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ORIGINAL INVESTIGATION

Acute serotonin depletion releases motivated inhibition of response vigour

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Abstract
Rationale The neurotransmitter serotonin has long been implicated in the motivational control of behaviour. Recent theories propose that the role of serotonin can be understood in terms of an interaction between a motivational and a behavioural activation axis. Experimental support for these ideas, however, has been mixed.
Objectives In the current study, we aimed to investigate the role of serotonin (5HT) in behavioural vigour as a function of incentive motivation.
Methods We employed dietary acute tryptophan depletion (ATD) to lower the 5HT precursor tryptophan during the performance of a speeded visual discrimination task. Feedback valence and feedback probability were manipulated independently and cued prior to target onset. On feedback trials, fast correct responses led to either reward or avoidance of punishment, while slow or incorrect responses led to reward omission or punishment.
Results We show that behavioural responding is inhibited under high incentive motivation (i.e. high-feedback probability) at baseline 5HT levels and that lowering these leads to behavioural disinhibition, while leaving accuracy unaffected. Surprisingly, there were no differential effects of motivational valence, with 5HT depletion releasing behavioural inhibition under both appetitive and aversive motivation.
Conclusions Our findings extend current theories on the role of 5HT in behavioural inhibition by showing that reductions in serotonin lead to increased behavioural vigour only if there is a motivational drive to inhibit behaviour at baseline.

Keywords Serotonin · Disinhibition · Motivation · Acute tryptophan depletion · Pavlovian bias · Behavioural control

Introduction

The prospect of receiving reward and punishment can strongly guide behaviour, and the neural processes underlying the influence of reinforcement on behaviour have been a major topic of interest. Serotonin (5HT) has been implicated in the motivational control of behaviour, yet the precise role is not completely understood. Low levels of 5HT have been associated not only with impulsivity and disinhibited behaviour (e.g. Evenden 1999; Soubrie 1986) but also with aspects of aversive processing as evident in anxiety and depression (e.g. Deakin 2013; Deakin 1998; Graeff et al. 1996).

A converging account of the role of 5HT has been put forward suggesting that the role of 5HT might lie specifically in behavioural inhibition to avoid punishment (Boureau and Dayan 2011; Cools et al. 2011; Dayan and Huys 2008; Dayan and Huys 2009). This behavioural inhibition in the face of punishment might be an expression of Pavlovian rather than

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instrumental mechanisms (Dayan and Huys 2009), a hypothesis that is supported by recent empirical data showing that depleting central 5HT removed Pavlovian inhibition under aversive motivation, while leaving Pavlovian activation under appetitive motivation unaffected (Crockett et al. 2012a; Geurts et al. 2013b).

This recent idea can account for apparently paradoxical findings of the role of 5HT in punishment processing on the one hand (Chamberlain et al. 2006; Deakin and Graeff 1991; den Ouden et al. 2013) and behavioural inhibition and impulsivity on the other hand (Cools et al. 2011; Dalley and Roiser 2012; Soubrie 1986). However, it appears at odds with other work suggesting that 5HT depletion impairs reward processing (Bari et al. 2010; Rogers et al. 2003). In addition, we have shown that 5HT depletion reduced rather than enhanced behavioural activation under high incentive motivation (Cools et al. 2005; Roiser et al. 2006). The paradigm used in these latter studies did not allow us to disentangle effects of reward and punishment motivation, and at the time we interpreted this motivation-related slowing as either reduced sensitivity to anticipated reward or enhanced sensitivity to anticipated punishment. This latter account is in direct opposition to the hypothesis that effects of 5HT depletion surface as behavioural disinhibition in anticipation of punishment. One possibility is that these previous studies did not show behavioural disinhibition after 5HT depletion, because there was no behavioural inhibition under baseline (placebo) conditions.

In the present study, we revisited and adapted our previous incentive motivation paradigm to assess separately performance in anticipation of reward, i.e. under appetitive motivation, and performance in anticipation of punishment, i.e. aversive motivation. We expected that at baseline 5HT levels, subjects would exhibit behavioural inhibition (i.e. response slowing) when anticipating punishment relative to reward. Furthermore, we hypothesised that 5HT depletion would disinhibit responding in anticipation of punishment, although not reward, in line with current ideas about 5HT and aversive inhibition. Thus, the task was designed to detect a form of behavioural inhibition elicited by punishment anticipation, rather than the more instrumental, instructed behavioural inhibition measured in classic go/no go or stop tasks. As in previous studies, 5HT levels were manipulated using acute tryptophan depletion (ATD), an effective procedure for lowering central 5HT levels through dietary depletion of the 5HT precursor tryptophan (Crockett et al. 2012b).

Materials and method

General procedure

The current study was part of a larger study, the results of which are reported elsewhere (Geurts et al. 2013b), approved by the local ethics committee in Nijmegen (CMO protocol NL29091.091.09) and in accord with the Helsinki Declaration of 1975. The study consisted of an intake session and two test days, which took place at least 1 week apart. During the intake session, participants received information about the experimental procedure and were screened for inclusion. Additionally, participants completed several questionnaires. On the two test days, participants were administered either a tryptophan-depleting drink (TRP−) or a balanced control amino-acid drink (TRP+), in a double-blind, placebo-controlled, cross-over design. Participants were asked to abstain from alcohol 24 h prior to testing and from food and caffeine from 10 p.m. on the night before test days. On the morning of each test day (between 8 and 10 a.m.), volunteers arrived at the research centre, where a blood sample was taken and participants ingested the amino-acid drink. Participants received a low-protein snack about 2.5 h later, and testing of the complete test battery started at about 5.5 h after drink intake to ensure stable and low TRP levels. To confirm TRP depletion, a second blood sample was taken approximately 7 h after amino acid (AA) ingestion. Participants performed the adapted version of the incentive motivation task (Cools et al. 2005) approximately 7.5–8 h after drink ingestion (mean, 7 h and 45 min). This is well within the peak of depletion as previously measured in the cerebrospinal fluid (Williams et al. 1999).

