at which subjects successfully stop on 50% of trials. The SSD was systematically varied in 50-ms increments using a tracking method to adjust the level of difficulty to the subject's stopping ability (Osman et al. 1990): a successfully inhibited response increased the SSD by 50 ms on the next trial (thereby making the next trial more difficult), whereas a failure to stop decreased the SSD by 50 ms on the next trial (making the next trial easier). The tracking procedure ensured that over time, the probability of successful inhibition reliably stabilised around 0.5 for each subject. To ensure unpredictability of the SSD, four overlapping staircase functions were used, starting at 100, 200, 400 and 500 ms. The SSRT was estimated by subtracting the average SSD after stabilisation (during the second half of the task, i.e. stop trials 41–80) from the average (median) Go reaction time (Go RT). The median Go RT was used to provide a superior estimate to the mean Go RT given the typical positive skew of rapid RT data.

Data analysis

Analysis is reported from 40 subjects: one subject was excluded for abnormal behavioural performance (see below), and 5-HTT genotype could not be determined for one subject.

Analysis of plasma samples

There were missing data points for four subjects due to difficulties with blood extraction. Blood samples (10 ml) were analysed to determine the total plasma TRP level and the TRP:ΣLNAA ratio. This ratio was calculated from the serum concentrations of total tryptophan divided by the sum of the large neutral amino acids (tyrosine, phenylalanine, valine, isoleucine, leucine) and is important because the uptake of TRP in the brain is strongly associated with the amounts of other competing LNAAs due to non-specific transport across the blood-brain barrier. 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) method of analysis. Plasma proteins were removed by precipitation with 3% trichlororacetic acid and centrifugation at 3,000 rpm, 4°C degrees for 10 min, 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.

Analysis of 5-HTT genotype

PCR was based on the protocol of Furlong et al. (1998) and was performed using the primers stpr5: 5′-ggc gtt gcc gct ctg aat gc-3′ and stpr3: 5′-gag gga ctg agc tgg aca acc ac-3′. Successful amplification was achieved using an initial denaturing step at 95°C for 4.5 min followed by 35 cycles of 95°C for 30 s, 61°C for 30 s, 72°C for 30 s and a final extension of 72°C for 10 min. The 25-μl reaction mixture consisted of 1 μl DNA (approximately 50–200 ng), 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MGCl2, 5% DMSO, 200 μM dNTPs (dGTP/7-deaza dGTP 1:1), 100 ng of each primer and 0.5 units *Taq* polymerase at pH 9.0. Products were analysed on 2% agarose gels and stained with ethidium bromide.

Analysis of behavioural SST data

Dependent variables from the SST were the median Go RT, the SSRT and the total number of discrimination errors. Behavioural data from the task were screened for outliers using a cut-off of 2.5 standard deviations from the average median Go reaction time in the placebo condition. One subject (male, short–long heterozygote) exceeded this threshold with a median reaction time of 609 ms (3.2 sd above the BAL mean). This slowing was associated with an abnormally high proportion of successful inhibitions (64 and 72% in the BAL and ATD conditions, respectively). This subject was judged to have delayed Go responding in order to improve stop signal detection. This invalidates the calculation of SSRT, as the race model assumes that the Go process starts on the presentation of the Go stimulus. Accordingly, the subject was excluded from all further analysis.

Data were tested for violation of the normality assumption using the Kolmogorov–Smirnov test. Discrimination errors were square-root-transformed to achieve suitability for parametric statistics; all other raw data were normally distributed. Stop signal variables were analysed using separate mixed-model analyses of variance (ANOVA) with condition (ATD, BAL) as a within-subjects factor, and 5-HTT genotype and gender as between-subjects factors. For the genotype factor, subjects carrying one (n=6) or two (n=19) copies of the short allele (n=25) were contrasted against subjects homozygous for the long allele (n=15) (Hariri and Weinberger 2003; Lesch et al. 1996). The probability of inhibition (P_{inhibit}) was plotted in Fig. 1 as a function of the SSD term. These P_{inhibit} data were analysed using a mixed-model ANOVA with SSD as a nine-level within-subjects factor (100–500 ms in 50-ms increments), condition as a two-level (ATD, BAL) within-subjects factor, and 5-HTT genotype and gender as between-subjects factors. All tests employed two-tailed statistics thresholded at $p<0.05$.

