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# Compromised decision-making and increased gambling proneness following dietary serotonin depletion in rats☆

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ABSTRACT

Psycho-genetic studies have revealed a role for the brain serotonin system in gambling proneness and poor decision-making. We assessed whether manipulation of brain serotonin levels in rats affected performance in operant-based tasks for decision-making and gambling proneness. Male Wistar rats were exposed to an L-tryptophan (TRP) deficient diet (0.0 g/kg; T− group) or to a control, L-tryptophan containing diet (2.8 g/kg; T+ group). The same rats were tested for decision-making performance in the rodent Iowa Gambling Task (rIGT) using home-cage operant panels, and subsequently for gambling proneness in a Probabilistic Delivery Task (rPDT) using classic Skinnerboxes. At sacrifice, monoamines and metabolites were evaluated with HPLC analysis, confirming a drastically reduced serotonin synthesis, as well as altered dopamine turnover in the prefrontal cortex of T− rats. As expected, control rats (T+) progressively chose the option with the best long-term payoff in the rIGT, and also shifted from "Large & Luck-Linked" (LLL) to "Small & Sure" (SS) reinforcers in the rPDT. In contrast, depleted animals (T−) exhibited a weaker improvement of performance in the rIGT and maintained a sub-optimal attraction for LLL reinforcer in the rPDT. Comparing individual performances in both tests, we found a significant correlation between the two tasks in control (T+) but not in depleted (T−) rats. The present study revealed that (1) brain 5-HT depletion leads to poor decision-making and to gambling proneness; (2) the relationship between these two traits, shown in the control group, was disrupted in 5-HT depleted rats. The data are discussed in terms of changes within forebrain loops involved in cognitive and motivational/affective processes.



## 1. Introduction

The rapid worldwide growth of legalised gambling opportunities has raised concerns over the impact of gambling and its consequences on public health (Carragher and McWilliams, 2011; Shaffer and Korn, 2002). Epidemiological data suggest that 27.1% of adult people gambled more than 100 times in their lifetime, whilst a 10.1% gambled more than 1000 times (Kessler et al., 2008). Although gambling may remain a recreational activity for some people, it may become an overt problem for others. Such problematic gambling behaviour may be maladaptive or pathological, and disrupt personal, family, professional or vocational pursuits (DSM-IV, A.P.A., 2000; Potenza, 2001). Problematic gambling behaviour is also associated with poor decision-making performance, as measured for instance by the Iowa Gambling Task (IGT; Brand et al., 2005; Cavedini et al., 2002; Goudriaan et al., 2005). The IGT measures decision-making processes by simulating real-life decisions involving reward, punishment, and uncertainty of outcomes (Bechara et al., 1994, 1999). In this task, poor decision-making performance is associated with a choice for long-term disadvantageous options. Here, we focus on the relationship between gambling proneness and (poor) decision-making, as measured by two rodent operant tasks exploiting reward uncertainty.

In general, the output of decision-making processes (i.e. which action is taken in the end), as well as the gambling temptations (caused by a lack of self-control abilities over impulsive attraction

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for "binging") are determined by an interaction between two different forebrain loops: a limbic (affective/motivational) loop, encompassing the prefrontal cortex (PFC) in its orbital sub-region (i.e. orbito-frontal cortex, OFC), the amygdala and ventral striatum, *versus* a cognitive (executive/motor) loop, encompassing the dorso-lateral prefrontal cortex (dlPFC) and dorsal striatum (Bechara, 2005; Doya, 2008; McClure et al., 2004; Tanaka et al., 2004, 2007; Canese et al., in press). These two loops exert different levels of control over decision-making behaviour. While the limbic loop is involved in immediate responding to (potential) rewards, losses or threats (i.e. impulsive behaviour) as well as in emotional control, the cognitive loop is more involved in long-term or future perspectives, i.e. in cognitive control (Bechara, 2005; Doya, 2008; McClure et al., 2004; Tanaka et al., 2004, 2007). Among others, serotonin (5-HT) plays a role in top-down control of behaviour, whereby these prefronto-cortical areas subserve inhibition of impulsive behavioural responses, through mediating the interplay between the limbic and cognitive loops (see reviews: Cools et al., 2011; Homberg, 2012; Rogers, 2011). For instance, low levels of 5-HT have been associated with poor decision-making and/or poor impulse control (Baldwin and Rudge, 1995; Daw et al., 2002; Doya, 2008; Homberg et al., 2008; Lucki, 1998; Owens and Nemeroff, 1994; Soubrié, 1986; Tanaka et al., 2007; Winstanley et al., 2004). Next to noradrenergic and dopaminergic dysfunction, serotonergic dysfunction has been reported as a key biological factor contributing to the pathophysiology of gambling proneness, which is characterized by a loss of impulse control (Ibanez et al., 2003; Pallanti et al., 2006, 2010). For instance, hypo-activity of the brain 5-HT system (Moreno et al., 2004) and low cerebrospinal-fluid levels of both L-tryptophan (TRP) and 5-HT have been found in pathological gamblers (Nordin and Sjodin, 2006). Furthermore, gambling proneness as well as poor decision-making in the IGT have been associated with the short (s/s) allele of the 5-HT transporter promoter length polymorphism (5-HTTLPR; da Rocha et al., 2008; He et al., 2010; Homberg et al., 2008; Ibanez et al., 2003; Must et al., 2007; Stoltenberg and Vandever, 2010; van den Bos et al., 2009). Given the considerations above, we experimentally manipulated the 5-HT brain availability, and investigated the consequences on decision-making and gambling proneness in rats.

Several methods exist to deplete central 5-HT function, such as 5-HT agonist and antagonist drugs (e.g. 8–OH–DPAT and WAY 100635; Mobini et al., 2000), lesions of the ascending serotonergic projection induced by intra-raphe injections of the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT; Fletcher et al., 2001) and systemic administration of the 5-HT synthesis inhibitor para-chlorophenyl-alanine (PCPA; Dringenberg et al., 1995; Fletcher et al., 2001). Another way of depleting central 5-HT is nutritional manipulation of TRP. Since brain 5-HT synthesis depends on the availability of its precursor, dietary TRP depletion is considered an effective method to substantially reduce plasma and cerebral TRP levels and consequently to reduce brain 5-HT synthesis (Biggio et al., 1974; Vergnes and Kempf, 1981). As acute L-tryptophan depletion (ATD) only leads to moderate, transient depletion of TRP levels in adult rats (Brown et al., 1998; Lieben et al., 2004), we applied long-term 5-HT depletion (Lee et al., 1999; Tanke et al., 2008; Uchida et al., 2005; Vergnes and Kempf, 1981) using a TRP-free diet, allowing us to investigate how hypo-activity of the 5-HT system affects decision-making and gambling proneness in rats.