Participants

We acquired full datasets for 15 participants (aged 18–25 years, mean=21.3, SD=2.5; 8 women; all right handed). Eight participants received TRP− on the first test day. Prior to participation, participants were screened for (and excluded based on the presence of) psychiatric and neurological disorders. Further exclusion criteria were a personal history of cardiac, hepatic, renal, pulmonary, gastrointestinal disorder, substance abuse, first-degree family history of mood disorders and current medication (apart from contraceptive pill) or regular recreational drug use. Participants signed informed consent prior to the start of the experiment, and upon completion they received a payment for participation.

Amino-acid mixtures

Central TRP was depleted by ingestion of a large dose of large neutral amino acids (LNAAs) (Reilly et al. 1997) that did not contain TRP, whereas in the control condition, this dose did contain TRP. This procedure results in reduced central TRP levels via two mechanisms. Firstly, intake of LNAAs increases amino acid turnover in the liver and subsequently decreases plasma TRP stores. Secondly, the increased amino-acid load of other LNAAs increases competition for the active transport system across the blood–brain barrier

relative to TRP. The quantities of amino acids in each drink were based on those used by Young et al. (1985), though a 78.2-g mixture was employed to minimise nausea. Both amino-acid mixtures (prepared by Nutricia, Liverpool, UK) had the following ingredients: L-alanine, 4.1 g; L-arginine, 3.7 g; L-cystine, 2.0 g; glycine, 2.4 g; L-histidine, 2.4 g; L-isoleucine, 6 g; L-leucine, 10.1 g; L-lysine, 6.7 g; L-methionine, 2.3 g; L-proline, 9.2 g; L-phenylalanine, 4.3 g; L-serine, 5.2 g; L-threonine, 4.9 g; L-tyrosine, 5.2 g; and L-valine, 6.7 g. The balanced amino drink contained additionally L-tryptophan, 3.0 g. Women received the same mixture but with a 20 % reduction in quantity to take into account lower body weight. The drinks were prepared by stirring the mixture into approximately 200 ml tap water with a choice of lemon–lime or grapefruit flavouring to mask the unpleasant taste. Quantitative amino-acid analysis was performed on the blood samples to confirm the effectiveness of the TRP depletion procedure, using high-performance liquid chromatography (Fekkes et al. 1995). The ratio total TRP/LNAA was calculated as the concentration of TRP divided by the summed concentrations of the other LNAAs, i.e. phenylalanine, tyrosine, valine, leucine and isoleucine (TRP/LNAA) (Fernstrom and Wurtman 1972).

Valenced cued-reinforcement reaction-time task

Participants completed an adapted version of the cued-reinforcement reaction-time task (CRRT) (Cools et al. 2005). The original CRRT is a speeded reaction-time task in which the probability of feedback is predicted by a cue. On feedback trials, correct responses are rewarded, where the speed of a correct response determines the magnitude of the reward (100 points or 1 for responses that are within or after a preset response window and 0 for incorrect responses). This task allows investigation of behavioural adaptation to varying incentive motivation, i.e. feedback probability signalled by the cue. We modified the CRRT in three ways. First, to study the effect of motivational valence in incentive motivation, we added an aversive component to the task, so that in separate blocks either correct behaviour was rewarded or inadequate behaviour punished. In the original study it was not possible to dissociate effects driven by negative feedback from those driven by positive feedback, because there were no-feedback trials with a neutral baseline outcome; on any feedback trial, the outcome was either positive (points+happy emoticon) or negative (no points+sad/angry emoticon). In contrast, in the current study outcome valence was presented in blocks with positive versus neutral outcomes and blocks with negative versus neutral outcomes. This allowed us to separate punishment from reward-related processes with respect to a neutral baseline. Secondly, to match the appetitive and aversive task conditions, we treated correct slow responses differently in the current versus the previous version of the paradigm. Specifically, correct slow responses were accompanied by either reward omission or punishment in the current paradigm, whereas in the previous paradigm they were rewarded with a small magnitude reward. Thirdly, we used primary (juice) and secondary (monetary) reinforcements rather than abstract points. We chose to combine the primary with secondary reinforcements to avoid confounding valence and motivational salience, based on pilot results that for punishment primary reinforcement (magnesium sulphate solution) was most effective, while for rewards, secondary reinforcement (money) was more effective.

Task details

On each trial, participants were presented with a target stimulus consisting of three adjoining circles, each containing a smaller inner circle (Fig. 1). Two of the circles were identical, and participants were instructed to identify the unique, 'odd-one-out', circle by pressing one of three adjacent keyboard buttons corresponding to the location of the odd-one-out circle. On some trials, the response was followed by feedback. The likelihood of feedback was indicated by a cue stimulus that preceded the target stimulus. This cue consisted of a rectangular frame in one of six colours, each colour associated with the valence (reward/punishment) and probability of feedback (10/50/90 %). After a randomised cue-target interval (CTI; ranging from 1100 to 1900 ms in steps of 200 ms), the target was presented in the centre of the frame cue.

