Psychopharmacology (2008) 196:29–38
DOI 10.1007/s00213-007-0920-y

ORIGINAL INVESTIGATION

The role of 5-HTTLPR in choosing the lesser of two evils, the better of two goods: examining the impact of 5-HTTLPR genotype and tryptophan depletion in object choice

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Received: 22 January 2007 / Accepted: 17 August 2007 / Published online: 17 October 2007
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Abstract
Rationale The serotonin (5-HT) system is considered important for decision-making. However, its role in reward- and punishment-based processing has not yet been clearly determined.
Objectives The present study examines the effect of 5-HTTLPR genotype and tryptophan depletion on reward- and punishment-related processing, using a task that considers decision-making in situations of subtlety of choice. Thus, it considers that response choice often occurs in situations where both options are desirable (e.g., choosing between mousse au chocolat or crème caramel cheesecake from a menu) or undesirable. It also considers that response choice is easier when the reinforcements associated with the options are far apart, rather than close, in value.
Materials and methods Healthy volunteers underwent acute tryptophan depletion (ATD) or control procedures and genotyping of the 5-HTTLPR for long and short allele variants. We then examined the effects and interactions of ATD and the serotonin promoter polymorphism genotype on two aspects of decision-making: (a) decision form, choosing between two objects to gain the greater reward or lesser punishment and (b) between-object reinforcement distance, the difference in reinforcements associated with two options.
Results ATD and LL homozygosity had comparable interactions with decision form and between-object reinforcement distance. Specifically, both modulated the effect of between-object reinforcement distance when deciding between objects both associated with punishment. Moreover, ATD and genotype interacted with ATD disproportionately affecting the performance of the LL homozygous group.
Conclusions These results suggest that serotonin is particularly associated with punishment, rather than reward-related processing, and that individual sensitivity to punishment-related information and tryptophan depletion varies with genotype.

Keywords Decision · Tryptophan depletion · 5-HTTLPR genotype · Reward · Punishment

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Introduction

The functional role(s) of serotonin remain unclear although abnormalities in this neurotransmitter are implicated in a variety of psychiatric conditions (Carver and Miller 2006;

Deakin 2003). One way in which they can be elucidated is through tryptophan depletion, a procedure which transiently lowers central nervous system serotonin levels by reducing serum and central nervous system levels of its precursor tryptophan (Krakowski et al. 1997; Williams et al. 1999). A second way is through examination of functional associations with alleles of the serotonin transporter (Hariri et al. 2002; Pezawas et al. 2005). In this study, we used tryptophan depletion and genetic assay of the long (L) and short (S) alleles of the 5-HT transporter-linked polymorphic region (5-HTTLPR). Our aim was to determine the impact of a hyposerotonergic state and polymorphisms of 5-HTTLPR, as well as their interactions, on a novel measure of stimulus–reinforcement guided decision-making, the differential reward/punishment learning task (DRPLT; Blair et al. 2006a, b).

There are strong indications that a major role of serotonin is in determining sensitivity to reinforcement information (Cools et al. 2005; Evers et al. 2005; Finger et al. 2007; Rogers et al. 2003) including social reinforcement information (Harmer et al. 2003b; Marsh et al. 2006). Thus, tryptophan depletion has been associated with reduced discrimination between magnitudes of expected gains in a decision-making paradigm (Rogers et al. 2003) and reduced reward-related speeding of response times (RTs) in a cued-reinforcement time task (Cools et al. 2005). Moreover, tryptophan depletion has been associated with an increased number of errors in response reversal (Park et al. 1994; Rogers et al. 1999) and global increases in RTs while performing such tasks (Evers et al. 2005; Murphy et al. 2002), although see Talbot et al. (2006). Similar response reversal impairments are seen in experimental animal studies after ablation of prefrontal serotonergic neurons (Clarke et al. 2004, 2005, 2007).

With respect to social reinforcers, the modulation of serotonin levels affects sensitivity to fear-relevant social cues (Harmer et al. 2003a, b, 2006; Marsh et al. 2006). Acute tryptophan depletion (ATD) is associated with decrements in recognition of fear facial expressions (Harmer et al. 2003b) while acute administration of tryptophan, by contrast, increases subjects' ability to recognize fear expressions (Attenburrow et al. 2003). Similar effects of fear recognition have been seen after administration of citalopram, an SSRI, which also increases serotonin levels (Harmer et al. 2003a). Moreover, neuroimaging studies indicate that 5-HTTLPR status affects the cortical response to aversive social reinforcers whereby LL homozygous individuals demonstrate reduced neural responses to fearful or angry faces compared to S-carriers (Hariri et al. 2002, 2005; Heinz et al. 2005; Pezawas et al. 2005).