Decision-making performance was assessed using a modified, automated version of a validated rodent version of the Iowa Gambling Task (rIGT, de Visser et al., 2011a; Homberg et al., 2008; van den Bos et al., 2006a): we developed an operant-based task using a modified home-cage operant panel (Koot et al., 2009, 2010). In this task, rats have to choose between a long-term advantageous option, containing a high probability of a small reward (two sugar pellets) and a low probability of punishments (two quinine pellets), *versus* a long-term disadvantageous option, containing a low probability of a large reward (four sugar pellets) and a high probability of punishments (four quinine pellets). After exploring the options, control rats normally develop a preference for the long-term advantageous one. Poor decision-making performance is thus characterized by a lack of developing this preference (de Visser et al., 2011a; Homberg et al., 2008; Koot et al., 2010; van den Bos et al., 2006a).

To assess gambling proneness in rats, we used the rodent Probabilistic Delivery Task (rPDT), recently developed from temporal-discounting operant tasks (Adriani and Laviola, 2006). In this task, rats learn to prefer a large (six pellets) over a small (two pellets) reward. Subsequently, the probability of occurrence of the large reward decreases progressively to very low levels (i.e. a risky choice). Control rats normally change their preference towards the certain ("Small & Sure", SS) reward, which is a "safer" option beyond the indifferent point (i.e. when the risky choice becomes mathematically disadvantageous in terms of total foraging; Adriani et al., in press). As such, gambling proneness in rats is associated with a maintained preference for the highly uncertain ("Large & Luck-Linked", LLL) reward, which becomes a sub-optimal option far beyond the indifferent point.

Depleted and control rats were tested in the rIGT and subsequently in the rPDT, allowing us to investigate (1) whether 5-HT depletion indeed disrupts rIGT performance and/or rPDT performance, and (2) whether poor decision-making in the rIGT is associated with gambling proneness in the rPDT, using correlational analysis.

## 2. Material and methods

All experimental procedures were approved by Institutional Animal Survey Board on behalf of the Italian Ministry of Health (licence to GL), and by the Animal Ethics Committee of Utrecht University. Procedures were in close agreement with the European Communities Council Directive (86/609/EEC) as well as with Italian and Dutch laws. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilise alternatives to *in vivo* techniques, if available.

### 2.1. Subjects

Twelve male adult Wistar rats (Charles River, Italy) were kept at the Istituto Superiore di Sanità (ISS, Rome, Italy) in an air-conditioned room (temperature 21 ± 1 °C) on a 12-h reversed light–dark cycle (lights off at 7:00 AM), where they were housed in pairs inside Makrolon III cages with sawdust bedding. Another twelve male adult Wistar rats (Harlan, The Netherlands) were kept in similar conditions at Utrecht University (UU, The Netherlands). The animals housed at UU were specifically intended to serve as an additional control group, in order to confirm the robustness of our novel operant version of rIGT. This is an adapted version of the validated maze-based protocol (de Visser et al., 2011a; Homberg et al., 2008; van den Bos et al., 2006a). Water was available *ad libitum*, whereas food (Rome: Altromin-R, A. Rieper S.p.A., Vandoies, Italy; Utrecht: 801730 CRM (E) Expanded, Special Diets Services, Witham, Essex, England) was available *ad libitum* unless stated otherwise.

After four weeks of habituation to the housing conditions and handling by the experimenters, rats were randomly assigned to one of two experimental groups: one group ($n = 6$ at ISS) received a TRP-free diet (T−), while the other group ($n = 6$ at ISS and $n = 12$ at UU) received a control diet (T+). The TRP-free diet (DP/1069 mod., A. Rieper S.p.A., Vandoies, Italy) had a standard nutritional value, but with the complete lack of TRP. The control groups (at ISS and UU) were fed a similar diet, containing a standard amount of TRP (2.8 g/kg diet). Rats were tested in an adjusted operant version of the rodent Iowa Gambling Task (rIGT) and subsequently in the rodent Probabilistic Delivery Task for gambling proneness (rPDT), followed by forebrain sample collection at sacrifice (see Fig. 1 for the entire experimental design). The rIGT test was presented first, involving a mild level of food restriction (95%), while the rPDT was presented afterwards, as a stronger food restriction was needed (85–90%). This order of testing was chosen since animals should proceed from less to more invasive behavioural tests, especially during dietary-manipulation periods (Zhang et al., 2006).

The present experiment exploited a nutritional-deprivation approach plus a food-restriction schedule, which were however devoid of overtly adverse

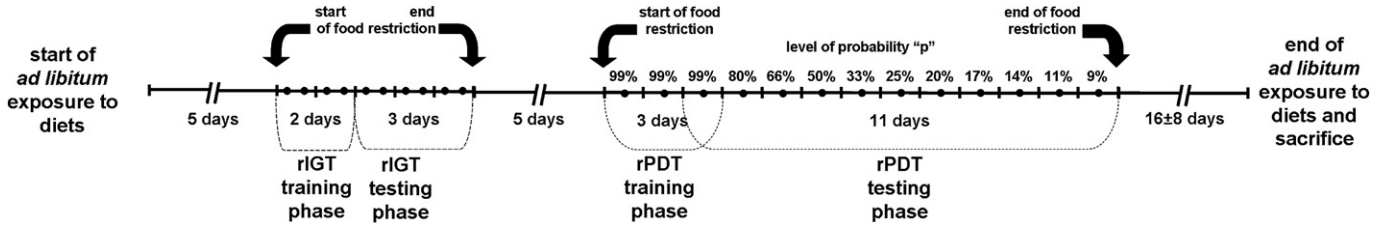


**Fig. 1.** Experimental layout.

consequences. Constant but informal observations of rats (in both the rIGT and rPDT apparatuses as well as when they returned to home-cages, until sacrifice) revealed no gross alterations in indexes of general welfare, nor emergence of stereotyped patterns of behaviour. Several experiments have indeed assessed parameters of behaviour in mice and rats consuming a TRP-free diet for one month or more (Vergnes and Kempf, 1981; Uchida et al., 2005; Zhang et al., 2006).

### 2.2. The rodent Iowa Gambling Task (rIGT) in the home-cage

#### 2.2.1. Apparatus

The operant-testing apparatus, consisting of a prototype computer-controlled panel (2f-HOP; PRS Italia, Rome, Italy), was placed in a Makrolon IV cage with sawdust bedding, in which rats were housed individually. The current 2f-HOP is an adjusted version of the apparatus which we used in an earlier study (Koot et al., 2009). The panel contained two nose-poking holes, hole lights, a chamber light, two feeder devices, a food magazine where sugar pellets (F0042) and quinine pellets (F06498, quinine 4.44 g/kg diet; 45 mg Dustless Precision Pellet; Bio-Serv, Frenchtown, NJ, USA) were delivered, a little trapdoor to remove uneaten pellets, and a magazine light. The panel was connected through an interface to a PC, where specific software (Ska020 3.2.3; PRS Italia, Rome, Italy) controlled and recorded all events. Four subjects were tested at the same time.

Nose-poking in the holes of the panel resulted in the delivery of sugar or quinine pellets (see below for ratio and amount). After nose-poking and before food delivery, the hole light was turned on for 1 s. Following food delivery, the magazine light was turned on for 15 s (timeout, TO), during which nose-poking was recorded but was without any scheduled consequence (i.e. inadequate nose-pokes). The trapdoor was opened 2 s before the end of the TO, to remove uneaten pellets from the food magazine. Then, the magazine light was turned off, the chamber light was turned on, and the system was ready for the next trial.