Participants were encouraged to respond both quickly and accurately, and based on their performance, participants could receive either reward or punishment in alternating blocks (Fig. 1). The response window was determined based on the individual speed in a practice block (see below). Feedback was presented after the response window on some trials, depending on the probability of feedback (signalled by the cue). The feedback was presented for 500 ms, after which the next cue was presented. Because feedback was presented directly after the end of the response window, it was clear to participants when their response was too late.

Participants completed four test blocks of 54 trials; two reward blocks and two punishment blocks (alternating and order counterbalanced between participants). On feedback trials in reward blocks, correct responses within the response window (*correct response*) were rewarded (positive feedback), whereas wrong responses (*incorrect response*) and responses outside the response window (*miss*) led to neutral feedback. Positive feedback consisted of a green, happy emoticon, accompanied by a flourish sound. Neutral feedback consisted of a grey circle, accompanied by a neutral beep. In case of a miss, the additional text 'too late' was presented. On feedback trials during the punishment blocks correct responses led to neutral feedback, whereas incorrect responses and misses were punished (negative feedback). Negative

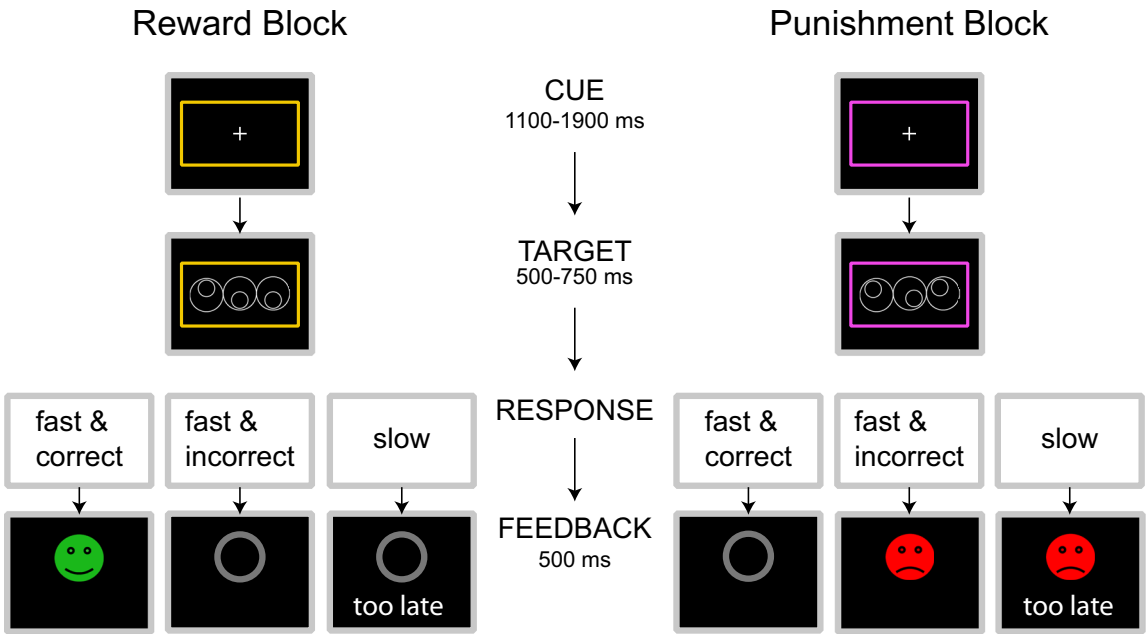

Fig. 1 Valenced cued-reinforcement reaction-time task. In the vCRRT, participants identify the 'odd-one-out' stimulus. By responding quickly and correctly, participants can receive reward during reward blocks (*left*) and avoid punishment during punishment blocks (*right*). The colour of the frame cue indicates the probability of receiving feedback (10, 50 or 90 %) on the current trial. On no-feedback trials, a fixation cross appeared after the target offset

feedback consisted of a red, sad emoticon, accompanied by a low buzz. Additionally, participants were instructed that they would accumulate a monetary gain (€0.10) and fruit juice (0.6 ml) for every positive feedback, which they would receive at the end of the task. Negative feedback led to an accumulating monetary loss (−€0.10) and bitter-tasting $MgSO_4$ solution (0.3 M; 0.6 ml). For the neutral feedback, no money or drink was lost or won. Participants tasted the bitter drink and two fruit juices before the start of the task and picked their preferred fruit juice. Upon completion of the task, participants consumed the amounts of fruit juice and $MgSO_4$ solution that they had gained/lost. Participants consistently rated the fruit juice as tasting nicer than the bitter solution ($F(1, 14)=207.7$, $p<.001$), independent of ATD ($p>.1$).

Two separate sets of three colours were used as cues, to avoid transfer effects of learning between the punishment and reward blocks. The colour sets were yellow/pink/blue and turquoise/purple/orange and were assigned to reward/punishment blocks counterbalanced across participants. Each cue was presented equally often, resulting in an average a priori feedback probability of 50 %. With respect to the target stimuli, we created 12 unique stimuli, equally divided across the three response options (left/middle/right). The target stimuli and required responses were counter balanced over cue types, so that no stimulus repetitions and more than two response repetitions occurred.

