Neuropsychopharmacology (2014) 39, 1519–1526

www.neuropsychopharmacology.org

Serotonin Depletion Induces 'Waiting Impulsivity' on the Human Four-Choice Serial Reaction Time Task: Cross-Species Translational Significance

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Convergent results from animal and human studies suggest that reducing serotonin neurotransmission promotes impulsive behavior. Here, serotonin depletion was induced by the dietary tryptophan depletion procedure (TD) in healthy volunteers to examine the role of serotonin in impulsive action and impulsive choice. We used a novel translational analog of a rodent 5-choice serial reaction time task (5-CSRTT)— the human 4-CSRTT—and a reward delay-discounting questionnaire to measure effects on these different forms of 'waiting impulsivity'. There was no effect of TD on impulsive choice as indexed by the reward delay-discounting questionnaire. However, TD significantly increased 4-CSRTT premature responses (or impulsive action), which is remarkably similar to the previous findings of effect of serotonin depletion on rodent 5-CSRTT performance. Moreover, the increased premature responding in TD correlated significantly with individual differences on the motor impulsivity subscale of the Barratt Impulsivity Scale. TD also improved the accuracy of performance and speeded responding, possibly indicating enhanced attention and reward processing. The results suggest: (i) the 4-CSRTT will be a valuable addition to the tests already available to measure impulsivity in humans in a direct translational analog of a test extensively used in rodents; (ii) TD in humans produces a qualitatively similar profile of effects to those in rodents (ie, enhancing premature responding), hence supporting the conclusion that TD in humans exerts at least some of its effects on central serotonin; and (iii) this manipulation of serotonin produces dissociable effects on different measures of impulsivity, suggesting considerable specificity in its modulatory role.

Neuropsychopharmacology (2014) **39,** 1519–1526; doi:10.1038/npp.2013.351; published online 22 January 2014

Keywords: serotonin; acute tryptophan depletion; impulsive action; premature responses; 4-CSRTT; attention

INTRODUCTION

Serotonin regulates diverse motor, cognitive, decisional, and affective brain functions (Homberg, 2012). Many studies in humans and animals have shown that reduction of serotonin (5-hydroxytryptamine, 5-HT) transmission in the brain promotes impulsive behaviors (Dalley *et al*, 2011; Evenden, 1999).

Impulsivity has been defined as 'a predisposition toward rapid, unplanned reactions to internal or external stimuli with diminished regard to the negative consequences of these reactions to the impulsive individual or to others' (Fineberg *et al*, 2010). Impulsivity may derive from one or more distinct neurocognitive mechanisms (Robbins *et al*, 2012) and can be broadly divided into 'waiting impulsivity' and 'stopping impulsivity'(Dalley *et al*, 2011), which refers to the inability to regulate responding in anticipation of reinforcement and following response initiation, respectively. 'Waiting impulsivity' can be further subdivided into impulsive action (the failure to suppress inappropriate actions) and impulsive choice (the choice of small, immediate rewards over larger, delayed rewards) (Winstanley *et al*, 2006).

The five-choice serial reaction time task (5-CSRTT) has been commonly used to measure impulsive action and sustained attention in rodents (Robbins, 2002). In this task, in order to obtain a food pellet, a rat makes a nose-poke response in one of five apertures in which a brief light stimulus has just been presented. A nose-poke response in any aperture prior to stimulus presentation is defined as a premature response, which results in time-out from positive reinforcement.

Administration of the 5,7-dihydroxytryptamine (5,7-DHT), which produces profound depletion ($>80\%$) of forebrain serotonin, increased premature responding in the 5-CSRTT (Harrison *et al*, 1997a; Winstanley *et al*, 2004a). Selective forebrain depletion produced by intra-raphe 5,7-DHT leads to different profiles of forebrain serotonin loss (Harrison *et al*, 1997b). Consistent with the neuroanatomical projection fields of the serotoninergic midbrain raphe nuclei, lesions to the dorsal raphe produced highly significant cortical and striatal (including nucleus accumbens)

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Received 5 September 2013; revised 16 December 2013; accepted 17 December 2013; accepted article preview online 2 January 2014

serotonin depletion, whereas median raphe lesions caused profound hippocampal and cortical serotonin depletion with much smaller impact on striatal serotonin levels.