#### 2.2.2. Protocol rIGT

One week before the start of the training/testing protocol, rats were handled for 2 min daily, their body weight was taken, and they were familiarised with the sugar pellets (two pellets per animal per day) and T− or T+ diet respectively (4 g per animal per day) in the home-cage. Five days before the start of the training/testing protocol the standard food was removed and animals received *ad libitum* the T− or T+ diet.

On the morning of the first day of training, rats were placed individually in the testing home-cages, with water available *ad libitum* but without food, where they were left undisturbed for an hour before the first session started. Two sessions were run per day, which took place around 9:00 AM and 5:00 PM respectively (for arguments, see Koot et al., 2009). Before each session, rats received 0.5 g of T− or T+ diet respectively, and after each session they received the rest of their diet needed to maintain them on 95–98% of *ad libitum* body weight (determined before the first magazine training session). Daily after the AM-session, animals' body weight was taken. Rats were food-restricted from the first day of training throughout the entire rIGT protocol in order to increase their motivation to work for food delivery.

The training phase consisted of three 15 min-sessions of magazine training and one session of alternation training (i.e. two days in total). During the magazine training two sugar pellets were delivered automatically in the magazine with an interval of 60 s, i.e. a total of 15 trials. The trapdoor was opened to remove the uneaten pellets after an interval which was fixed for each session and progressively decreased (time to eat, TTE, 20–15–10 s for each magazine training session, respectively). Pilot experiments showed that these sessions were sufficient for rats to reliably learn to consume pellets within 10 s. During the magazine training, nose-poking holes were permanently covered by Plexiglas plates. During the entire experiment, the food magazine was covered by an aluminium plate which was only removed during sessions, to protect the underlying mechanics of the trapdoor against sawdust entering the food magazine.

Subsequently, rats were trained to nose-poke in both holes, to prevent the development of a bias for one hole (alternation training, 10 trials per hole). This was done by training rats to emit alternating responses: just one hole was active while the other was inactive, during alternate trials. Nose-poking in the active hole led to the delivery of the reinforcer, whilst nose-poking in the inactive hole was without any consequence. One session was sufficient for rats to learn to nose-poke equally in both holes.

After the training phase, the test phase started. The rIGT in the home-cage was based on the rIGT performed in a maze as described previously (de Visser et al., 2011a; Homberg et al., 2008; van den Bos et al., 2006a), modified to be adapted in the home-cage operant panels. Rats received two sessions per day, 40 trials per session for a total of 240 trials; thus this phase lasted 3 days. Each session started with switching on the chamber light accompanied by the free delivery of two sugar pellets. Nose-poking in either hole led to the delivery of reward and/or punishment, followed by the TO (15 s, including a TTE of 13 s before the uneaten pellets were removed). Rewards were represented by sugar pellets, punishments were represented by quinine pellets that were unpalatable but not uneatable. The (in the long run) disadvantageous hole presented occasionally large rewards (four sugar pellets, probability 30%) among series of quinine pellets (four, probability 70%), i.e. 12 sugar pellets per 10 trials. The (in the long run) advantageous hole presented regularly small rewards (two sugar pellets, probability 80%) among quinine pellets (two, probability 20%), i.e. 16 sugar pellets per 10 trials. The entire training and testing protocol lasted five days. After the last session, rats were housed again in their former pairs, and had *ad libitum* access to their diets (T− and T+ respectively) for five days until the rPDT protocol started.

### 2.3. The rodent Probabilistic Delivery Task (rPDT)

#### 2.3.1. Apparatus

Computer-controlled operant chambers (Coulbourn Instruments, Allentown, PA, USA) were placed in an experimental room, adjacent to the animal room. The operant chambers were provided with two nose-poking holes, two chamber lights, two feeder devices, two food magazines where control (F06555, T+; TRP 2.8 g/kg diet) and depleted pellets (F06554, T−; TRP 0.0 g/kg diet; Dustless Precision Pellet 45 mg, Bio-Serv, Frenchtown, NJ, USA) were delivered, each with a magazine light, and five infrared photobeams on the bottom of the chamber (spaced apart 3 cm) to measure locomotor activity. Nose-poking in either hole was detected by a photocell. A computer, with custom-made software, controlled and recorded all events.

#### 2.3.2. Protocol rPDT

Five days after the last rIGT session, the rPDT protocol started. Daily sessions lasted 25 min and were run between 11:00 AM and 3:00 PM, for which animals were transported to the experimental room and placed in the operant chambers. After each session, animals were returned to their home-cage, where they were given an appropriate amount of their diet (4.5 g each) previously titrated in order to complete their daily caloric intake. During daily sessions, rats were able to eat (on average) 9 g of precision pellets, i.e. about two thirds of their daily needs to maintain their body weight on 85–90% of their *ad libitum* body weight (determined before the first rPDT training session). Animals were food restricted in order to increase their motivation to work for food delivery. After the final rPDT session, rats had again *ad libitum* access to their diets (T− and T+) until sacrifice (on average 16 ± 8 days after the last rPDT session).

During the training phase (3 days) nose-poking in one of the two holes resulted in the delivery of two pellets, whereas nose-poking in the other hole resulted in the delivery of six pellets. After nose-poking and before food delivery, the chamber light above the nose-poked hole was turned on for 4 s. Following food delivery, the magazine light was turned on for 15 s (timeout, TO), during which nose-poking was recorded but was without scheduled consequences (i.e. inadequate nose-pokes). The magazine light was then turned off, and the system was ready for the next trial. The total number of completed trials and the inter-trial interval were not fixed, since rats were free to express nose-poking for food at their own, individually-variable rate during the 25 min session.

During the testing phase (10 days) a probabilistic dimension was associated with the delivery of the large reward. The chamber lights were switched on after nose-poking following the usual schedule. However, sometimes the delivery of a large reward was omitted, while the magazine light was still switched on for 15 s, according to a given level of probability ($p$ = percentage of actual food delivery over total demands). The small reward delivery was unchanged. Hence, animals had a choice between a "Large & Luck-Linked" (LLL) or a "Small & Sure" (SS) reward. The probability level was kept fixed for each daily session, and was decreased progressively over days (from $p$ = 99% to 80%, 66%, 50%, 33%, 25%, 20%, 17%, 14%, 11% and finally $p$ = 9%). One session was run for each $p$-level, which was changed daily. The indifferent point (at which either choice was mathematically identical in terms of

total foraging) was $p = 33\%$. We initially imposed a range of $p$-values before the indifferent point (99%, 80%, 66%, 50%) when LLL was always the optimal choice. Rats were then tested far beyond the indifferent point (25%, 20%, 17%, 14%, 11%, 9%) when LLL became a sub-optimal option.

### 2.4. Monoamines and their metabolites: HPLC determination

Rats were decapitated, their brains removed and rapidly dissected on ice to obtain the PFC, striatum, and hippocampus for HPLC analysis. We did not subdivide these areas into subnuclei, and thus the brain areas were collected as a whole. All samples were immediately flash frozen on dry ice, then stored at −80 °C until further processing. Brain samples of six T− and T+ rats were then analysed by determination of monoamines and their metabolites, according to previously published protocols (Cassano et al., 2009). In particular, each brain region was weighed and ultrasonicated in percloric acid (PCA) 0.1 M. Samples were centrifuged for 20 min at 15,000 g (4 °C), then the supernatant was used for monoamine and monoamine metabolite assays.