To determine the response window and ensure basic task understanding, participants started with two practice blocks of 20 trials. In these practice blocks, cues were omitted and feedback was provided on every trial, indicating whether the response was correct, incorrect or too slow, using a generous response window of 5 s. Participants were encouraged to respond as quickly and accurately as possible and were required to have at least 85 % correct during the second practice block, otherwise the second practice block was repeated (average performance, 96 % correct; range=(85–100 %)). The response window for the test blocks was defined as the mean reaction time for the second practice block minus one standard deviation, with a minimum of 500 ms and a maximum of 750 ms (mean=535 ms, 25–75 % range=(500–534 ms)). In order to account for potential speeding effect, the response window was allowed to differ between the first and the second test day. The response window was shorter for the second test day ($F(1, 13)=5.9$, $p=.03$), but this effect was independent of ATD (main effect of ATD: $F(1, 13)=2.2$, $p=.17$; *treatment*×*session*: $F(1, 13)=0.6$, $p=.44$).

Upon task completion, participants were asked to estimate the feedback probability associated with each cue colour and rated their confidence of the estimated feedback probabilities on a scale of 0 to 100.

Statistical analysis

We verified the effectiveness of the ATD procedure using two repeated measures analysis of variance (ANOVA), with dependent variables TRP concentration in the plasma and the percentage TRP/LNAA and with within-subject factors treatment (TRP+/−) and time (a.m./p.m.).

The aim of our analyses was to assess whether ATD released behavioural inhibition in anticipation of high probability of punishment, but not reward. To this end, we analysed behavioural slowing in terms of (i) the proportion of misses as a function of valence and (ii) changes in reaction times of on-time responses. For completeness, we also analysed the accuracy of on-time responses. We report significant effects at a Bonferroni-corrected alpha of .05/3=.017.

We first established that each of the dependent measures was normally distributed using the Kolmogorov-Smirnov test. Reaction times (RTs) were cut off within the response window so their distribution did not include the commonly observed long tail. Accordingly, it was not necessary to log-transform the RTs. RTs were *z*-score transformed within participants (across TRP+/−) to improve normality (Crockett et al. 2009). Next, we analysed proportion of misses, standardised RTs and accuracy using three repeated measures (rm)ANOVA with the within-subject factors treatment (TRP+/−), *p(feedback)* (10/50/90 %) and *valence* (punishment/reward). Significance is inferred using a Bonferroni-corrected *p* value of 0.017 (uncorrected *p* values are reported). Significant effects were further investigated by breaking them down into simple (interaction) effects. Greenhouse-Geisser corrections were applied when the assumption of sphericity was not met and corrected degrees of freedom are reported.

In supplementary analyses, we also assessed the total of received rewards and avoided punishments, again using an rmANOVA with the same within-subject factors. Note that this analysis is not independent of the ANOVAs above, because it is a function of the total number of correct, on-time responses.

Finally, to rule out the possibility that the observed effects of ATD on performance were due to differences in explicit awareness of the cue-feedback contingencies, we analysed whether participants had explicitly learned the feedback probabilities. For each cue, participants were asked to rate the probability that this cue resulted in feedback, and rate their confidence in this estimate. Estimated feedback probabilities and confidence ratings were analysed with a multivariate analysis of variance (MANOVA) using Pillai's trace and the within-subject factors treatment, *p*(feedback) and valence. Significant effects were further analysed using rmANOVA. Two participants did not complete these ratings and were excluded from this analysis.

Self-report measures

In order to assess any global effects of tryptophan depletion on mood, we administered the Positive Affect Negative Affect Scale (PANAS) (Watson et al. 1988) and the Bond and Lader Visual Analogue Scales (calmness, contentedness, alertness; Bond and Lader 1974) on four occasions each test day. We compared these ratings at baseline and directly prior to testing to assess effects of ATD treatment on mood, using a repeated measures MANOVA with treatment (TRP−, TRP+) and time point (baseline, prior to testing).

To assess effects of ATD on global cognitive status, a background neuropsychological test battery was administered after the testing session, consisting of a number cancellation task (Lewis and Kupke 1977), a box completion test (Salthouse 1996), a digit span test (forward and backward) (Wechsler 2008) and a verbal fluency task (Spreen and Strauss 1991).

To measure relevant trait demographics in the testing population, participants completed the following questionnaires during the screening sessions: Barratt Impulsiveness Scale (BIS-11) (Patton et al. 1995), Behavioural Inhibition/Behavioural Activation Scale (Carver and White 1994), Sensitivity to Punishment and Sensitivity to Reward Questionnaire (Torrubia et al. 2001), Eysenck Personality Questionnaire (Eysenck and Eysenck 1975), Beck Depression Inventory II (Beck et al. 1996), Spielberger Trait Anxiety Inventory (Spielberger et al. 1983), Hamilton Rating Scale for Depression (Hamilton 1960), the Dutch reading test (Schmand et al. 1991) and Kirby Questionnaire (Kirby et al. 1999).

Results

Serotonin manipulation

The total TRP levels (Table 1) varied over time depending on treatment ($F(1, 14)=329.4$; $p<.001$), showing that after drinking the AA mixture, for the TRP+ mixture total TRP plasma concentration increased ($t(14)=4.8$, $p<.001$), while for TRP− it decreased ($t(14)=32.4$, $p<.001$), in the absence of any differences in TRP levels at baseline ($t(14)=-0.1$, $p=.89$). The same ATD-dependent decrease over time was present in the percentage TRP/LNAA ($F(1, 14)=178.3$, $p<.001$). Together, these results confirm successful depletion of tryptophan in the TRP− relative to TRP+ condition.