These different patterns of depletion were associated with remarkably different profiles of effects on 5-CSRTT performance (Harrison *et al*, 1997b). Although both dorsal and median raphe 5,7-DHT lesions led to enhanced premature responding, the effects of dorsal raphe lesions were much more consistent over different manipulations of the task parameters. Although median raphe lesions produced consistent speeding of the latency to collect earned food pellets, suggestive of a primary motivational effect, the dorsal raphe lesions had no such effect, suggesting that the impulsivity engendered by this manipulation did not result from primary motivational changes such as hunger for food. Remarkably, the dorsal raphe lesion also led to improved attentional accuracy in terms of both errors of commission and omission; in addition, this lesion was also associated with speeded decisional response latencies, though not to collect the earned food pellets. However, global 5-HT depletion has no effect on attentional accuracy (Harrison *et al*, 1997b; Winstanley *et al*, 2004b).

The overall interpretation of these findings is that serotonin has important roles in the control of a form of waiting impulsivity, as well as attention and reward processing that cannot simply be attributed to general response disinhibition. Premature responding on the 5-CSRTT was also linked to effects of either systemically or centrally administered 5HT2A/C receptor antagonists or the 5-HT1A receptor partial agonist 8-OH-DPAT (Fletcher *et al*, 2007; Winstanley *et al*, 2004b). However, it should be noted that other studies (Dalley *et al*, 2002) found that high impulsivity was linked to increased levels of 5-HT as measured by *in vivo* dialysis in the rat prefrontal cortex.

Recently, a 4-choice human analog of the 5-CSRTT has been introduced (Voon *et al*, 2014), which compared with previous CANTAB version (Sahakian *et al*, 1993) was specifically designed to measure premature responses. This task has shown parallel effects for human substance abusers to those of high impulsive rats that compulsively self-administer cocaine (Belin *et al*, 2008). Indeed, 'waiting impulsivity' has been associated with pathological behaviors such as gambling and substance addictions (Leerman and Potenza, 2012).

In this study, we sought to determine effects of manipulating serotonin during performance of the human 4-CSRTT to provide a parallel to the rodent experiments on the 5-CSRTT reviewed above and thus provide further evidence of the translational utility of the human 4-CSRTT.

In human experimental studies, the dietary acute tryptophan depletion (TD) procedure has been commonly used to reduce central serotonin transmission (Carpenter *et al*, 1998). As tryptophan is the amino-acid precursor of serotonin, its depletion by dietary means causes a rapid decrease in brain serotonin level (Biggio *et al*, 1974; Carpenter *et al*, 1998). However, the validity of acute TD as a means of manipulating central serotonin has been recently questioned (van Donkelaar *et al*, 2011). Although this view has been rebutted on the basis of considerable evidence (Crockett *et al*, 2012), the present experiment also tests the validity of the TD technique itself as a viable means of manipulating serotonin.

We therefore hypothesized that TD in healthy volunteers would produce a qualitatively similar profile of deficits as observed in rodents performing the 5-CSRTT following 5,7-DHT-induced serotonin depletion, with a prominent effect to enhance premature responding. We also administered a delay-discounting questionnaire (Kirby *et al*, 1999) before and after serotonin depletion to measure the parallel effects on impulsive choice as distinct from impulsive action.

MATERIALS AND METHODS

Participants' Inclusion Criteria

The East of England-Essex Research Ethics Committee approved this study. Participants were recruited from university-based advertisements and from the Cambridge BioResource (www.cambridgebioresource.org.uk) and gave informed consent prior to participation. The inclusion criteria were age 18–45 years, no history of neurological or psychiatric disorders as assessed with the Mini International Neuropsychiatric Inventory (Sheehan *et al*, 1998), no regular or recreational use of drugs including nicotine, no significant physical illness and not currently taking any type of regular medication (except contraceptive pills for women).