There is considerable variation in individual responses to ATD (Booij et al. 2003). This may relate to genetic factors (Finger et al. 2007; Neumeister et al. 2002; Roiser et al. 2006). There has thus been growing interest in examining whether the effects of 5-HT depletion are differentially affected by genotype at the 5-HTTLPR polymorphism (Finger et al. 2007; Marsh et al. 2006; Neumeister et al. 2002; Roiser et al. 2006). There has been suggestion that women who are homozygous for the S allele at the 5-HTTLPR were vulnerable to mood change after ATD, while those homozygous for the L allele did not show mood change (Neumeister et al. 2002); although see Roiser et al. (2007). With respect to the impact of reinforcement, the attenuation of motivationally speeded action on the cued-reinforcement reaction time task by ATD was only seen in subjects with the SS genotype and not those with the LL genotype (Roiser et al. 2006). Moreover, tryptophan-depleted individuals homozygous for the long allele failed to appropriately use punishment information to guide responding during performance on a response reversal paradigm after ATD (Finger et al. 2007).

While there is relatively good support that serotonin plays a role in determining sensitivity to reinforcement information, it remains relatively unclear whether it determines sensitivity to reward or punishment or both. Data from a decision-making paradigm (Rogers et al. 2003) and studies examining reward-related speeding of response times in a cued-reinforcement time task (Cools et al. 2005) suggest reduced sensitivity to reward information. In contrast, data on the impact of serotonergic manipulations on the response reversal paradigm (Evers et al. 2005; Finger et al. 2007; Park et al. 1994; Rogers et al. 1999) and social reinforcement where the processing of fearful expressions is particularly affected (Harmer et al. 2003a, b, 2006; Marsh et al. 2006) suggest reduced sensitivity to punishment information.

One way of examining this issue further is to use paradigms that provide a more graded measure of sensitivity to reward and punishment information. We examined the impact of ATD and LL-homozygous relative to S-allele carrier state, as well as interactions between these variables, on performance on the DRPLT (Blair et al. 2006a, b). This task requires the subject to choose between two objects associated with different levels of reward, two objects associated with different levels of punishment, or one object associated with reward and one object associated with punishment. The subject has to choose the object that will gain the most points or, on trials involving two punishing objects, lose the least points. As the objects are associated with different levels of reward, response choice indexes not only reward/punishment sensitivity but also sensitivity to the numerical distance, or between-object reinforcement distance, between the reward/punishment levels associated with the two objects presented (see below). Thus, previous work with this task has shown that it is easier to choose between objects associated with

reinforcements far apart in numerical value (e.g., choosing between objects associated with 100 vs 900 points) than objects associated with reinforcements close together in numerical value (e.g., choosing between objects associated with 100 vs 300 points; Blair et al. 2006a, b). Moreover, recent functional magnetic resonance imaging (fMRI) work has shown the involvement of the amygdala and ventromedial prefrontal cortex (vmPFC) in the representation of reinforcement information necessary for choice selection, but that dorsal anterior cingulate cortex is particularly sensitive to between-object reinforcement distance (presumably reflecting its role in resolving increasing response conflict as distance decreases; Blair et al. 2006b).

In short, examination of performance on the DRPLT allows identification of whether the individual shows selective impairment for choices based on either reward or punishment information or both. If ATD or LL homozygosity particularly affects sensitivity to reward information then ATD/LL homozygosity will be associated with significantly poorer performance on trials where subjects must choose between objects that are both associated with reward. In contrast, if ATD or LL homozygosity particularly affects sensitivity to punishment information, then ATD/LL homozygosity will be associated with significantly poorer performance on trials where subjects must choose between objects that are both associated with punishment. The current study tests these predictions.

Materials and methods

Subjects

Twenty-four subjects (13 females; aged 20–47, mean age= 27.67) from the Washington, DC, metropolitan area took part in the study (Table 1). Before the study subjects underwent a screening visit at the National Institutes of Health, which included a medical history and physical exam performed by a physician, a structured Clinical Interview for DSM-IV performed by a clinician, and blood and urine screening tests. In addition, the matrix reasoning and vocabulary subtests of the Wechsler Abbreviated Scale of Intelligence were administered to obtain an estimated IQ score. Exclusion criteria included current medical illness or major psychiatric disorders, a lifetime history of mood disorders or psychosis, or relatives with a history of mood disorders. In addition, no subject was currently taking psychotropic medications. All subjects gave written informed consent to participate in the study, which was approved by the National Institute of Mental Health Institutional Review Board.

Table 1 Subject characteristics: SD in parentheses

	Placebo (N=13)	ATD (N=11)
Age	29.00 (8.70)	26.09 (7.01)
Gender	6 F/7 M	7 F/4 M
IQ	111.91 (12.42)	117.22 (8.17)
VAS	7.75 (1.68)	6.88 (1.83)

F Female; *M* male

Fifteen of the 24 subjects were S carriers, and nine were LL-homozygous. Following a double-blind procedure, 11 subjects (7 females; 8 S carriers) received the TD (tryp-) capsules and 13 subjects (6 females; 7 S carriers) received placebo capsules. There was no significant drug group (ATD, placebo) or genotype group (LL homozygous, S carriers) difference in age, IQ or VAS mood ratings following ATD/placebo procedure (F=0.79, 1.21 and 1.40 respectively; ns; and F=3.07, 1.74, and 0.43 respectively; ns).