Table 1 Mean (standard error of the mean) total TRP plasma concentration and percentage TRP/ΣLNAA before and after the AA mixture intake

	Total TRP (μM)		TRP/ΣLNAA (%)	
	Prior to AA intake	After AA intake	Prior to AA intake	After AA intake
TRP−	43.1 (1.4)	7.0 (0.8)	8.5 (0.3)	0.7 (0.1)
TRP+	43.4 (2.1)	57 (3.4)	8.2 (0.2)	5.8 (0.3)

AA amino acid, *TRP* tryptophan

Serotonin modulates behavioural inhibition under high incentive motivation

ATD affected the influence of feedback probability on the proportion of misses (p(feedback)× treatment: $F(2, 28)=10.0$, $p=.001$, $\eta^2=.42$; Fig. 2a; Table 2). Note that the proportion of misses is large by design of the study and does not signal bad performance. In the control condition, participants made fewer misses when feedback probability was at chance (50 %) than when it was either low or high (quadratic polynomial contrast: $F(1, 14)=10.4$, $p=.006$). In contrast, after TRP− participants showed a linearly decreasing miss rate with increasing probability of feedback (linear polynomial contrast: $F(1, 14)=6.6$, $p=.022$). Next, we analysed the simple interactions of p(feedback)× treatment. These ANOVAs confirmed that the effects of tryptophan depletion were driven by the high-feedback probability condition; the p(feedback)× treatment interaction was most strongly significant for the 50 vs. 90 % cue ($F(1, 14)=24.6$, $p<.001$), was also significant for 10 vs. 90 % ($F(1, 14)=11.4$, $p=.005$), but there was no difference for the 10 vs. 50 % p(feedback)× treatment interaction ($F(1, 14)=1.5$, $p=.25$). Further breakdown into paired t tests showed that participants made more misses for the 90 % cue relative to the 50 % cue ($t(14)=3.6$, $p=.003$) under TRP+, while this difference was abolished under TRP− ($t(14)=1.6$, $p=.14$). Thus, ATD released the inhibition of behavioural responses to high-feedback target stimuli. This effect, however, did not interact with valence ($F(1, 14)=0.01$, $p=.99$, $\eta^2=.001$). There were also no main effects of p(feedback) ($F(2, 28)=1.9$, $p=.17$, $\eta^2=.12$), treatment ($F(1, 14)=0.01$, $p=.92$, $\eta^2=.001$), valence ($F(1, 14)=0.1$, $p=.74$, $\eta^2=.01$), nor an effect of *ATD* treatment on valence ($F(1, 16)=0.26$, $p=.62$, $\eta^2=.02$) or any other interactions ($p>.1$, $\eta^2<.03$). Note that the null results for the valence effects are not due to the relatively small sample size in this study; the required sample size to achieve a power of 80 % to detect a significant effect at the specified alpha level (alpha=.017) for the contrast of interest (valence× p(feedback)× treatment) given the observed effect size ($\eta^2=.001$) was >6000 (Campbell and Thompson 2012). Similarly small effect sizes were observed for the other valence contrasts, requiring similarly large sample sizes to be rendered significant (valence: required $N=352$; valence× p(feedback): required $N=1730$; valence× treatment: required $N=738$).

The ATD-specific effect of feedback probability in the proportion of misses was not accompanied by a similar treatment× p(feedback) effect on the accuracy ($F(2, 28)=0.4$, $p=.67$, $\eta^2=.03$) or RTs ($F(2, 28)=1.9$, $p=.17$, $\eta^2=.12$) of the on-time responses (Fig. 2b, c). There were also no effects of treatment (RT: $F(1, 14)=2.5$, $p=.14$, $\eta^2=.15$; accuracy: $F(1, 14)=0.3$, $p=.61$, $\eta^2=.02$), p(feedback) (RT: $F(2, 28)=0.7$, $p=.48$, $\eta^2=.05$; accuracy: $F(2, 28)=1.1$, $p=.36$, $\eta^2=.07$), valence (RT: ($F(1, 14)=.6$, $p=.44$, $\eta^2=.04$; accuracy: $F(1, 14)$ <.1, $p=.93$, $\eta^2=.001$) or any interactions (all $p>.1$). We confirmed that the RT effects were also non-significant for the non-normalised RT values (treatment: $F(1, 14)=3.3$, $p=.09$, $\eta^2=.19$; p(feedback): $F(2, 28)=0.6$, $p=.54$, $\eta^2=.04$; valence: $F(1, 14)=0.05$, $p=.83$, $\eta^2=.004$; treatment× p(feedback):