Experimental Procedure

Participants were assigned to receive either the tryptophan depleting drink (TD) or the placebo mixture in a randomized, placebo-controlled, double blind order. They were asked to abstain from food and alcohol 12 h before the testing session.

In the TD procedure, tryptophan was depleted by ingestion of a liquid amino-acid load that did not contain tryptophan but did include other large neutral amino acids (LNAAs). The biochemical composition of drinks is provided in Supplementary Materials.

At baseline, the first blood sample was taken and we administrated the Beck Depression Inventory (BDI) (Beck *et al*, 1996), the Spielberg State-Trait Anxiety Inventory (STAI) (Spielberger, 1989), the Barratt Impulsivity Scale (BIS-11) (Patton *et al*, 1995), and a delay-discounting questionnaires. The STAI-state and the delay-discounting questionnaire were also administered at additional time points of the study. An intelligence quotient (IQ) was measured using the National Adult Reading Test (NART) (Bright *et al*, 2002). Behavioral testing was performed and the second blood sample taken after a resting period of approximately 4.5 h to ensure stable and low tryptophan levels.

Biochemical Analysis

Immediately after venipuncture, blood (7 ml) was centrifuged at 2000 r.p.m. for 20 min, and serum was separated by centrifugation and stored at $-80\,^{\circ}C$. Plasma total amino-acid concentrations (tyrosine, valine, phenylalanine, isoleucine, leucine, and tryptophan) were measured by means of high-performance liquid chromatography with fluorescence end-point detection and precolumn sample derivatization. The tryptophan/large neutral amino-acid

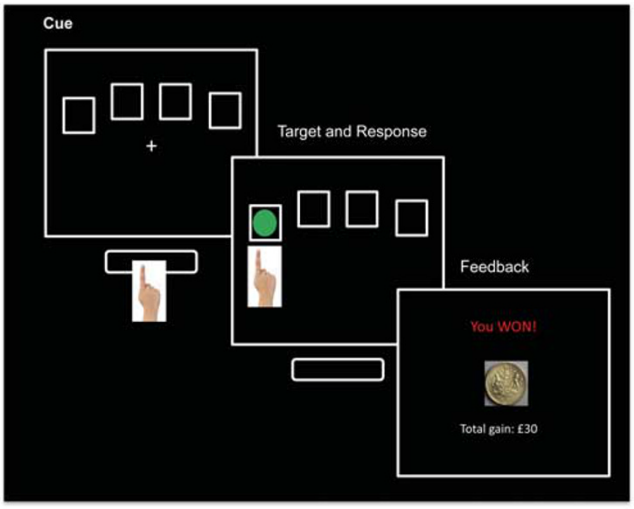

Figure 1 Four-choice serial reaction time task (4-CSRTT): Participants were instructed to press and hold down the space bar. The press of the space bar indicated 'cue onset' and four empty boxes appeared on the screen. The participants were instructed to release the space bar and touch the box in which the target (green circle) had appeared. They were asked to respond as quickly as possible. Premature release of the space bar prior to onset of the target (premature responses) was a primary outcome measure of the task.

(TRP:ΣLNAAs) ratio was calculated as an indicator of central serotoninergic function (Carpenter *et al*, 1998).

4-Choice Serial Reaction Time Task

The task was programmed with Visual Basic using Visual Studio 2005 and Microsoft.NET Framework 2.0 with a total task duration of 20 min.

Participants were seated in front of a touch-screen computer (Figure 1 and Supplementary Materials) and were instructed to press and hold down the space bar with the index finger of their dominant hand. A press of the space bar indicated the 'cue onset' and four boxes appeared on the screen. After a random (2–10 s) cue-target interval, a green circle target appeared briefly (32–64 ms) in one of the boxes. Participants released the space bar and touched the box on the screen in which the target had appeared. They were asked to respond as quickly as possible.