Amino acid mixtures

The composition of the mixtures ingested by the subjects was as that used in Finger et al. (2007). The amino-acid mixtures of the 70 capsules ingested by the ATD subjects were L-isoleucine (4.2 g), L-leucine (6.6 g), L-lysine (4.8 g), L-methionine (1.5 g), L-phenylalanine (6.6 g), L-threonine (3.0 g), and L-valine (4.8 g). The placebo consisted of 70 capsules containing a total of 31.5 g of lactose. All capsules were taken with water (Wolfe et al. 1995).

Total tryptophan determination

Serum was collected in pre-chilled ethylenediaminetetraacetic acid (EDTA) tubes and, immediately after collection, was then centrifuged for 15 min at 300 rpm and 4°C. Subsequent storage was at −70°C. Plasma tryptophan concentrations were determined by reverse-phase high performance liquid chromatography (HPLC) in conjunction with fluorescence end-point detection. For total tryptophan, plasma proteins were removed by precipitation with 3% trichloroacetic acid followed by centrifugation. For the estimation of free tryptophan, protein-bound TRP was separated from free by filtration through 10K cutoff micro-filters via a centrifugation process. The LNAAs (total tryptophan/large neutral amino acids ratio) were analyzed via gradient HPLC with utilization of pre-column derivatization and fluorescence end-point detection.

DNA extraction

DNA for each subject was prepared from 16 ml of peripheral blood and approximately 300 μg were obtained for each subject. Using HPLC, all 5-HTTLPR analyses

were accomplished via amplification of the region followed by size separation of the alleles. Positive (known genotype) and negative control (no DNA) standards were run with each assay. Genotyping was performed by a contractor blind to subject identities.

Procedure

Subjects arrived for testing at 8:30 A.M. having fasted since midnight. In addition, they had been instructed to follow a low-protein diet (10–15 g protein) on the day before testing. Serum was drawn on admission and then again 5 h after ingestion of the last capsule for analysis of free tryptophan and total tryptophan: large neutral amino acids (LNAA) ratios. Serum was also collected on admission for 5-HTTLPR genetic analysis. After capsule ingestion, two separate meals, consisting of a low tryptophan breakfast and lunch (containing a total of 60 mg of tryptophan) were served. Five hours after the last capsule ingestions, subjects completed a cognitive test battery, including the DRPLT. Mood changes were monitored by mood visual analog scales (VAS) and verbal report. The duration of the entire battery was approximately 90 min.

Differential reward/punishment task and experimental procedure

The stimuli were a set of ten line drawings from the Snodgrass and Vanderwart (1980) picture set. Each stimulus depicted a common object: house, cup, fork, duck, pineapple, necklace, raccoon, door, flashlight, or shoe. To prevent systematic task interference from any existing valence attached to the objects (e.g., pineapple might have a pre-existing positive valence), each object was randomly assigned a value (−900, −700, −500, −300, −100, 100, 300, 500, 700, or 900 points) by the program when the subject began the task (i.e., not every participant received 900 points for choices of the shoe for example). On each trial, objects were presented in pairs, appearing in two of four (left-hand top, left-hand bottom, right-hand top, and right-hand bottom) screen locations. The subject was told that on each trial, one of the two objects must be chosen and that some objects would result in losing points and that some objects would result in winning points. After object selection (with the click of a mouse), its assigned value was revealed. Thus, choosing the object assigned with the value 100 resulted in the feedback: 'You have WON 100 points'. Conversely, choosing the object assigned with the value −100 resulted in the feedback: 'You have LOST 100 points'. Feedback stayed on the screen for 1,000 ms and was then replaced by the two objects for the subsequent trial (see Fig. 1 for example trial). Subjects did not receive feedback as to whether or not their choice on each trial was

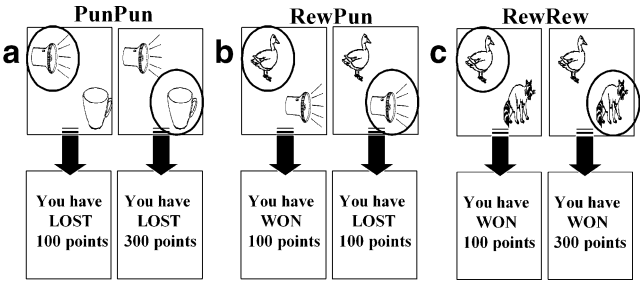

Fig. 1 Sample stimuli of **a** PunPun, **b** RewPun, **c** RewRew trials

the more advantageous. They also did not see a running tally as to how many points they won/lost overall; however, they were given their overall score at the end of the session. There was no time limit for making a response.