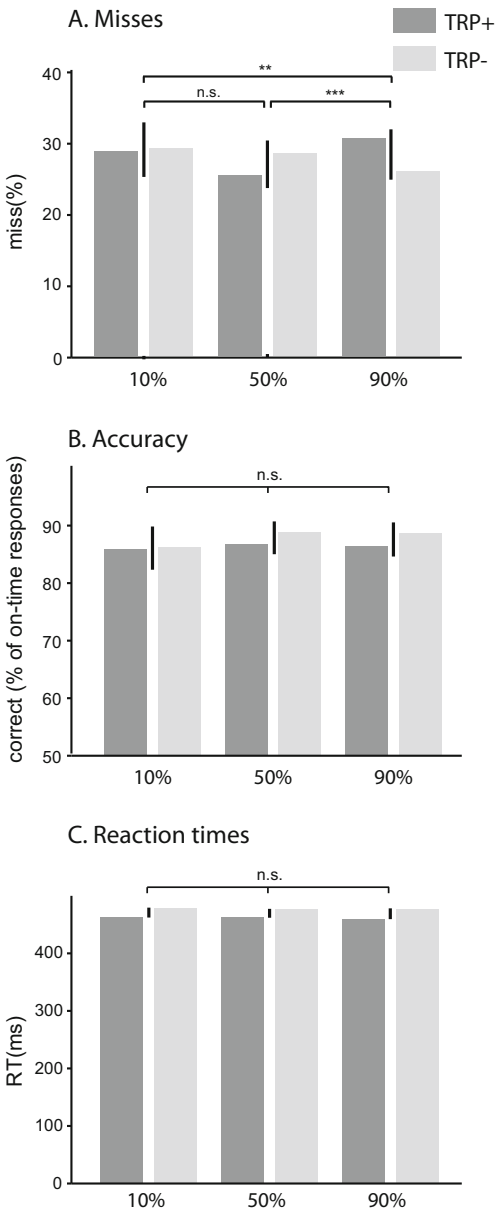

Fig. 2 Behavioural performance. The proportion of misses increased under TRP+ for higher feedback probabilities, indicating behavioural slowing under high incentive motivation. The proportion of misses did not increase under TRP− (**a**). The effect of ATD was specific for the 90 % cue relative to the other two cues (50 %: $F(1, 14)=24.6$, $p<.001$; 10 %: $F(1, 14)=11.4$, $p=.005$), while there was no significant differential effect of the 50 % cue relative to the 10 % cue ($F(1, 14)=1.5$, $p=.25$). With respect to reaction times (**b**) and accuracy of on-time responses (c), there was no influence of feedback probability nor an interaction with ATD. Plotted are mean±2 standard error of the difference (*SED*, *vertical lines*) for the TRP+/− simple contrasts. ***$p<.001$; **$p<.005$; *n.s.* non-significant

Table 2 Mean (standard error of the mean) behavioural performance

	Punishment		Reward	
	TRP+	TRP−	TRP+	TRP−
Miss[a] (%)				
10 %	26.9 (3.8)	27.8 (4.2)	27.5 (3.1)	27.6 (3.8)
50 %	24.3 (3.3)	27.6 (4.9)	23.9 (3.4)	26.3 (3.4)
90 %	29.3 (3.7)	25.7 (4.0)	28.7 (3.1)	23.5 (4.0)
Accuracy[b] (%)				
10 %	85.3 (3.0)	86.0 (3.2)	84.6 (2.1)	84.8 (4.2)
50 %	85.6 (2.7)	86.0 (3.4)	86.2 (2.4)	90.1 (2.5)
90 %	87.3 (3.5)	87.7 (3.2)	83.9 (2.6)	87.6 (2.9)
Reaction time[c] (ms)				
10 %	456 (7)	468 (12)	454 (8)	469 (13)
50 %	452 (7)	465 (12)	455 (7)	470 (12)
90 %	452 (7)	470 (13)	450 (7)	466 (13)

[a] Per cent of failure to respond before target offset

[b] Per cent correct of on-time responses

[c] Computed across both correct and incorrect on-time responses

$F(1.4, 19.5)=0.6$, $p=.50$, $\eta^2=.042$; all other interactions: $p>.1$) nor when only correct responses were analysed (treatment: $F(1, 14)=2.2$, $p=.16$, $\eta^2=.14$; p(feedback): $F(2, 28)=0.7$, $p=.52$, $\eta^2=.05$; valence: $F(1, 14)=0.4$, $p=.52$, $\eta^2=.03$; treatment × p(feedback): $F(2, 28)=1.3$, $p=.28$, $\eta^2=.09$; all other interactions: $p>.1$). The observed ATD-induced abolition of behavioural slowing under high-feedback probability in the absence of an ATD-induced influence on accuracy suggests that the ATD effect is not simply an altered speed-accuracy trade-off.

In addition, we quantified the influence of ATD on the outcome score. This analysis showed that the relative slowing under high-feedback probability for TRP+ was detrimental to performance (p(feedback) × treatment: $F(1.3, 18.5)=5.1$, $p=.027$). Specifically, under TRP+ participants received fewer rewards and avoided fewer punishments for 90 % relative to 50 % cues compared with under TRP− ($t(14)=3.3$, $p=.005$).

In a final control analysis, we checked whether the results were affected by the order in which participants were tested. There were no effect of testing order (proportion of misses ($F(1, 13)=0.3$, $p=.86$), RTs ($F(1, 13)<0.1$, $p=1.0$), accuracy of on-time responses ($F(1, 13)=1.1$, $p=.32$). In addition, the significance of all reported results remained unaltered independent of in or exclusion of testing order as a factor.