Results

Stop signal performance

Behavioural data from the SST are displayed in Table 1. A mixed-model ANOVA of SSRT data indicated no significant main effect of treatment ($F_{1,36}$=0.00, p=0.996), 5-HTT genotype ($F_{1,36}$=0.538, p=0.468) or gender ($F_{1,36}$=0.435, p=0.514), and no significant interaction terms (all $F<1.0$). There was no effect of treatment ($F_{1,36}$=1.43, p=0.239), genotype ($F_{1,36}$=0.206, p=0.653) or gender ($F_{1,36}$=0.077, p=0.783) on median Go RT. The gender × genotype

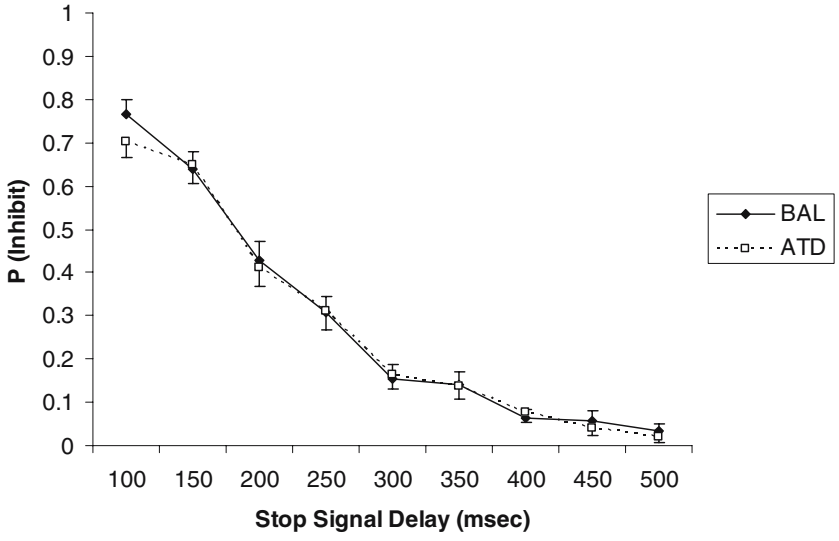

Fig. 1 The probability of successfully inhibiting a response on a stop trial (P_{inhibit}) varies as a function of the delay between the presentation of the Go stimulus and presentation of the Stop Signal (the *Stop Signal Delay*, SSD). There was no difference in inhibitory capacity in subjects in the acute tryptophan depletion (*ATD*) and balanced (*BAL*) conditions. *Error bars* indicate standard errors of the mean

Table 1 Performance [mean (sd)] on the stop signal task following placebo (BAL) and serotonin-depleting (ATD) mixtures

	BAL	ATD		
Go RT	378.7 (61.2)	371.9 (56.6)		
SSRT	189.0 (55.5)	190.8 (52.6)		
Errors	5.7 (7.3)	5.3 (5.7)		
	Male		Female	
	BAL	ATD	BAL	ATD
Go RT	373.3 (63.6)	368.6 (63.2)	389.9 (56.4)	378.6 (40.9)
SSRT	183.7 (48.4)	186.4 (52.6)	200.1 (68.7)	200.0 (53.6)
Errors	6.3 (7.7)	6.1 (5.9)	4.6 (6.4)	3.7 (5.1)
	Short carriers (ss/sl)		Long homozygotes (ll)	
	BAL	ATD	BAL	ATD
Go RT	371.5 (62.1)	363.6 (57.4)	390.6 (59.7)	385.5 (54.3)
SSRT	180.2 (46.4)	187.5 (51.0)	203.8 (67.1)	196.3 (56.6)
Errors	5.4 (7.5)	4.4 (4.7)	6.3 (7.1)	6.9 (6.8)

interaction for Go RT approached significance ($F_{1,36}$=3.85, p=0.057), but simple effects analysis collapsing across treatment revealed no significant differences (all p>0.10). The other interaction terms involving treatment were not significant (all F<1.0). Analysis of discrimination errors indicated no effect of treatment ($F_{1,36}$=0.123, p=0.728), genotype ($F_{1,36}$=1.56, p=0.220) or gender ($F_{1,36}$=2.55, p=0.119), and no significant interaction terms (all F<1.0).