The study involved a 3 (decision form: PunPun, RewRew, and RewPun) by 3 (between-object reinforcement level distance [Distance]: close, medium, and far) design. RewRew trials involved two objects both associated with a reward (e.g., [100 vs 300] [100 vs 500] [300 vs 700]). On these trials, response choice of either object would result in a point gain, although one of the objects would result in the greater point gain (see Fig. 2). PunPun trials involved two objects both associated with a punishment (e.g., [−100 vs −300] [−100 vs −500] [−300 vs −700]). On these trials, response choice of either object would result in a point loss, but one of the objects would result in the greater point loss. Thus, the appropriate strategy on these trials would be to select the object associated with the smaller point loss. RewPun trials involved one object associated with reward and one object associated with punishment (e.g., [100 vs −100] [100 vs −300] [100 vs −500]). On these trials, response choice of one of the objects would result in a point gain, whereas the response choice of the competing object would result in a punishment; see Fig. 1 for example trials.

The "close" between-object reinforcement distance trials involved two objects associated with values that were close together in value (e.g., [−900 vs −700] [900 vs 700] [300 vs −100]. The "far" between-object reinforcement distance trials involved two objects associated with values that were

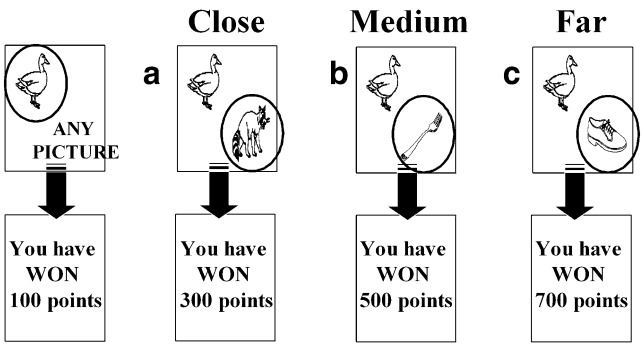

Fig. 2 Sample stimuli of **a** close, **b** medium, or **c** far between-object reinforcement distance

far apart in magnitude (e.g., [−900 vs −100] [900 vs 100] [300 vs −900]); see Fig. 2 for example trials.

On any trial, selecting the superior choice over the inferior choice was scored as 'correct'. Thus, on PunPun trials where both objects represented a point loss (e.g., −100 vs −300) selecting the object representing the smaller loss of −100 was scored as correct and on RewRew trials where both objects represented a gain (e.g., 100 vs 300), selecting the object representing the greater gain of 300 was scored as correct. On RewPun trials where one object represented a gain and one object represented a loss (e.g., −100 vs 100), selecting the rewarding object was scored as correct. For analysis purposes, subjects' error scores were converted into error percentages. The task involved 234 trials and was programmed in VisualBasic.

Results

Biochemical results

A 2 (drug: ATD, placebo) by 2 (time point: pre- vs 5 h post-capsules) analysis of variance (ANOVA) was conducted on the serum free tryptophan levels. There was a main effect of drug (F[1,22]=8.10; p<0.01) (M [free tryptophan in μg/ml pre-capsules]=1.02; SE=0.06; M[free tryptophan in μg/ml post-capsules]=0.47; SE=0.04; as expected subjects had a significant reduction in free tryptophan levels after capsule ingestion. There was also a significant drug by time point interaction (F[1,22]=19.42; p<0.001); there was no significant difference in baseline free tryptophan levels between the drug groups but a significant difference in the free tryptophan levels between the two groups after capsule ingestion (F<1 and 6.15; n.s. and p<0.001, respectively) (M [free tryptophan in μg/ml post-capsules in the ATD group]=0.23; SE=0.06; M[free tryptophan in μg/ml post-capsules in the placebo group]=0.71; SE=0.05. This represented a reduction of 88% in free tryptophan levels in the ATD group compared to a 30% reduction in the placebo group. Similarly, there were no significant differences between groups at baseline in the LNAA, while at 5 h post-capsule ingestion, there was a significant group difference in LNAA ratio (F<1 and 3.13; n.s. and p< 0.01, respectively; M [LNAA ratio post-capsules in the ATD group]=0.03; SE=0.01 M[LNAA ratio post-capsules in the placebo group]=0.07; SE=0.01.

5-HTTLPR results

Two 2 (Drug: ATD, placebo) by 2 (genotype: LL homozygous, S carriers) ANOVAs conducted on tryptophan levels demonstrated no significant effect of genotype on baseline or 5 h post-capsule free tryptophan levels or LNAA ratios and no significant genotype by drug group interactions.

Differential reward/punishment learning task results

A mixed ANOVA with decision form (PunPun, RewPun, and RewRew) and between-object reinforcement distance (distance: close, medium, and far) as within-subjects variables, and genotype (LL homozygous, S carriers) and drug (ATD, placebo) as between-subjects variables was applied to the data. There was a significant main effect of decision form (F[2,40]=12.70; p<0.001); subjects were less accurate at selecting the correct object on PunPun trials relative to RewRew trials and on RewRew trials relative to RewPun trials (F=3.21 and 1.72; p<0.005 and 0.05 one-tailed, respectively; M [PunPun]=15.12; SE=2.53; M[RewRew]= 9.81; SE=2.02; (M[RewPun]=6.84; SE=1.36). There was also a significant main effect of distance (F[2,40]=16.72; p<0.001) with the linear contrast being significant (F[1,20]= 28.43; p<0.001); subjects were less accurate at selecting the correct object as between-object reinforcement distance decreased (M[close]=14.55; SE=2.28; M[medium]=9.76; SE=1.68; (M[far]=7.46; SE=1.76). In addition, there was a significant decision form by distance interaction (F[4,80]= 3.69; p<0.01); the increase in error rates across between-object reinforcement distance was greater for RewRew and RewPun trials relative to PunPun trials (p<0.01 and 0.05 one-tailed, respectively).