No effect of serotonin on explicit learning or mood

To rule out the possibility that the observed effects of ATD on performance were due to differences in explicit awareness of the cue-feedback contingencies, we analysed the estimated feedback probabilities and the corresponding confidence ratings. The experienced feedback probabilities reflected the actual feedback probabilities ($V=.4$, $F(4, 48)=2.7$, $p=.039$). Both the confidence ratings ($F(2, 24)=4.5$, $p=.023$) and the probability estimates (*trend:* $F(1.3, 15.3)=3.7$, $p=.065$) depended on p(feedback) in a linear relation fashion (confidence ratings: $F(1, 12)=6.0$, $p=.031$; probability estimates: $F(1, 12)=4.5$, $p=.056$). Note that the effects of the estimated feedback probabilities were only a trend, which is likely due to the difficulty of the task. In comparison to the previous version of the CRRT (Cools et al. 2005), this valenced task involved double the number of cues signalling feedback probability (three for each valence). Importantly, there were no differences in terms of explicit learning for either treatment ($V=.05$, $F(2, 11)=0.3$, $p=.78$) or valence ($V=.1$, $F(2, 11)=0.6$, $p=.56$). All higher order interactions were also non-significant ($p>.1$).

There were no significant effects of ATD treatment on mood (calmness, contentedness, alertness, positive and negative affect; MANOVA, using Pillai's trace, $V=.6$, $F(5, 7)=2.1$, $p=.18$). With respect to global cognitive status (number cancellation and box completion times; total digit span and verbal fluency scores) a MANOVA of these test scores indicated an effect of ATD ($V=.7$, $F(4, 11)=5.4$, $p=.012$). Subsequent Bonferroni-corrected repeated measures ANOVAs with ATD treatment as factor (BF corrected alpha=.013) showed an effect of treatment on the digit span score ($F(1, 14)=10.8$, $p=.005$), which the digit span was larger under TRP+ compared with TRP−. Verbal fluency ($F(1, 14)=4.8$, $p=.046$), number cancellation ($F(1, 14)=1.1$, $p=.32$) and box completion times ($F(1, 14)=3.2$, $p=.10$) were not significantly altered by ATD. Given the ATD-induced effect on digit span score and trend on verbal fluency score, we verified that inclusion of these measures as covariates in the above described analyses did not affect the results. Finally, we report relevant neuropsychological trait assessment scores of the testing population in Table 3.

Discussion

In the current study, we investigated the role of 5HT in incentive motivation, using ATD and a speeded incentive motivation task. Specifically, we aimed to assess whether 5HT depletion releases behavioural inhibition in anticipation of high probability of punishment, but not reward, in line with current theoretical proposals (Boureau and Dayan 2011; Cools et al. 2011; Dayan and Huys 2008) as well as recent empirical findings (Crockett et al. 2009; Geurts et al. 2013b).

Results revealed that under baseline conditions, participants showed an increased number of misses due to maladaptive behavioural slowing on high (90 %) feedback probability trials compared with lower (50 or 10 %) feedback probability trials. This motivationally induced behavioural slowing did

Table 3 Demographic and trait characteristics

Questionnaire	Range	Mean (SD)
Barratt impulsiveness scale	45–70	56.3 (8.65)
BIS	11–25	17.7 (3.89)
BAS	19–34	25.9 (4.24)
SPSRQ—reward	2–17	9.8 (4.18)
SPSRQ—punishment	0–10	4.4 (3.36)
EPQ—psychoticism	0–4	1.3 (1.28)
EPQ—extraversion	7–12	9.9 (1.41)
EPQ—neuroticism	0–6	1.9 (1.94)
EPQ—lie	2–12	7.3 (3.22)
BDI	0–5	1.0 (1.46)
STAI	20–41	32 (5.89)
Ham-D	0–3	0.5 (0.83)
NLV	92–115	104.3 (6.04)
Kirby	0.001–0.070	0.015 (0.018)

Abbreviations: *BIS* behavioural inhibition system score from the BIS/BAS scale, *BAS* behavioural activation system score, *SPSRQ* the Sensitivity to Punishment and Sensitivity to Reward Questionnaire, *EPQ* Eysenck Personality Questionnaire, *BDI* Beck Depression Inventory, *STAI* Spielberger Trait Anxiety Inventory, *Ham-D* Hamilton depression scale, *NLV* Nederlandse Lees Vaardigheid-Dutch reading test.

not simply reflect an adaptively altered speed-accuracy trade-off, because it was not accompanied by an improvement in accuracy on on-time trials. The fact that subjects slowed down for the high-feedback probability trials despite negative consequences suggests that this reflects an automatic behavioural bias rather than an explicit adaptive strategy.

This behavioural slowing under high incentive motivation was abolished when 5HT levels were lowered. Indeed, overall performance as indexed by both the number of misses and the total amount of reward and punishment received, improved after the administration of ATD. The observed abolition of behavioural slowing under low levels of 5HT is generally consistent with earlier work demonstrating behavioural disinhibition under depletion of 5HT (Crockett et al. 2012a; Soubrie 1986). We show additionally that this disinhibition is specific to high incentive salience, supporting previous research indicating 5HT is involved in the motivational value of actions rather than in general response inhibition (Bari and Robbins 2013). Thus, these data strengthen the hypothesis that 5HT plays an important role in the coupling between motivation and behavioural inhibition (Boureau and Dayan 2011; Cools et al. 2011).