The tracking algorithm successfully stabilised stopping performance over the task, with an average probability of inhibition of 0.45 (sd 0.04) in the BAL condition and .45 (sd 0.04) in the ATD condition. A mixed-model ANOVA revealed a highly significant effect of SSD (Greenhouse–Geisser correction, $F_{3.63,130.6}$=129.7, p<0.0001), such that stopping (in terms of $P_{inhibit}$) was more successful at shorter SSDs (see Fig. 1). The main effects of condition ($F_{1,36}$=0.040, p=0.843), genotype ($F_{1,36}$=0.043, p=0.838) and gender ($F_{1,36}$=0.001, p=0.975), and the interaction terms (all p>0.10) did not attain significance.

Three post hoc analyses were performed to verify the absence of effects. First, a between-subjects analysis of SST data from the first session only (18 ATD vs 22 BAL) detected no effect of treatment on SSRT (ATD mean=197, sd=61; BAL mean=206, sd=52; t_{38}=0.504, p=0.617) or median Go RT (ATD mean=379, sd=64; BAL mean=394, sd=65; t_{38}=0.730, p=0.470), but in the ATD condition, subjects made marginally more discrimination errors (ATD mean=7.1, sd=6.8; BAL mean=3.2, sd=3.5; t_{38}=1.97, p=0.059). Second, the lack of effect of genotype was unchanged when the six heterozygote subjects were excluded from the analysis (main effect of genotype $F_{1,30}$=1.12, p=0.299; condition × genotype interaction $F_{1,30}$=0.412, p=0.526). Third, the 30 volunteers prescreened for the 5-HTT polymorphism displayed a similar lack of effect of tryptophan depletion to the participants from our earlier experiment that included an fMRI scan (Cools et al. 2005; Evers et al. 2005) [SSRT group 1 (n=30): ATD mean=189, sd=53; BAL mean=192, sd=60; SSRT group 2 (n=10): ATD mean=197, sd=54; BAL mean=180, sd=39; all F< 1.49], indicating that the two data subsets are comparable.

Biochemical analysis of plasma levels

The ATD procedure achieved a significant depletion of total plasma TRP levels and the TRP: ΣLNAA ratio. Repeated-measures ANOVA revealed a significant condition (ATD, BAL) by time point (baseline, +6 h) interaction on plasma TRP levels ($F_{1,32}$=114.4, p<0.0001) and the TRP:ΣLNAA ratio ($F_{1,32}$=70.6, p<0.0001), with no significant effect (or interaction terms) of gender or 5-HTT polymorphism (all p>0.10). There was a highly significant decrease in plasma TRP levels (baseline mean=10.7, sd=2.9, time 2 mean=4.2, sd=2.2; t_{36}=14.5, p<0.0001) on the ATD session, averaging 61%. There was a highly significant decrease in the TRP:ΣLNAA ratio (baseline mean=0.12, sd=0.05, time 2 mean=0.04, sd=0.03; t_{36}= 10.7, p<0.0001), averaging 68%. On the BAL session, the TRP: ΣLNAA ratio increased by an average of 13% (baseline mean=0.13, sd=0.07, time 2 mean=0.15, sd=0.09; t_{38}=−2.25, p=0.030) and the plasma tryptophan level increased by 68% (baseline mean=9.7, sd=3.7, time 2 mean=16.3, sd=8.6; t_{38}=5.5, p<0.0001). To test the possibility that ATD-induced changes in response inhibition were most pronounced in subjects showing the greatest biochemical depletion, the change in TRP: ΣLNAA ratio (BAL minus ATD) was correlated with the change in SSRT (BAL minus ATD). There was no significant association between these change scores (r_{36}=0.202, p=0.236).

The influence of trait impulsivity

The effect of ATD on response inhibition as a function of trait impulsivity was investigated using self-report ratings on the BIS 11. Subjects reported an average BIS 11 total score of 63.5 (sd=9.2) (motor subscale mean=23.4, sd=3.4; attentional subscale mean=16.6, sd=4.1; non-planning subscale mean=23.4, sd=4.2), which is within the range of healthy subjects in existing normative data (Patton et al. 1995). SSRT was modestly associated with the BIS attentional subscale in the placebo condition (r_{40}=0.323,

p=.042) (ATD condition r_{40}=0.291, p=0.069). However, BIS ratings were not associated with the change in SSRT (BAL minus ATD) following ATD (total BIS r_{40}=0.114, p=0.484, all subscales p>0.10).