There was a significant distance by genotype by drug interaction (F[2,40]=3.41; p<0.05). As can be seen in Fig. 3, ATD had a disproportionate effect on the distance performance of the LL homozygous group, although it should be noted that follow-up tests indicated only weak gene and gene by distance interaction effects for the ATD group (F[1,9]= 2.75 and F[2,18]=3.33; p=0.133 and 0.101, respectively).

There was a significant decision form by distance by genotype interaction (F[4,80]=2.82; p<0.05); see Fig. 4. Follow-up tests showed that the S carriers group showed a significant effect for decision form (F[2,28]=5.64; p<0.01) and a very significant effect for distance (F[2,28]=15.53; p<0.001) but no significant decision form by distance interaction (F<1; n.s.). In contrast, the LL homozygous group showed a significant effect for decision form (F[2,16]=6.42; p<0.01) but no significant effect for distance (F[2,16]=2.82; n.s.). However, they did show a significant decision form by distance interaction (F[4,32]= 2.82; p<0.05); in short, the LL homozygous group only showed a significant distance effect for RewRew trials (F[2,16]=4.56; p<0.05); see Fig. 4. However, it should be noted that a 2 (genotype: LL homozygous, S carriers) by 3 (distance: far, medium, and close) ANOVA for the PunPun trials indicated only a weak trend for a genotype by linear distance interaction (F=2.12; p=0.11).

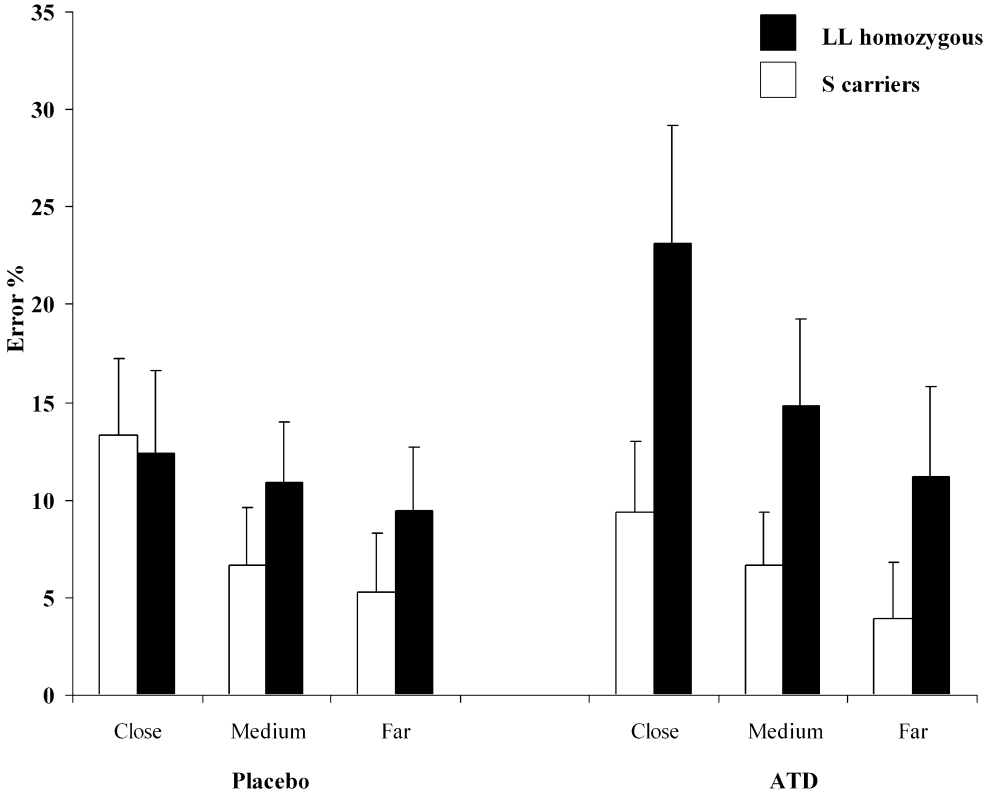

Fig. 3 Error rates (mean±SE) to between-object reinforcement distance as a function of genotype and drug

There was a significant decision form by distance by drug interaction (F[4,80]=2.64; p<0.05). Follow-up tests showed that the placebo group showed a significant effect for decision form (F[2,24]=6.10; p<0.01) and a significant effect for distance (F[2,24]=5.91; p<0.01) but no significant decision form by distance interaction (F<1; n.s.). The ATD group showed a significant effect for decision form (F[2,20]=7.37; p<0.005) and a significant effect for