The endogenous levels of 5-HT and 5-HT metabolite (5-hydroxyindolacetic acid, 5-HIAA), of dopamine (DA) and final DA metabolite (homovanillic acid, HVA), of noradrenaline (NA) and NA metabolite (4-hydroxy-3-methoxyphenyl-glycol, MOPEG) were assayed by microbore HPLC using a SphereClone 150-mm × 2-mm column (3-μm packing). The detection was accomplished with a Unijet cell (BAS) with a 6-mm-diameter glassy carbon electrode at +650 mV *versus* an Ag/AgCl reference electrode, connected to an electrochemical amperometric detector (INTRO, Antec Leyden, The Netherlands). For each analysis, a set of standards containing various concentrations of each compound (monoamines and their metabolites) was prepared in the acid solution, and calibration curves were calculated by a linear regression. The retention times of calibration standards were used to identify peaks, and areas under each peak were used to quantify monoamine levels. Results were normalised to the weight of wet tissue.

### 2.5. Data analysis

Significance level was set at $p \leq 0.05$, ns = not significant; all statistics are two-tailed. Statistical analyses were performed using Statview II (Abacus Concepts, CA, USA), STATA Release 8.0 (Stata Corporation, College Station, TX, USA) and SPSS v16.0 for Windows (IBM corporation, Armonk, NY, USA). Data are expressed as mean ± SEM, unless otherwise indicated. Multiple comparisons were performed with the Tukey HSD Test.

#### 2.5.1. Home-cage rIGT experiment

Exclusion criteria were (1) rats that did not reliably eat the rewards, (2) rats that did not reliably nose-poke for pellet delivery, and (3) rats that consistently ate quinine pellets. The following variables were recorded automatically: adequate nose-poking (nose-pokes after a TO, resulting in the delivery of the pellets), inadequate nose-poking (nose-pokes during a TO interval, which were recorded but were without any consequences), and time needed to complete the session.

As measure of decision-making performance, the dependent variable was the choice preference (calculated as the percentage of choices at the [in the long run] disadvantageous hole for each block of 40 trials). As measures of (motor) impulsivity (Sagvolden, 2000; Sagvolden and Sergeant, 1998), the dependent variables were the average frequency of inadequate nose-pokes per trial, calculated for each block of 40 trials, as well as the time needed to complete a trial. For all parameters, repeated measure ANOVAs were performed. Within-subject factor was trial block; the between-subjects factors were location (when comparing UU and ISS controls) or treatment (when comparing T+ and T− groups on the whole experiment). As the two diets had differential effects on rats' body weight, to account for a possible bias due to gross differences, body weight changes were included as a covariate in the ANOVAs. When these covariates yielded an effect on the statistical analysis, a Pearson's correlational analysis was done to further reveal the biasing effect.

#### 2.5.2. Standard-Skinnerbox rPDT experiment

The inclusion criterion was defined as a preference for LLL of more than 60% at $p = 66\%$, and was applied independently from rIGT exclusion criteria. As measure of gambling proneness, the dependent variable was the choice preference (%) for the large-unlikely reward, i.e. the percentage of LLL over total choices expressed. As a measure of (motor) impulsivity (Sagvolden, 2000; Sagvolden and Sergeant, 1998), the dependent variable was the average frequency of inadequate nose-pokes per trial, calculated for each session, as well as the time needed to complete a trial. Locomotor activity inside operant chambers (measured as photobeam interruptions) was also measured to reveal general changes in motility. For all parameters, repeated measure ANOVAs were performed: day (i.e. session) was the within-subject factor; treatment was the between-subjects factor. As the two diets had differential effects on the rats' body weight, to account for a possible bias due to gross differences, body weight changes were included as a covariate in the ANOVAs. When these covariates yielded an effect on the statistical analysis, a Pearson's correlational analysis was done to further reveal the biasing effect.

#### 2.5.3. The relationship between rIGT and rPDT

The relationship between rIGT and rPDT was studied using correlational analysis. The last three sessions of both tasks were analysed, since they were characterized by similar conditions (stable performance in both experiments; probability values in the rIGT in the range of those in the rPDT). Moreover, these data are good indicators of decision-making performance (de Visser et al., 2011a) and gambling proneness (Adriani et al., 2009, 2010), respectively. Specifically, the individual values for choice preference (%) in the two tasks were correlated using Pearson linear correlation.

Four Pearson's $R$ values were obtained by comparing the last three experimental sessions from rPDT (taken individually or as mean) with the mean value of the second half (last three experimental sessions) from rIGT (see Table 3). A further approach was undertaken for the purpose of graphical representation (see Fig. 4). Each of the last three experimental sessions of the rPDT was combined with all the three rIGT sessions. We only showed those pairings of sessions that maximized the number of significant $R$ values, avoiding day repetitions. This was done within each treatment separately, to avoid a bias introduced by the treatment itself.

#### 2.5.4. Monoamines, monoamine metabolites and their ratios

HPLC data were analysed by a set of two-tailed unpaired Student's $t$-tests, to evaluate differences between experimental groups. The threshold for statistical significance was set at $p \leq 0.05$.

## 3. Results

### 3.1. rIGT

The control curves on decision-making performance were obtained from two independent locations: the labs and animal facilities at ISS and UU. One T+ subject at ISS and four T+ subjects at UU were excluded from further rIGT testing as they did not reliably nose-poke for pellet delivery. To assess the robustness of the rIGT protocol and whether the data of the control rats of ISS and UU could be pooled, these two locations were compared (see Table 1). Rats shifted their choice behaviour (across trial blocks) towards the long-term advantageous option, in an indistinguishable way (trial block × location: $F(5,55) = 0.568$, ns; location: $F(1,11) = 0.259$, ns). Therefore, results indicate that the rIGT protocol is robust and fully replicable across laboratories, allowing us to collapse these two locations into a single control group. Hence, data of six T− and thirteen T+ rats ($n = 5$ at ISS; $n = 8$ at UU) were analysed.

The day before the start of the special diets, no difference was found in body weight between the groups (T+ rats, $n = 13$: 377.6 ± 7.5 g *versus* T− rats, $n = 6$: 367.9 ± 8.4 g; Student's $t = -0.776$, df = 17, ns). After five days of *ad libitum* special diet, T+ rats (390.6 ± 6.8 g; 103.5 ± 0.6% relative to the start of diets) were heavier than T− rats (341.2 ± 8.4 g; 92.8 ± 1.5% relative to the start of diets; Student's $t = -4.295$ (absolute values) and −7.809 (relative values), df = 17, $p < 0.001$). However, when the experiment-induced decrease was calculated on the last day of the rIGT (relative to the beginning of rIGT), no difference was found between the groups: the food restriction-induced body weight decrease (i.e. restriction level) was similar in T+ and T− rats across the five-day rIGT (T+ rats: 95.1 ± 0.3% *versus* T− rats: 96.0 ± 0.5% at the end of rIGT relative to the beginning; Student's $t = 1.474$, df = 17, ns).