Discussion

The present data do not support a role for 5-HT neurotransmission in stop signal response inhibition. ATD did not impair the ability of healthy subjects to suppress prepotent motor responses following presentation of an auditory countermand. Using a powerful crossover design in a group of 40 subjects, the core measure of inhibitory performance (SSRT) differed by less than 2 ms on average in the placebo (BAL) and ATD conditions. This lack of effect confirms our previous report in a subset of these volunteers (n=12) who were all male (Cools et al. 2005). The present data indicated no differences between male and female participants, and the influence of ATD on stop signal performance was unrelated to trait impulsivity, measured by self-report ratings on the Barratt Impulsivity Scale (BIS) (Patton et al. 1995). The attentional subscale of the BIS was correlated significantly with SSRT in the BAL condition (and at trend in the ATD condition), which supports the construct validity of the stop signal task as a laboratory measure of impulsivity (see also Logan et al. 1997). Whilst our observations fail to support the long-standing hypothesis that reduced 5-HT neurotransmission predisposes impulsive behaviour, these data relate to a specific component of inhibitory control in the motor domain. As such, these findings do not refute the involvement of the 5-HT system in other facets of impulsivity, such as delayed choice behaviour (Mobini et al. 2000; Wogar et al. 1993) or reversal learning (Clarke et al. 2004), or an association between reduced 5-HT function and clinical disorders characterised by impulsivity (Asberg et al. 1976; Linnoila et al. 1983).

In addition to the acute effects of a 5-HT challenge, the present study investigated the efficiency of response inhibition in relation to a functional polymorphism of the serotonin transporter (5-HTTLPR). The short (s) allele of this polymorphism is associated with reduced 5-HTT expression and efficiency (Lesch et al. 1996), with concomitant effects on 5-HT neurotransmission: s carriers display reduced levels of the serotonin metabolite 5-hydroxyindole acetic acid (Williams et al. 2001), attenuated neuroendocrine responses to stimulation of the 5-HT system by fenfluramine or clomipramine (Reist et al. 2001; Whale et al. 2000) and reduced 5-HT_{1A} receptor binding (David et al. 2005). In the present study, s carriers performed similarly in the placebo (BAL) condition to subjects homozygous for the long allele on the SST. There was also no evidence that s carriers were disproportionately affected by the ATD procedure, contrary to our hypothesis. Restricting this analysis to the two homozygote groups (i.e. excluding the six s–l heterozygotes) did not alter these findings. These data provide further support for our conclusion that the 5-HT system is not implicated in this form of response inhibition in healthy subjects.

There was no effect of ATD on the speed of Go responding or on the number of Go discrimination errors on the SST. These variables do not provide a measure of inhibition but a more general index of arousal/sedation and attentional capacity, which may be affected by active pharmacological challenge. The results of the repeated-measures analysis of crossover data were largely supported by a between-subjects analysis of subjects' first test session only. These two groups displayed similar SSRT and Go RT, although the ATD group showed marginally inflated discrimination errors compared to the BAL group. In a previous work, ATD has been shown to actually improve focussed attention on the Stroop test and dichotic listening (Schmitt et al. 2000). The trend effect in the present data should be treated with caution, given that it was not apparent in the crossover data and that the overall rate of discriminative errors was very low (<5%) in both groups.

The lack of effect on response inhibition in the present study is fully consistent with a recent study using the selective 5-HT neurotoxin 5,7-DHT on an SST analogue developed for use in rodents (Eagle and Robbins 2003). That study revealed that global 5-HT depletion in rats did not affect stop task performance (Eagle et al. unpublished observations). Previous investigations of ATD effects in healthy human subjects have yielded more equivocal findings. Walderhaug et al. (2002) reported increased impulsiveness after ATD in normal individuals performing a continuous performance task with 'catch' trials inserted to encourage false responding. The ATD treatment did not significantly increase the rate of false alarms on catch trials, in either a verbal or non-verbal condition, but there was a significant reduction in response bias in the verbal condition, indicating that subjects were more likely to make a response to any stimulus. In contrast, Crean et al. (2002) reported that ATD decreased SSRT (i.e. improved inhibition) in healthy volunteers with no family history of alcoholism. The present data are more consistent with work using the Go–No Go paradigm, where several studies corroborate the lack of any disinhibitory effect (LeMarquand et al. 1999; Murphy et al. 2002; Rubia et al. 2005; Rubinsztein et al. 2001).