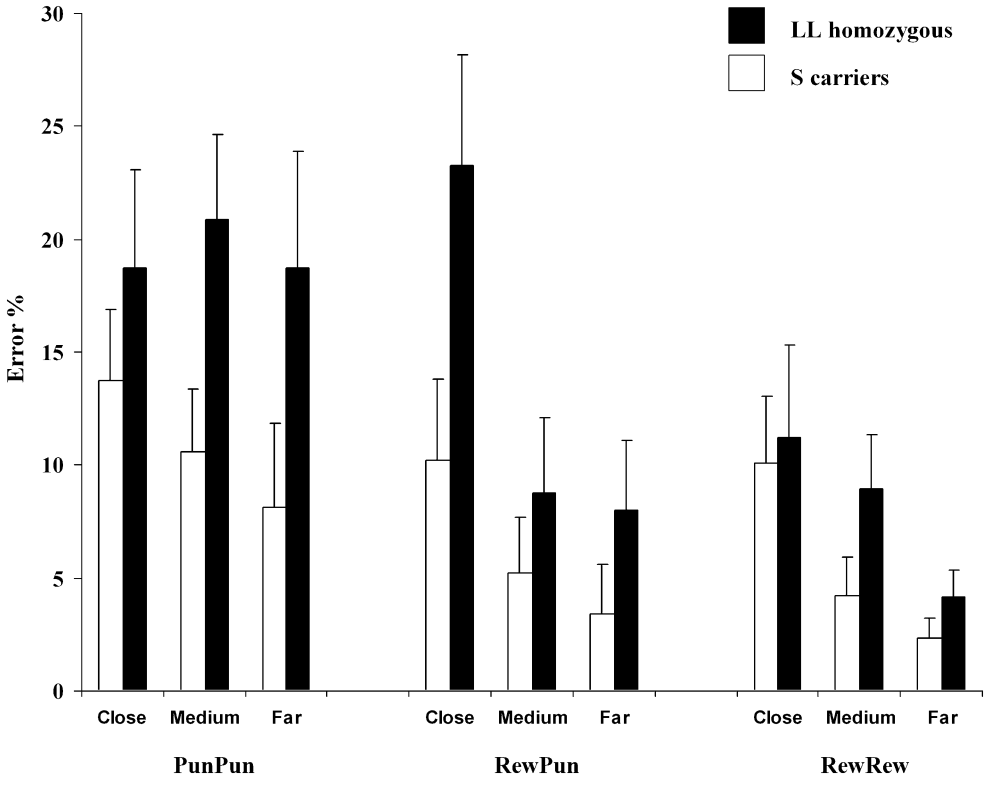

Fig. 4 Error rates (mean±SE) to between-object reinforcement distance conditions as a function of genotype and decision form

distance (F[2,20]=9.51; p<0.001), but they also showed a significant decision form by distance interaction (F[4,40]= 2.34; p<0.05 one-tailed). On post-hoc tests, the ATD group showed a significant distance effect for both the RewRew and RewPun trials (F[2,20]=5.75 and 7.85, p<0.05, respectively) but not the PunPun trials (F[2,20]=1.05; n.s.); see Fig. 5. However, it should be noted that a 2 (Drug: ATD vs placebo) by 3 (distance: far, medium, and close) ANOVA for the PunPun trials did not reveal a drug by linear distance interaction (F=<1; n.s.).

In our previous work with this task, we found a linear correlation between total available reinforcement (sum of the values of both objects) and activation in vmPFC. On the basis of a reviewer's suggestion, given previous findings of ATD on mPFC in a learning task (Evers et al. 2005), we applied a mixed ANOVA with genotype (LL homozygous, S carriers) and drug (ATD, placebo) as between-subjects variables and total available reinforcement (mean: −1,500, −1,100, −700, −300, 0, 300, 700, 1,100, and 1,500) as within-subjects variable to the data. There was a significant main effect of total available reinforcement (F[8,160]= 5.70; p<0.001) with the linear contrast being significant (F[1,20]=7.85; p<0.05); subjects were less accurate at selecting the correct object as the total available reinforcement level decreased. In addition, there was a significant drug by total available reinforcement interaction (F[8,10]= 1.78; p<0.05); the ATD committed a greater number of errors on trials involving a negative total available reinforcement, and a smaller number of errors on trials involving a positive total available reinforcement (see Fig. 6). However, it should be noted that follow-up tests on this interaction did not reach significance. There were no significant interactions with genotype.

Discussion

The current study investigated the impact of ATD and polymorphisms of 5-HTTLPR, as well as their interactions, on a novel measure of stimulus-reinforcement guided decision-making, the differential reward–punishment task (Blair et al. 2006a,b). We found that ATD had a disproportionate effect on the performance of the LL homozygous group. In addition, there were some indications that LL homozygosity and perhaps ATD disrupted the effect of between-object reinforcement distance for PunPun decisions.