Surprisingly, however, the observed behavioural slowing at baseline was observed independent of cue valence. We had expected the pattern of behavioural inhibition at baseline and associated disinhibition under lower 5HT levels to be specific to the punishment condition. Given the statistics of the environment, reward and punishment predicting stimuli require different behavioural strategies, namely approach/vigour and avoidance/inhibition, respectively (Boureau and Dayan 2011; Dayan and Huys 2008), and accordingly, it has been shown that humans are more likely to express behavioural activation under reward and behavioural inhibition under punishment (Cavanagh et al. 2013; Geurts et al. 2013a; Guitart-Masip et al. 2012). This bias to avoid punishment and approach reward has been suggested to rely on innately specified, Pavlovian control of behaviour (e.g. Huys et al. 2011). Based on the specific involvement of 5HT in inhibition under punishment, 5HT is thought to promote these aversive Pavlovian tendencies (Dayan and Huys 2009). Indeed, several recent studies point at the involvement of 5HT in promoting behavioural inhibition in the face of expected punishment (Cools et al. 2011; Crockett et al. 2012a, b; Dayan and Huys 2009; Geurts et al. 2013b; Huys et al. 2011), including a study of our own that reports data of an overlapping set of subjects (Geurts et al. 2013b). Why then, did we not observe valence-specific baseline inhibition in this paradigm?

One possibility is that the absence of valence specificity is the result of the blocked presentation of the reward and punishment trials in this task. The blocked design might have resulted in a different a priori expected outcome and thus a different baseline within each block (positive for reward blocks, negative for punishment blocks; Dayan 2012; Niv et al. 2007). If observed outcomes were encoded with respect to these differential baselines, then punishments and missed rewards might have been equally punishing (Amsel 1958; Mowrer 1951). Under the assumption that there is such a baseline reset, the observed maladaptive inhibition under baseline conditions might be best explained by aversive Pavlovian biases induced by the cues. Cues predictive of high-feedback probability, i.e. increasing incentive salience, likely activate Pavlovian biases more strongly than do cues predictive of low feedback probability. When 5HT levels are intact, this aversive Pavlovian bias would lead to stronger inhibition for higher feedback probabilities. When 5HT levels are lowered, the aversive Pavlovian bias would be weakened and would result in behavioural disinhibition, consistent with the current observations. In order to assess this hypothesis, future studies should use a design in which punishing and rewarding stimuli are interleaved.

Another possibility is that the behavioural inhibition under high motivation is indeed independent of valence in this paradigm. This option should be considered seriously given that valence-specific disinhibition following a reduction in 5HT has been observed in prior studies using blocked designs (e.g. Crockett et al. 2009). In other words, the disinhibitory effects of serotonin depletion might not be restricted to aversive contexts but might in some circumstances extend to appetitive contexts. It has indeed been suggested that 5HT specifically affects behavioural inhibition when there is a motivational drive to inhibit behaviour (Chase et al. 2011).

Thus, perhaps the effects of 5HT on behavioural vigour are not dependent on valence per se, but rather depend on whether a particular stimulus/situation induces slowing. Then, in rarer situations where appetitive stimuli induce slowing, such as in the current paradigm, a reduction of 5HT will also release this baseline inhibition. In short, lowering 5HT level will only disinhibit behaviour in situations where inhibition of behaviour is induced at baseline.

We think that the observation of valence-independent baseline inhibition under high incentive motivation may also help understand the discrepancy of the current findings with our previously reported effect of ATD on a similar speeded incentive motivation task (Cools et al. 2005). In that study, we observed that ATD reduced motivation-related speeding rather than reducing motivation-related slowing. We hypothesise that this discrepancy reflects recruitment of distinct performance strategies under baseline (TRP+) conditions. In our prior study, participants speeded responding when anticipating high-feedback probability, and this strategy was adaptive (led to more reward). In the current study, by contrast, participants slowed responding when anticipating high-feedback probability, even though this strategy was maladaptive.

A number of features of the two tasks may have encouraged this differential tendency to speed/activate or slow/inhibit responding with increasing motivation under baseline. We discuss the differences between these tasks in the Electronic supplementary material but will briefly highlight them here. Firstly, speeding in the prior study was encouraged by the structure of the task, in which all accurate responses were followed by a happy green emoticon, but were rewarded with only 1 point for slow responses, yet with 100 points for fast responses. Thus, speed was strongly emphasised. This asymmetry was absent in the current paradigm where speed and accuracy were rewarded equally. Second, in the prior study participants received positive feedback on average on an average of 91 % of the trials (for both slow and fast correct responses), while here participants received positive feedback on only 64 % of the trials (correct, on-time responses only). Thus, substantially more negative feedback was received, making it plausible that the behaviour was overall more driven by the possibility of getting the negative outcome. This shift towards more aversive outcomes may have elicited innately specified behavioural inhibition at baseline (Dayan and Huys 2009). Third, explicit subjective awareness of the feedback probabilities for the different cues was much lower in the current study than in the previous study. Unlike in the present study, high awareness in the previous study might have encouraged participants to recruit an adaptive speeding strategy, thus overruling any maladaptive, possibly innately specified slowing response.

In conclusion, we show that lowering 5HT levels leads to behavioural disinhibition, consistent with previous research and ideas on the role of 5HT (Cools et al. 2011; Dayan and Huys 2009; Soubrie 1986). This disinhibition was not global but specific to high incentive motivation, supporting the role of 5HT in the motivational value of actions rather than in general response inhibition (Bari and Robbins 2013). This idea helps understand the inconsistency of effects of lowering 5HT on behavioural vigour. We posit that perhaps we should understand the effects of 5HT on inhibition not as valence-dependent but rather driven by general motivation. If 5HT affords motivationally driven behavioural inhibition, regardless of whether the inhibition is driven by appetitive or aversive cues, then in these circumstances reductions in 5HT will lead to increased behavioural vigour.

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