The task included two baseline blocks (20 trials per block; at the start and after the first test block) without monetary feedback and four test blocks (40 trials per block) with monetary feedback. To increase premature responding, the testing blocks included decreasing target time, variable cue-target intervals and introduction of a distractor (Robbins, 2002).

For each participant, the standard deviations (SD) and mean reaction time (RT) in each baseline block were used to set individualized feedback in the test blocks. If response RT was less than mean RT − 0.5 SD, the subject earned £1. The responses with RT equal to mean RT ± 0.5 SD were followed by feedback of 50 pence and those less than mean RT + 1.5 SD, by 10 pence. The responses with RT more than mean RT + 1.5 SD were followed by loss of £1. For no responses, the feedback was a loss of 50 pence. Following a premature response, subjects touched the screen to complete the trial and earned nothing. The total won was also specified on the feedback display.

The premature release of the space bar prior to onset of the target (premature responding) was a primary outcome measure of the task. Other outcome measures included the total amount of money won, mean RT of baseline and of testing blocks and late responses (>1.5 SD). The accuracy was measured as a ratio of correct responses divided by the sum of correct and incorrect responses. The trials in which the subject touched the wrong box after the target onset were defined as incorrect responses. Motivation Index or motivation to reward feedback was defined as the response to reward feedback and assesses reaction time in extinction after instrumental conditioning with monetary feedback. The baseline blocks without feedback had similar characteristics, but were separate from the first testing block with feedback.

Subjects were paid £60 for their participation in the study and were told that they could receive an additional £10 depending on their performance in the task.

Reward Delay-Discounting Questionnaire

Impulsive choice was assessed using a self-administered Monetary Choice Questionnaire, containing 27 items (Kirby *et al*, 1999), in which participants choose between smaller immediate or larger delayed monetary rewards. The discounting rate (k) was calculated separately for small, medium, and large reward magnitudes as follows: $V = A/(1 + kD)$, where V is a present reward value, A is a delayed reward value and D is a delay. Impulsive choice is characterized by the greater values of k, which corresponds to a steeper discounting rate.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 21 (SPSS, Chicago, IL). Prior to analysis, all variables were tested for Gaussian distribution (Shapiro–Wilk test, $p<0.05$) and were transformed with square root transformation if necessary. Outliers (2SD > group mean) were removed (three subjects in total). Behavioral data were analyzed using a multivariate ANOVA with group as a fixed factor and the 4-CSRTT outcome measures as dependent factors.

Demographic data were analyzed using one-way ANOVA. Data from biochemical analyses were performed using mixed measures ANOVA with time as a dependent factor and group as a fixed factor. Correlation analysis was performed amongst relevant neurochemical and behavioral variables using Pearson's correlation coefficient r. We used the FDR correction for multiple comparisons after Fisher's z-transformation of correlation coefficient r (Benjamini *et al*, 2001).

RESULTS

Subjects

Twenty-two placebo (Con) and 22 TD subjects were included in the study that were matched by age, gender, and NART score (IQ). The groups' demographic data are reported in Table 1. There was no significant difference

Table 1 Participants' Demographic and Behavioral Data

	Controls ($n=22$)	TD group ($n=22$)	F	p
Age	27.181 ± 1.650	30.750 ± 1.684	2.283	0.139
Men (number)	8	9	0.095[a]	0.756[a]
IQ	120.687 ± 1.916	120.200 ± 1.642	0.037	0.879
BDI	3.545 ± 0.915	3.045 ± 0.790	0.171	0.682
STAI, trait	46.8400 ± 1.294	44.875 ± 0.995	1.432	0.237
STAI, state	44.227 ± 1.044	45.619 ± 0.967	0.951	0.335
BIS, total	69.545 ± 6.456	70.571 ± 5.153	0.330	0.569
BIS, motor impulsivity	22.727 ± 3.194	23.333 ± 3.864	0.315	0.577
BIS, attentional impulsivity	17.0454 ± 3.015	17.190 ± 2.441	0.030	0.864
BIS, nonplanning impulsivity	22.727 ± 3.194	23.333 ± 3.864	0.315	0.577

Abbreviation: TD, tryptophan depleted group.
[a]χ^2 test; Reported as mean ± SEM values.

between groups in baseline impulsivity, mood, or anxiety trait-state evaluation.