The current data add to previous work indicating that manipulations of serotonergic function interact with genotype at the 5-HTTLPR polymorphism. In vitro studies of 5-HTTLPR function have demonstrated increased reuptake of extracellular serotonin in platelets by the LL-homozygous status compared to the SS- or SL-variants (e.g., Greenberg et

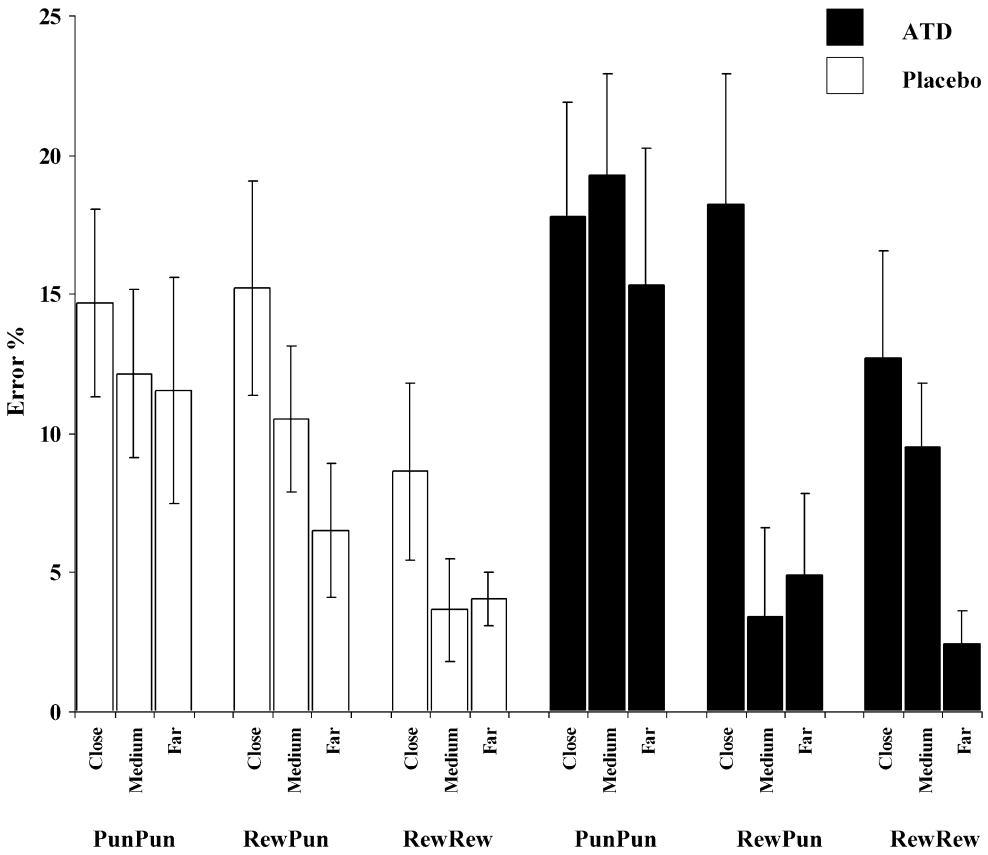

Fig. 5 Error rates (mean±SE) to between-object reinforcement distance as a function of drug and decision form

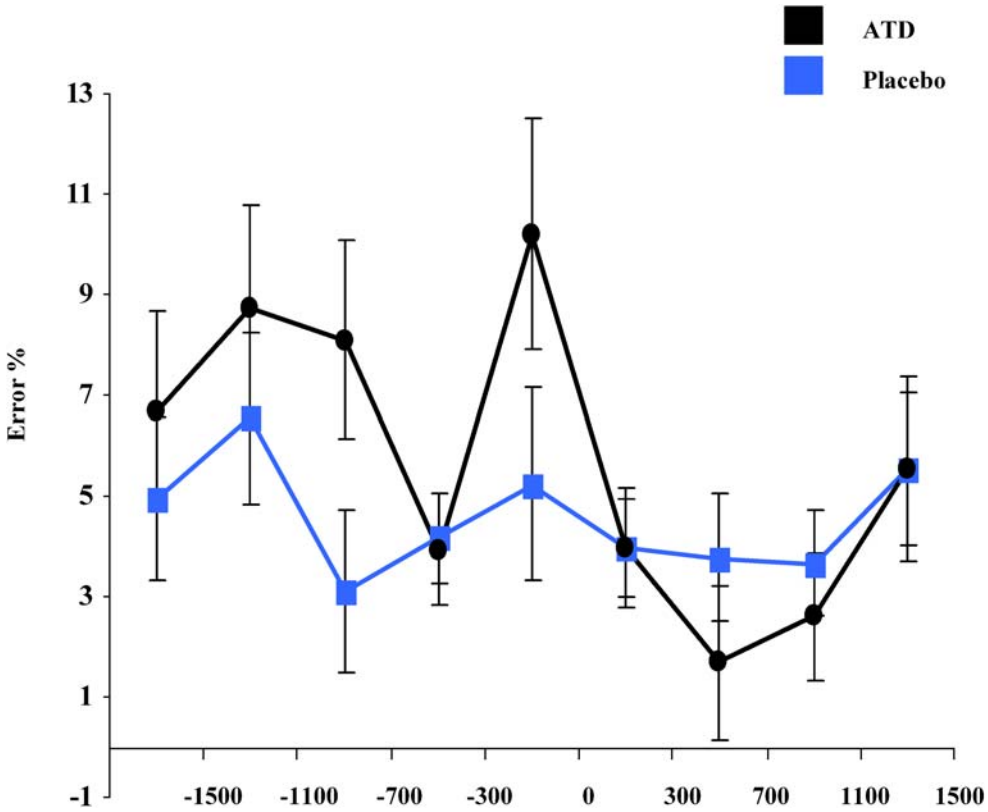

Fig. 6 Error rates (mean±SE) to total available reinforcement as a function of drug