**Table 1**
Performance of control rats at ISS ($n = 5$) and UU ($n = 8$) as tested in the home-cage rIGT. Shown are the mean (±SEM) proportions (%) of "bad" choices (for the disadvantageous hole) per block of 40 trials.

| Trial blocks | 1–40 | 41–80 | 81–120 | 121–160 | 161–200 | 201–240 |
|---|---|---|---|---|---|---|
| T + ISS (control) | 47.00 ± 7.64 | 29.50 ± 5.39 | 32.00 ± 12.58 | 17.50 ± 5.18 | 15.00 ± 5.48 | 13.13 ± 2.89 |
| T + UU (control) | 35.94 ± 6.70 | 28.13 ± 3.77 | 27.19 ± 4.05 | 20.31 ± 5.38 | 11.25 ± 3.63 | 14.69 ± 3.55 |

**Table 2**
Locomotor activity inside the operant chambers during the last three sessions of rPDT. Shown are the mean ± SEM amount of photobeam interruptions per session. Rats are the same as in Fig. 3.

| Probability level | $p = 14\%$ | $p = 11\%$ | $p = 9\%$ |
|---|---|---|---|
| T+ | 50.82 ± 5.19 | 58.54 ± 5.63 | 64.45 ± 6.89 |
| T− | 65.11 ± 4.68 | 53.00 ± 4.52 | 61.44 ± 6.82 |

Hence, the absolute and relative weights of rats differed at the end of the rIGT: T+ rats (371.5 ± 6.1 g, 98.5 ± 0.6% relative to the start of diets) were heavier than T− rats (327.6 ± 7.9 g; 89.1 ± 1.4% relative to the start of diets; Student's $t = -4.169$ (absolute values) and −7.051 (relative values), df = 17, $p < 0.001$).

Independent of treatment, all subjects improved their performance over trials (see Fig. 2; trial block: $F(5,85) = 9.344$, $p < 0.001$; trial block × treatment: $F(5,85) = 0.919$, ns). However, visual inspection of data strongly suggests that T− rats showed an overall weaker improvement across trials than T+ rats. Indeed, a near significant (treatment: $F(1,17) = 3.828$, $p = 0.067$) difference emerged. When considering the two halves of the rIGT ("exploration *versus* exploitation"; de Visser et al., 2011a) separately, clearly the difference between the depleted and the control groups was significant in the second half of the test, i.e. the last three sessions of the rIGT (treatment: $F(1,17) = 5.143$, $p < 0.05$). Accordingly, 5-HT depletion leads to a poor performance in the rIGT, specifically during the second half of the test ("exploitation" phase).

To account for body weight differences, we included the rIGT-beginning and rIGT-end body weights (%, relative to *ad libitum* weight at start of the special diets) as covariates in the repeated measure ANOVA. It turned out that the treatment effect (without covariates: $p = 0.067$, see above) became significant, $F(1,15) = 4.862$, $p < 0.05$, indicating that relative body weight differences masked, but only slightly so, the observed test effect (main effect of rIGT-beginning relative weight covariate: $F(1,15) = 1.407$, ns; main effect of rIGT-end relative weight covariate: $F(1,15) = 0.351$, ns). No significant correlations were observed between the choice scores at each trial block and the relative body weight at the start or the end of the rIGT ($R$ values between +0.112 and −0.383, $n = 19$, ns). Therefore, covariate and correlation approaches indicate that relative body weight differences if anything slightly masked behavioural differences.

Neither group showed a change across trials (trial block: $F(5,9) = 0.581$, ns; trial block × treatment: $F(5,45) = 1.281$, ns): T− rats showed more inadequate nose-pokes per trial (frequency: 3.18 ± 0.26) than T+ rats (1.80 ± 0.19; treatment: $F(1,9) = 7.071$, $p < 0.05$) throughout the experiment.

While T+ rats decreased time needed to complete their trials across the sessions, T− rats showed the same score across trials, as per a floor effect (trial block × treatment: $F(5,45) = 4.244$, $p < 0.01$; data not shown). In fact, T− rats consistently needed less time to complete their trials (minutes needed per trial: 0.34 ± 0.01) than T+ rats (0.55 ± 0.04; treatment: $F(1,9) = 49.56$, $p < 0.001$).

### 3.2. rPDT

Before the rPDT started, all animals were weighed. On this baseline day, T− rats showed a markedly lower body weight compared to T+ controls (T+: 381.3 ± 9.5 g, 104.3 ± 1.9% relative to start of diets; T−: 326.8 ± 11.5 g, 89.3 ± 1.2% relative to start of diets; treatment: $F(1,18) = 13.61$, $p < 0.01$). However, when the task-induced decrease was calculated as percentage of the *ad libitum* baseline, taken just before the start of the rPDT, food restriction had a similar impact on body weight in either group (treatment: $F(1,18) = 0.02$, ns; day × treatment: $F(6,108) = 1.39$, ns). On average, the restriction level maintained during the rPDT was similar in T+ and T− rats (T+: 90.8 ± 1.0%; T−: 91.2 ± 1.0%; pool: 91.0 ± 0.7% of the *ad libitum* body weight, taken just before the start of rPDT). Hence, the weight of rats still differed at the end of the rPDT, with T+ rats (325.9 ± 13.4 g, 88.6 ± 2.4% relative to the start of diets) being heavier than T− rats (280.4 ± 5.3 g; 76.2 ± 1.5% relative to the start of diets; treatment: $F(1,18) = 48.19$, $p < 0.001$).

In contrast to T+ rats, T− rats showed only a slight reduction in their LLL preference, when the large reward decreased in probability. Specifically, a clear preference for SS was not developed by the T− group, even beyond the indifferent point (see Fig. 3; treatment: $F(1,18) = 0.39$, ns; day × treatment: $F(10,180) = 5.72$, $p < 0.001$). At the lowest probability values, LLL choices were significantly higher in T− rats compared to T+ animals. Tukey post-hoc showed a significant difference between groups on $p = 80\%$, 14%, 11% and 9%, i.e. specifically during the final "gambling" part of the test. A separate ANOVA performed on the last three task sessions yielded a nearly significant treatment effect ($F(1,18) = 4.25$, $p = 0.054$).

To account for body weight differences, we included as covariates the rPDT-mean weight (for the main effect of treatment) and the weights of each rPDT session (for the repeated measures factor). This ANCOVA revealed a completely similar profile (treatment: $F(1,17) = 0.88$, ns; day × treatment: $F(10,179) = 5.97$, $p < 0.001$; main effect covariate: $F(1,17) = 0.99$, ns; repeated measures covariate: $F(1,179) = 2.22$, ns), indicating that body weight differences between T+ and T− rats did not contribute to the observed effect. No significant correlations were observed between subject's choice scores at each session and the corresponding body weight at each session ($R$ values between −0.400 and +0.347, $n = 20$, ns), with the only exception of the second testing session ($p = 80\%$). For this session, in which the decrease of probability was first introduced, a positive correlation was found ($R$-value = +0.528, $p < 0.05$). This significant correlation is in agreement with the significant group difference, revealed indeed at this session through Tukey post-hoc (see above). Therefore, covariate and correlational approaches confirm that body weight differences did not contribute at all to the behavioural profiles observed.