Biochemical Measures

Tryptophan depletion robustly decreased the TRP:ΣLNAAs ratio relative to the control depletion group (Main effect of Group: $F_{(1,42)}=30.218$, $p<0.0001$; Main effect of time: $F_{(1,42)}=10.080$, $p=0.003$; Group × Time interaction: $F_{(1,42)}=44.291$, $p<0.0001$). The mean (±SEM) baseline and post-procedure values of this measure were as follows: baseline—Con (0.153 ± 0.011), TD group (0.138 ± 0.007); post-procedure—Con (0.187 ± 0.015), TD group (0.042 ± 0.012).

Previous studies reported no effect on mood in healthy volunteers following the TD procedure (Crockett *et al*, 2012), although induced anxiety state could be one of the adverse effects of this procedure (van Donkelaar *et al*, 2011). To assess this side effect, we administered the STAI-state questionnaire at baseline and at 4, 6, and 8 h after drink intake. There was no significant effect of TD on anxiety state: main effect of time ($F_{(1,42)}=2.349$, $p>0.05$), main effect of group ($F_{(1,42)}=2.579$, $p>0.05$). We also found no correlation of TRP:ΣLNAAs levels with anxiety state (all $p>0.05$).

Effect of Tryptophan Depletion on 4-CSRTT Performance

There was a significant overall main effect of TD ($F_{(8,35)}=3.475$, $p=0.005$). Figure 2 shows that TD subjects made significantly more premature responses (Number of premature responses, mean ± SEM, Con: 5.045 ± 0.755, TD: 8.818 ± 1.632, $F_{(1,42)}=4.375$, $p=0.042$), had superior attentional accuracy (Score, mean ± SEM, Con: 0.909 ± 0.016, TD: 0.947 ± 0.008, $F_{(1,42)}=4.462$, $p=0.041$) and a higher motivational index score (Score, mean ± SEM, Con: 0.118 ± 0.021, TD: 0.180 ± 0.018, $F_{(1,42)}=4.666$, $p=0.037$) (Figure 2). There was no effect of TD on total win, late response or RT (all $p>0.05$) (Supplementary Table 1).

There was no correlation of the premature responses with a BIS total score and sub-scores in the controls (all $p>0.05$). In the TD group, there was a positive correlation of motor impulsivity sub-score with premature responses ($r=0.461$, $p=0.023$, FDR corrected) but no correlation with other scores (all $p>0.05$).

We also found a positive significant correlation between the percentage of TD, calculated as the ratio of TRP:ΣLNAAs level change between baseline and post-procedure, and premature responding ($r=0.600$, $p=0.002$, FDR corrected). There was no correlation between percentage of TD and discounting rate (k) (all $p>0.05$).

Effect of Tryptophan Depletion on Impulsive Choice

TD had no significant effects (see Table 2 for mean (SEM) data). Mixed ANOVA, using time and group (TD *vs* controls) as inter-subject factors, showed a main effect of the discounting parameter k ($F_{(1,42)}=22.992$, $p<0.0001$) as a measure of impulsivity, and a main effect of time ($F_{(1,42)}=5.857$, $p=0.020$), but no effect of TD or K × time, time × TD or TD × K interactions (all $F_{(1,42)}<1.0$, all $p>0.05$).