A defining feature of the stop signal procedure is the absence of any feedback in relation to stopping behaviour. The only feedback provided in the SST is a graphical representation of Go RT between task blocks to maintain fast motor responding in accordance with the race model. Feedback in the form of either reward and/or punishment is a central component to many of the cognitive paradigms modulated by 5-HT manipulations. With respect to reward and reinforcement, 5-HT depletion has been shown to attenuate the reinforcing effects of cocaine (Aronson et al. 1995) and to impair choice between large and small rewards on a gambling task (Rogers et al. 2003). Our previous research has described an abolition of reward-related speeding following ATD in high-impulsive subjects (Cools et al. 2005) on a task that disproportionately

reinforces fast responding. With respect to punishment, 5-HT depletion attenuates punishment-induced suppression of motor behaviour on a rodent paradigm where behavioural inhibition is a normal response Soubrié (1986; Tye et al. 1977; Wise et al. 1973). It is an empirical question whether ATD could affect motor inhibition if trial-by-trial performance was associated with motivational consequences in terms of either reinforcement or punishment.

To confirm that the sample size in the present study was sufficient to detect an effect of tryptophan depletion, power was calculated based on SSRT change (30 ms) from a previous repeated-measures design in ADHD adults following a single dose of methylphenidate (Aron et al. 2003a). The standard deviation of SSRT change (46.7) was taken from the present data. Using an effect size calculation from Altman (1991) ($2\times\text{Difference}/\text{SD}_{\text{change}}=1.28$), this study had a power of approximately .98 to detect a significant effect of tryptophan depletion on SSRT (two-tailed, $p=0.05$). It is therefore unlikely that the present negative result could be attributed to a small sample and insufficient power to test the null hypothesis. However, the effect size for the influence of genotype on response inhibition is likely to be smaller and therefore greater numbers may be required to fully assess the role of the 5-HTT polymorphism on response inhibition.

Analysis of biochemical data indicated a highly significant depletion of central 5-HT neurotransmission. In this study, we employed a 75-g amino acid load (Sobczak et al. 2002), which is slightly smaller than the widely used 100-g technique developed by Young et al. (1985). With the 75-g procedure, the incidence of nausea and vomiting post-ingestion was negligible. Nonetheless, the ATD procedure had a marked effect on plasma tryptophan levels and the TRP:ΣLNAA ratio, causing an average decrease of 61 and 68%, respectively. These values fall within the range of previous studies using the 100-g technique: Booij et al. (2002) reported ATD-induced decreases in total tryptophan varying from 25–96% (see also Klaassen et al. 1999). It is clearly established that reduced tryptophan availability in the brain significantly attenuates brain 5-HT levels (Biggio et al. 1974; Carpenter et al. 1998; Williams et al. 1999), although one limitation of the present study is the absence of any biomarkers directly related to 5-HT function (e.g. 5-HIAA or prolactin levels) distinct from its precursor. The TRP:ΣLNAA ratio is the more sensitive index of brain tryptophan availability (Fernstrom 1981), as this ratio indicates the rate of transport of amino acids across the blood-brain barrier via a common transporter protein. After the BAL mixture, the TRP:ΣLNAA ratio increased by a modest 13%, suggesting that this treatment is appropriate as a placebo condition.

It has been suggested previously (Spillmann et al. 2001) that a threshold of 5-HT depletion must be achieved in order to trigger effects on psychological function. However, it seems unlikely that a putative threshold can account for the lack of effects in the present study, given that ATD significantly affected performance in these subjects on another cognitive task measuring reward sensitivity (Cools et al. 2005; Roiser et al. unpublished observations). We conclude that 5-HT neurotransmission is not implicated in response inhibition in healthy human subjects. Future research is required to assess whether these null effects in response inhibition extend to clinical populations with impulse control disorders.

Acknowledgements This work was supported by a Wellcome Trust programme grant to T.W.R., B.J.S., B.J. Everitt and A.C. Roberts and completed within the MRC Centre for Behavioural and Clinical Neuroscience. All testing was conducted at the Wellcome Trust Clinical Research Facility at Addenbrooke's Hospital, Cambridge. D.C.R. is a Wellcome Trust Senior Fellow in Clinical Science. We wish to thank Dr. M. Franklin and Dr. J. Odontiadis (University of Oxford) for analysis of plasma data, Dr. J. Whitaker for DNA extraction and Dr. A. Aron for programming assistance and helpful comments on the manuscript. This article was presented as a poster at the Cognitive Neuroscience Society annual meeting in New York, April 10–12, 2005.

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