al. 1999). However, it remains uncertain whether S carriers or LL-homozygous individuals are the more sensitive to changing serotonin levels. In some work, ATD has had a greater impact on S carriers. Thus, the attenuation of motivationally speeded action on the cued-reinforcement reaction time task by ATD was only seen in S carriers but not in LL homozygotes (Roiser et al. 2006). Similarly, ATD impaired fearful expression recognition in S carriers but not LL homozygotes (Marsh et al. 2006). In contrast, ATD disrupted the use of punishment information during performance on a probabilistic response reversal paradigm only in individuals with the LL genotype and not in S carriers (Finger et al. 2007). The current study, similar to Finger et al. (2007), found that ATD particularly disrupted the performance of LL homozygotes rather than S carriers.

As noted above, although there are strong indications that serotonin has a major role in determining sensitivity to reinforcement information (Cools et al. 2005; Evers et al. 2005; Finger et al. 2007; Rogers et al. 2003), it has remained relatively unclear whether it determines sensitivity to reward or punishment or both. The current study was more suggestive of a role for serotonin in determining sensitivity to punishment information. This is consistent with data on the impact of serotonergic manipulations on the response reversal paradigm (Evers et al. 2005; Finger et al. 2007; Park et al. 1994; Rogers et al. 1999) and social reinforcement paradigms involving the processing of fearful expressions (Harmer et al. 2003a, b, 2006; Marsh et al. 2006). Moreover, it is also consistent with data showing that LL homozygotes show particular difficulty learning to avoid responding to stimuli associated with punishment in the human passive avoidance learning paradigm (Finger et al. 2007). It appears less consistent with data from the Rogers' decision-making study (Rogers et al. 2003) and studies examining reward-related speeding of response times in a cued-reinforcement time task (Cools et al. 2005; Roiser et al. 2006). However, it should be noted that there is considerable variety in these paradigms. Potentially importantly, in the studies by Rogers et al. (2003) and Cools et al. (2005), the trials presented to participants were broadly and consistently appetitive. In contrast, in the current study, the PunPun trials involved choices between two negative outcomes. It is only after additional work and more formal models of the processing involved in these tasks that a detailed understanding of serotonin's role in determining sensitivity to reinforcement information will emerge.

A couple of caveats should be noted with respect to the current results. The dosage of amino acids was lower than that used in many other studies, although it did produce relative reductions in free tryptophan and the tryp/LNAA ratio comparable to that of other studies of response reversal (Evers et al. 2005; Rogers et al. 1999; Talbot et al. 2006). However, we also observed relatively greater decreases in these parameters under the placebo-control condition than was

observed in these studies (Evers et al. 2005; Rogers et al. 1999; Talbot et al. 2006). This may have resulted from the use of lactose capsules as the placebo, coupled with the ingestion of low-tryptophan meals, producing a decrease in the tryp/LNAA ratio in the control group. The relative reduction in group differences at the biochemical level is an important consideration in the interpretation of our negative results regarding serotonin's role with respect to sensitivity to reward information, although it does not affect our conclusion that serotonin's role may be greater for punishment information. Further studies would be strengthened by use of a control mixture containing tryptophan to avoid generation of a non-neutral control condition. In addition, interpretation of the genotype results in the current study might be limited by the potential implication of recent data suggesting that a triallelic model may apply to the 5-HTTLPR (Hu et al. 2006; Parsey et al. 2006). While it was not possible within the current design to apply this triallelic model, future studies using a triallelic model will be important for the future constraint in the interpretation of interactions of ATD and 5-HTTLPT genotype. Finally, while the sample size in the current study is comparable to that of other studies that have examined the effects of ATD and 5-HTTLPT genotype, the results of future studies will be important in further interpreting the results from these smaller sample size studies.

In summary, in this paper, we found that both ATD and LL homozygosity were associated with a reduced sensitivity to punishment-based information. Moreover, ATD and genotype interacted with ATD disproportionately affecting the performance of the LL homozygous group. These results suggest that serotonin is particularly associated with determining sensitivity to punishment, rather than reward information, and that individual susceptibility to tryptophan depletion varies according to the 5-HTTLPR polymorphism status. These results may help resolve how serotonin and genotype can interact in psychiatric conditions, and why, for some, it is possible to choose the better of two goods, but difficult to choose the lesser of two evils.

Acknowledgements This research was supported by the Intramural Research Program of the National Institutes of Health: National Institute of Mental Health.

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