DISCUSSION

Using a novel translational version of the rodent 5-CSRTT in healthy volunteers, we showed that dietary TD, leading putatively to central serotonin loss, produced significantly increased levels of premature responding correlated with the decline in plasma tryptophan levels.

TD was also associated with improved accuracy and a higher motivational index on the 4-CSRTT. The similar results on performances in 5-CSRTT have been found in selective lesions of raphe nucleus in rats, but not with a global serotonin depletion procedure. This discrepancy between human and animal performance in the task could result from the subtle difference in the task versions and from the level of serotonin depletion as 5,7-DHT induces much more massive loss of central 5-HT than TD does.

In contrast, TD had no effect on delayed reward discounting as measured by Kirby questionnaire. These findings suggest a dissociation of effects of a central serotonin manipulation on impulsive action and impulsive choice.

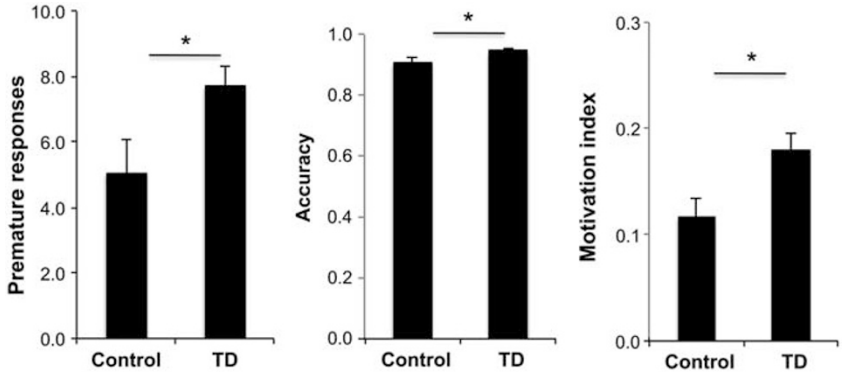

Figure 2 Significant main outcome measures of the 4-CSRTT: premature responses, accuracy, and motivational index. Reported as Mean ± SEM values. *$P < 0.05$; Con, control group; TD, tryptophan depleted group.

Table 2 Outcome Measures for Reward Delay-Discounting Questionnaire

	Baseline	F	*p*	Post procedure	F	*p*
K small controls	0.064 ± 0.022	0.961	0.332	0.101 ± 0.026	0.366	0.566
K small TD	0.098 ± 0.027			0.123 ± 0.028		
K medium controls	0.104 ± 0.025	0.294	0.591	0.125 ± 0.027	0.100	0.753
K medium TD	0.125 ± 0.028			0.137 ± 0.028		
K large controls	0.157 ± 0.024	0.721	0.401	0.181 ± 0.024	0.548	0.463
K large TD	0.187 ± 0.024			0.207 ± 0.025		
K mean controls	0.108 ± 0.019	0.533	0.469	0.125 ± 0.021	0.604	0.441
K mean TD	0.131 ± 0.023			0.149 ± 0.022		

Abbreviation: TD, tryptophan depleted group.
Reported as Mean ± SEM values.

Serotonin Depletion Promotes Impulsive Action

The effect of serotonin manipulation on impulsive action in humans has been addressed in only a limited number of studies that have also used different measures of impulsivity, including the Go/NoGo paradigm and the stop-signal reaction-time task.

Both acquisition and performance of NoGo responding has been shown to be greatly impaired by central serotonin depletion in rats (Harrison *et al*, 1999) but it has been proven to be more difficult to observe such effects using TD in humans. Thus, TD was found to affect Go/NoGo responding to a greater extent in males with a history of alcoholism (LeMarquand *et al*, 1999) or in healthy volunteers where responding was suppressed by punishment (Crockett *et al*, 2009). By contrast, TD had no effect on stop-signal reaction-time performance in healthy subjects (Clark *et al*, 2005; Cools *et al*, 2005) in parallel with a similar lack of effect of serotonin depletion in rats (Eagle *et al*, 2009).