While T+ rats showed, with decreasing levels of probability, a progressive increase in the total number of inadequate nose-pokes performed per trial, T− rats did not (data not shown). This was reflected by a near significant interaction term

**Table 3**
R values of correlations between performance within each treatment group: for the rPDT, choice preference (%) for the LLL hole in each of the last three daily sessions, and their mean; for the rIGT, mean choice preference (%) for the disadvantageous hole calculated on the task's second half ("exploitation", last three experimental sessions). Threshold $R$ value = 0.878 ($n = 5$).

| | | | Choice (%) for LLL hole in rPDT | | | |
|---|---|---|---|---|---|---|
| | | | $p = 14\%$ | $p = 11\%$ | $p = 9\%$ | Mean |
| Choice (%) for disadvantageous hole in rIGT | Mean of the second half | T+ | 0.397 | 0.742 | 0.755 | 0.702 |
| | | T− | −0.174 | −0.237 | −0.311 | −0.238 |

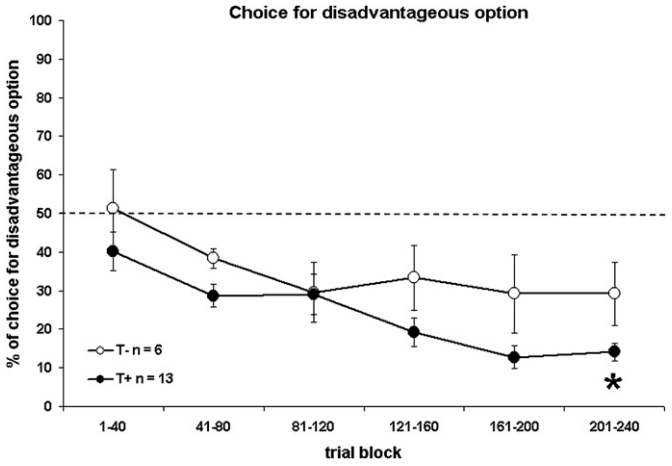


**Fig. 2.** Performance of depleted (T−) and control (T+) rats, as tested at ISS (Rome, Italy) and at UU (Utrecht, NL), in the home-cage rIGT. Shown are the mean (±SEM) proportions of "bad" choices (for the disadvantageous hole) per block of 40 trials. Rats could choose between two food pellets (the advantageous option: quinine at low probability, sugar at high probability) or four food pellets (the disadvantageous option: sugar at low probability, quinine at high probability). The test was conducted in the rats' home-cages, with operant panels provided inside. $^{*}p < 0.05$ Student *t*-test; depleted *versus* control rats.

(day × treatment: $F(10,180) = 1.87$, $p = 0.052$). Overall, however, T− rats (frequency: 1.12 ± 0.04) did not differ from T+ rats (1.10 ± 0.03; treatment: $F(1,18) = 0.05$, ns) in the total number of inadequate nose-pokes performed per trial.

T+ rats exhibited progressive reductions in time needed to complete their trials across daily sessions, whereas T− rats barely did so because of a floor effect (day × treatment: $F(10,180) = 5.29$, $p < 0.001$; data not shown). Moreover T− rats consistently needed (on average) less time to complete their trials (seconds needed per trial: 30.43 ± 0.49) than T+ rats (34.76 ± 0.90; treatment: $F(1,18) = 10.49$, $p < 0.01$), especially at the beginning of the task. Indeed, post-hoc comparisons evidenced a significant difference between T+ and T− on $p = 99\%$, 80%, 66% and 33%. In contrast, no significant difference was found between animal groups when considering the post-hoc comparisons performed on the last three task's sessions (data not shown).

No difference was observed between T− and T+ rats throughout the entire experiment for locomotor activity inside the operant

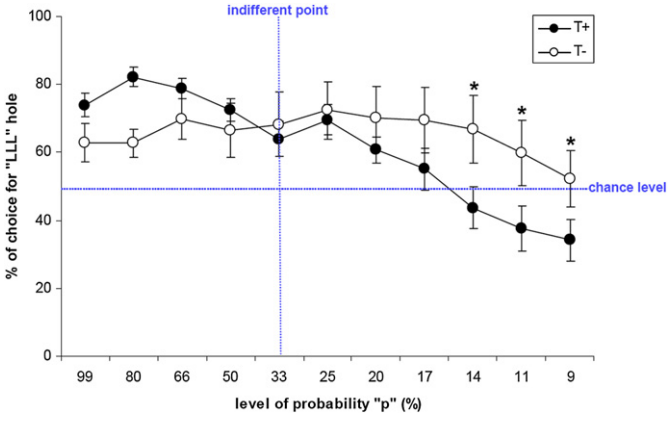


**Fig. 3.** Performance of control and depleted rats as tested in the rPDT. Shown are the mean (±SEM) proportions of "risky" choices (for the LLL hole) per daily session. Rats could choose between two food pellets (small and sure, SS) or six uncertain food pellets (large and luck-linked, LLL). The test was conducted in classical operant (i.e. standard Skinnerbox) cages, with subjects daily transported from the housing room. Tukey HSD post-hocs: $^{*}p < 0.05$; depleted *versus* control rats.

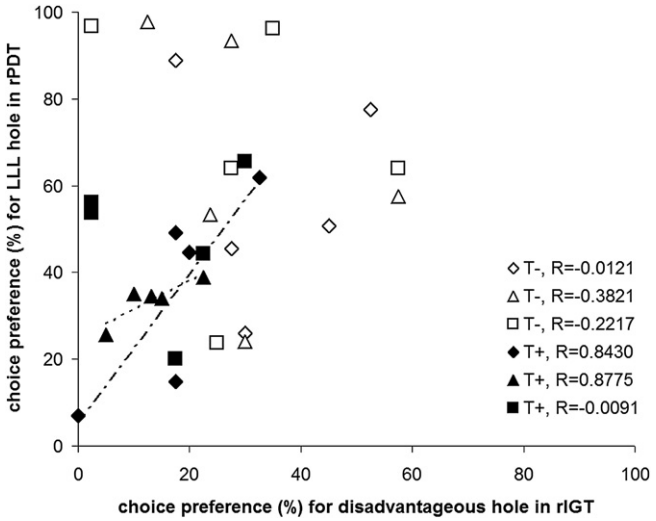


**Fig. 4.** In the cloud configuration, each individual rat of the depleted (T−, white symbols) and the control (T+, black symbols) group is represented by three symbols at the following coordinates: the parameter from the rIGT on *X*-axis and the parameter from the rPDT on *Y*-axis. The different shapes of the symbols (rhomb, triangle, square) identify the three selected comparisons, between pairs of sessions, ordered based on decreasing *R* values. The square refers to the third last ($p = 14\%$), the triangle to the second last ($p = 11\%$) and the rhomb to the last session ($p = 9\%$) of the rPDT, for which the specific level of reward uncertainty is of particular relevance. The dotted lines, delineate significant correlations (within black triangles and within black rhombs) in the linear regression between rIGT and rPDT.

chamber (treatment: $F(1,18) = 0.99$, ns; day × treatment: $F(10,180) = 1.38$, ns). Interestingly, a gradual increase of locomotor activity was observed over daily sessions, but similarly for both groups (day: $F(10,180) = 11.27$, $p < 0.001$). Noteworthy, the 5-HT depletion did not affect locomotion during the last three task sessions (see Table 2).

### 3.3. Relationship between rIGT and rPDT performance

In the rIGT, the probability level was kept fixed throughout the experiment, while it was decreased progressively over daily sessions in the rPDT. Therefore, performance during the last three sessions of the rIGT was represented by the mean value, while the use of individual values from each session was more appropriate in the case of rPDT. A clear difference emerged between T+ and T− rats. A strong tendency for positive correlations emerged in T+ rats (see Table 3): slightly poorer decision-makers were likely to be more prone to gambling-like behaviour. In contrast, no correlations were found within the T− group.

This result is also supported by a scatter plot of the last three task sessions, showing the correlations of individual performance parameters (within each treatment): the choice preference (%) for the disadvantageous hole in rIGT and for the LLL hole in rPDT (see Fig. 4). For T+ rats, the relative choice for the disadvantageous hole in the rIGT was directly proportional to the relative choice for the LLL hole in the rPDT, that is, subjects behaving as good decision-makers in the rIGT were scoring lower for gambling proneness in the rPDT. In contrast, no correlations were found in the T− group. These data are suggesting a dissociation between decision-making and gambling proneness, at least when these emerge prominently as a consequence of 5-HT depletion in T− individuals.

### 3.4. Monoamine concentrations in forebrain areas

HPLC data (see Table 4) demonstrated that dietary TRP depletion drastically reduced brain 5-HT synthesis, having marked

consequences on levels of 5-HT and 5-HIAA in all brain areas analysed. Indeed, decreased 5-HT levels were observed in the PFC ($t = 6.894$, df = 9, $p < 0.001$, $n = 5$–6), striatum ($t = 3.957$, df = 9, $p < 0.01$, $n = 5$–6) and hippocampus ($t = 3.000$, df = 10, $p < 0.05$, $n = 6$) of T− rats compared to T+ animals. A comparable effect in the same direction was found on levels of the 5-HT metabolite 5-HIAA in the PFC ($t = 11.90$, df = 10, $p < 0.001$, $n = 6$), striatum ($t = 3.799$, df = 9, $p < 0.01$, $n = 5$–6), and hippocampus ($t = 6.981$, df = 9, $p < 0.001$, $n = 5$–6). The 5-HT turnover (calculated as the 5-HIAA/5-HT ratio) was significantly different only in the PFC ($t = 7.022$, df = 9, $p < 0.001$, $n = 5$–6), whereas no significant changes were observed in the striatum ($t = 1.409$, df = 8, ns, $n = 4$–6) and hippocampus ($t = 0.6933$, df = 9, ns, $n = 5$–6).

No significant effect on dopaminergic and noradrenergic parameters was present in the striatum or hippocampus (data not shown). Moreover, no significant difference was observed in DOPAC concentrations detected in the PFC ($t = 1.505$, df = 10, ns, $n = 6$) (Table 4), the striatum ($t = 1.710$, df = 10, ns, $n = 6$), or the hippocampus ($t = 0.4423$, df = 10, ns, $n = 6$) (data not shown). However, in the PFC, a trend towards a decrease of DOPAC was observed (−53% in T− compared to T+ group).

In the PFC of T− rats (see Table 4), both the levels of HVA and the DA turnover (calculated as the HVA/DA ratio) were significantly decreased ($t = 11.93$, df = 9, $p < 0.001$, $n = 5$–6; $t = 2.684$, df = 9, $p < 0.05$, $n = 5$–6, respectively), in the absence of significant alterations for levels of DA ($t = 0.8576$, df = 10, ns, $n = 6$). Furthermore, in the PFC of T− rats, the level of NA was significantly decreased ($t = 2.309$, df = 10, $p < 0.05$, $n = 6$), in the absence of significant alterations for levels of MOPEG and NA turnover ($t = 1.315$, df = 10, ns, $n = 6$; $t = 0.5875$, df = 10, ns, $n = 6$, respectively).

## 4. Discussion

The present study confirmed that serotonergic transmission was changed in PFC, hippocampus and striatum of TRP depleted subjects (Lee et al., 1999; Tanke et al., 2008; Uchida et al., 2005; Vergnes and Kempf, 1981), and revealed (1) that dietary 5-HT depletion in rats leads to both poor decision-making and gambling proneness; (2) that poor decision-making is related to gambling proneness in control but not in 5-HT depleted rats.

### 4.1. rIGT

Reduced brain 5-HT synthesis, induced by dietary TRP depletion, disrupted rIGT performance in the home-cage setting, in that it led to a weaker improvement of decision-making performance over subsequent trials. Furthermore, it led to higher levels of motor impulsivity, as measured by inadequate nose-pokes and time needed per trial. The comparison of control groups run in Rome (ISS) and in Utrecht (UU) confirmed the robustness and replicability of the rIGT. These data also indicate that the home-cage operant task, which has been developed on the basis of a validated rIGT protocol (de Visser et al., 2011a; Homberg et al., 2008; van den Bos et al., 2006a), is well suited to study the decision-making performance in rats (Koot et al., 2010). Indeed, such home-cage panels are in general well-suited tools to measure operant behaviour-based performance (see also Koot et al., 2009).

In the first half of the IGT, subjects explore the available options, while in the second half of the task they switch to the option(s) which are advantageous in the long run. It has been hypothesized that the cognitive system, in which 5-HT plays a major role for the control of behaviour, is mainly activated during the second half of the task (de Visser et al., 2011a,b; Homberg et al., 2008; van den Bos et al., 2006b, 2007). Recently, it was shown that the reduced rIGT performance of female rats, compared to male rats (van den Bos et al., 2006a, 2007), could be enhanced by increasing 5-HT levels through genetic engineering, i.e. in serotonin transporter knock-out rats (SERT KO; Homberg et al., 2008). Although data in the SERT KO rats may also be explained by 5-HT induced neuro-developmental changes (Homberg et al., 2008), the fact that enhanced performance was particularly expressed in the second half of the task may suggest a specific contribution of increased 5-HT levels. In line with the idea that 5-HT levels *per se* do matter, we show here that decreasing 5-HT levels in male rats led to exactly the opposite: a poorer performance in the second half of the task. Furthermore, after administering 8−OH−DPAT (a mixed 5-$HT_{1A/7}$ receptor agonist), hence decreasing 5-HT release and acutely replicating a low 5-HT function, the subjects' performance was impaired in an another IGT-like rat gambling task (where the duration of time-out periods was used as punishment, Zeeb et al., 2009). These data in rats collectively indicate that 5-HT levels do modulate decision-making performance as measured in IGT-like tasks (see de Visser et al., 2011b, for review).