We found a similar parallel in the effects of serotonin manipulations on premature responding in the 5-CSRTT in humans and rodents but in this case to increase impulsivity rather than having no effect. Therefore, it appears that TD only induces certain forms of impulsivity, both in humans and rodents, in particular when the subject is required to inhibit responding prior to an expected outcome, as in the rat 5-CSRTT (Baarendse and Vanderschuren, 2012). Interestingly, increased premature responding in TD correlated significantly with individual differences on the motor impulsivity subscale of the Barratt Impulsivity Scale. Other studies also pointed to the relationship to some extent between the capacity of inhibition of prepotent response and psychometric impulsivity trait measured by Barratt scale (Aichret *et al*, 2012).

These data support the hypothesis that central serotonin loss through TD selectively impacts impulsive action during anticipation of responding or 'waiting impulsivity' (Dalley *et al*, 2011), rather than when stopping an already initiated response. This is consistent with electrophysiological recordings from the raphe nucleus in awake animals showing that increased firing of serotoninergic neurons in rodent facilitated waiting behavior for future rewards (Miyazaki *et al*, 2012).

In contrast to impulsive action, there was no effect in this study of TD on impulsive choice as measured by the Kirby questionnaire. Previous studies have shown mixed effects of serotonin manipulations on reward delay discounting (Baarendse and Vanderschuren, 2012; Paterson *et al*, 2012). Recent translational analyses pointed to the lack of correlation between measures of impulsive action and impulsive choice both in animals and humans (Broos *et al*, 2012). Therefore, our findings of increased 4-CSRTT premature responding following serotonin depletion in humans suggest specific roles of forebrain serotonin in the mediation of certain forms of impulsivity.

Effect of Serotonin Depletion on Accuracy and Motivational Index

TD had other behavioral effects on human 4-CSRTT performances. Notably TD improved attentional accuracy in terms of percentage correct responses, similarly to the effects of dorsal raphe 5,7-DHT lesions in the rat (Harrison *et al*, 1997b). Nonetheless, global serotonin depletion in rats produced by intraventricular 5,7-DHT, probably most similar to the procedure used here, showed no effect on attentional accuracy in two studies (Harrison *et al*, 1997b; Winstanley *et al*, 2004b).

However, the present results are of particular interest in demonstrating that increased impulsive responding does not necessarily impair attentional function. The result in

this case argues that 5-HT depletion is not likely to provide an accurate model of the combined impairments of attentional dysfunction and loss of response control evident in attention deficit hyperactivity disorder. Other rodent data suggest that 5-HT depletion can actually enhance visual discrimination learning as long as the task does not require behavioral inhibition (Harrison *et al*, 1999; Ward *et al*, 1999). These findings suggest that serotonin loss enhances salience of stimuli associated with reward as in the case of both the rodent and human 5-CSRTT. This general conclusion appears valid for the effects of TD in other test situations in humans, as, for example, TD-enhanced performance in Stroop test (Scholes *et al*, 2007).

A further effect of TD in the human 4-CSRTT was to increase a 'motivational index', which was derived from the difference in RT of baseline test blocks separated by the block where error feedback was provided. There is no direct parallel in the rodent 5-CSRTT paradigm, although there is a primary measure of motivation in terms of latency to collect the earned food pellets (sometimes following satiation induced by prior feeding). Harrison *et al* (1997b) found that although both median and dorsal raphe 5,7-DHT lesions increased impulsive behavior in terms of premature responding, only median raphe lesions speeded this magazine latency. This suggests that sensitivity to reinforcement may contribute to enhanced impulsivity following serotonin depletion, although this factor seems unlikely to account for impulsivity overall.

Again, this is broadly in agreement with what has been shown here. TD clearly affected both sensitivity to reinforcement and response control factors, although it is of course a limitation of the TD methodology in humans that the serotonin depletion cannot be localized to particular regions as is feasible in the rodent model. It should also be noted that we cannot completely rule out the possibility that the higher motivational index in the TD group resulted from effects on appetitively framed punishment (ie, missed opportunities to win) or from avoidance of aversive outcomes (Delgado *et al*, 2009). In general, these findings for sensitivity to reinforcement are consistent with previous studies in humans pointing to the role of serotonin in reward processing and learning (Kranz *et al*, 2010; Seymour *et al*, 2012).

The main finding of this study suggests that dietary TD in humans induces premature responses in this task, which is a similar profile of behavioral effects to those in rodents with profound forebrain serotonin loss induced by 5,7-DHT. This behavioral similarity strongly suggests that TD does produce similar neurochemical effects to those of 5,7-DHT, a relatively selective 5-HT neurotoxin. Moreover, animal (Ardis *et al*, 2009) and human (Cox *et al*, 2011) studies also suggested a selective TD effect on serotonin, with no effect on dopamine and norepinephrine neurotransmission. Contrary to the view of van Donkelaar *et al* (2011), we thus argue that TD in humans probably exerts at least some of its effects on central serotonin.

Limitations

One limitation of our comparison between premature responding on the 4-CSRTT as a measure of 'impulsive action' and delayed discounting as a measure of 'impulsive choice' is that the former was measured behaviorally, whereas the impulsive choice with a hypothetical Kirby questionnaire. A recent review of the studies of both experimental and hypothetical versions of delay-discounting task has shown similar results (Odum, 2011). Moreover, global depletion of 5-HT has been shown to produce large increases in premature responding on the rat 5-CSRTT, but no large effects by itself on delayed discounting (Winstanley *et al*, 2004b).

Another limitation for translation of the human 4-CSRTT is a possible difference in the nature of the premature response measure between rats and humans in both tasks. For the humans, premature responding is measured as the premature release of a space bar in order to make a touch-screen response. It is therefore different from the rodent task where premature responding is at the non-illuminated apertures. However, premature responses at the touch screen are much rare in humans and space bar release is evidently a much more sensitive measure. Nevertheless, both versions are clearly measuring response initiation at the beginning of the trial, in anticipation of the visual target stimuli. Moreover, recent data on 5-CSRTT have shown that rats begin to scan the array just prior to the emission of a premature response (Donelly *et al*, 2012) and so this may be functionally equivalent to humans initiating the trial with an initial press of the space bar. The fact that both responses exhibit similar effects following serotoninergic manipulations as shown in this study, however, supports the conclusion that they are both indexing aspects of 'waiting impulsivity'. Further studies will determine the similarities and differences between the two procedures.

CONCLUSIONS

We have shown here that manipulation of serotonin by TD produces dissociable effects on different measures of impulsivity in humans, as well as in the rat, suggesting considerable specificity in its modulatory role. The human 4-CSRTT will be a valuable addition to the tests already available to measure impulsivity in humans in a direct translational analog of a test extensively used to measure attention and impulsivity in rodents.

FUNDING AND DISCLOSURE

TWR consults for Cambridge Cognition, E. Lilly, Lundbeck, Merck, Sharpe and Dohme, Teva, Shire Pharmaceuticals and Chempartners and has received research grants on the E. Lilly, Lundbeck and GlaxoSmithKline. EFE consults for Roch Pharmaceuticals. YW received support from the Fyssen Foundation. VV is a Wellcome Trust Intermediate Fellow in Clinical Neurosciences. GS declares nothing to disclosure.

ACKNOWLEDGEMENTS

This work was supported by a Wellcome Trust programme grant to TWR (089589/z/09/z). The Behavioural and Clinical Neuroscience Institute is jointly funded by the MRC and the Wellcome Trust (G00001354). We wish to thank Dr M Franklin (University of Oxford) for analysis of plasma data, Dr Sanja

Abbott for the help with the computer programming of the human 4-CSRTT and Dr Mercedes Arroyo for the help with administrative support of the study. We thank Cambridge Bioresource for providing volunteers.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)