Data from human studies are in agreement with the present preclinical findings, in that differences in 5-HT transmission affect IGT performance. Healthy subjects being homozygous for the 5-HT transporter short allele (s/s) performed worse on the IGT than l/l carriers (He et al., 2010; Homberg et al., 2008; Stoltenberg and Vandever, 2010). The same was found in clinical patients with depression (Must et al., 2007), obsessive-compulsive disorder (da Rocha et al., 2008), borderline personality disorder (Maurex et al., 2009), especially in case of a TPH-1 (tryptophan hydroxylase-1, the rate-limiting enzyme in 5-HT bio-synthesis) haplotype gene (Maurex et al., 2009).

Our data on motor impulsivity are in line with numerous studies that support the idea of an inverse relationship between 5-HT function and impulsivity, as was shown not only through a variety of human disorders, but also in a series of animal studies (see Bizot et al., 1999; Clarke et al., 2004; Evenden, 1999; Leyton et al., 2001; Rogers et al., 1999; Soubrié, 1986). Cools et al. (2011) have pointed out that manipulating 5-HT levels seems to affect performance only on impulsivity tasks that have a clear affective (reward *versus* punishment) component (e.g. reversal learning, conditioned suppression, premature responding and inter-temporal choice), while the performance on tasks without a clear affective component (e.g. the stop-signal reaction-time task and the go-nogo task) was not affected by 5-HT manipulation. The rIGT clearly belongs to the first category.

### 4.2. rPDT

Our results demonstrate that a reduced brain serotonergic function strongly elicits a trait of gambling proneness, as evidenced in the rPDT. At the lowest probability values, far beyond the indifferent point, depleted rats showed only a slight reduction in their

**Table 4**
Levels of monoamines and their metabolites as detected ex-vivo in the PFC (mean ± SEM; pg/mg of wet tissue). $^{*}p < 0.05$, $^{***}p < 0.001$ between T− and T+ rats ($n = 5$–6).

| | 5-HT | 5-HIAA | 5-HIAA/5-HT | DA | DOPAC | HVA | HVA/DA | NA | MOPEG | MOPEG/NA |
|---|---|---|---|---|---|---|---|---|---|---|
| T+ | 23.3 ± 1.4 | 271.9 ± 16 | 11.6 ± 0.6 | 16.9 ± 2.6 | 1.8 ± 0.6 | 31.2 ± 2.8 | 2.6 ± 1.0 | 172.8 ± 3.9 | 47.8 ± 1.8 | 0.28 ± 0.01 |
| T− | 9.0 ± 1.5*** | 52.9 ± 9.1*** | 5.9 ± 0.5*** | 19.3 ± 1.1ns | 0.8 ± 0.3ns | 1.2 ± 0.4*** | 0.06 ± 0.02* | 153.1 ± 7.6* | 44.4 ± 1.9ns | 0.30 ± 0.03ns |

LLL preference: these rats were not only tolerant to reward uncertainty, but also sub-optimally attracted by its random-and-binge delivery.

Although brain 5-HT depletion is known to affect processing of delayed reinforcement (temporal-discounting), so far it does not appear to consistently influence choices involving uncertain reinforcement (probabilistic discounting; Cardinal, 2006; Mendelsohn et al., 2009). Indeed, experimental evidence demonstrating a link between 5-HT and risky decision-making is rather contradictive, in part due to the lack of a good animal model (Long et al., 2009). Similarly, a study performed in rats did not seem to support a role for central 5-HT in sensitivity to probabilistic reinforcement (Mobini et al., 2000). However, other experimental studies in both humans, monkeys and rats have demonstrated the opposite (Long et al., 2009; Murphy et al., 2009; Rogers et al., 1999; Zeeb et al., 2009).

Recently, Mendelsohn et al. (2009) have reviewed the existing literature on the effects of acute L-tryptophan depletion (ATD) on memory, attention and executive functions in humans. Distinctively, decreasing central 5-HT by means of ATD can both improve (Rogers et al., 2003; Talbot et al., 2006) and decrease (Rogers et al., 1999), or even has no effect on (Anderson et al., 2003) probabilistic discounting. ATD may subtly alter the ability to discriminate differences in the magnitude of rewards, but not of losses (Rogers et al., 2003). More recently, other authors concluded that 5-HT activity plays a significant role in irrational, risky decision-making under conditions of uncertainty. Indeed, it has been found that the subjective value of the risky option increased (or the subjective value of the safe option decreased) following serotonin depletion (Long et al., 2009). The role of 5-HT in decision-making (under conditions of reward uncertainty) can also be investigated by means of dietary TRP supplementation instead of depletion. Such supplements were associated with alterations in the impact of gains and losses and with a marked unbalance between risk-averse and risk-seeking choices (Murphy et al., 2009).

In line with findings in the rIGT, 5-HT depleted rats needed less time per trial than control rats at the beginning of the rPDT, i.e. before the indifferent point. They also tended to show slightly more inadequate nose-pokes. Apparently, 5-HT depletion leads to motor impulsivity and quick choice selection in both tasks. However, in the rPDT beyond the indifferent point (i.e. in the final "gambling" part), 5-HT depleted and control rats did not differ in time needed to complete a trial. Furthermore, when considering only the SS hole (data not shown), the number of inadequate nose-pokes increased more in controls than in the 5-HT depleted rats with progressive reward rarefaction, suggesting that the former may have been disturbed by punishment (consisting in reward-delivery omission) while the latter did not. Finally, locomotor activity increased over sessions, which could be interpreted as a reaction to the progressive rarefaction of rewards in both groups.

### 4.3. Roles of serotonin and PFC in decision-making/gambling proneness

The notion that 5-HT depletion modulates both decision-making and gambling proneness may be related to reward sensitivity (Hayes and Greenshaw, 2011), punishment sensitivity (Cools et al., 2008, 2011) or behavioural flexibility, and points to the interplay among different cortico-basal ganglia loops (Bechara, 2005; Doya, 2008; Homberg, 2012; Tanaka et al., 2004, 2007, 2009).

Serotonin plays an important role in the regulation of reward-related processing, as it is involved in natural reward-related physiology and behaviour, from feeding and sexual activity to emotional regulation. Altered reward processing has been proposed in many psychiatric disorders (see for review Hayes and Greenshaw, 2011). In the present study, data obtained in the rIGT suggest that the reward-system might have been affected by TRP depletion. However, this does not appear the case for the performance in the rPDT, as no significant difference between T+ and T− rats was observed in learning curves during the training phase and the initial testing phase when uncertainty level was low (see Fig. 3, sessions until $p = 33\%$). That is, both control and depleted rats were perfectly able to differentiate large from small rewards. Accordingly, we conclude that differences in reward sensitivity *per se* do not seem to account for the differences between 5-HT depleted and control rats in rIGT and rPDT performance.

Serotonin plays also a role in the perception of punishment and in negative reinforcement, as depletion of 5-HT has been suggested to enhance (see Cools et al., 2008, 2011) and/or to decrease (Graeff, 2002; Soubrié, 1986; see also Crockett et al., 2009) the behavioural and brain responsiveness to punishment. According to the first suggestion, a reduction of 5-HT levels could enhance aversive processing (Cools et al., 2008, 2011). However, in both rIGT and rPDT the 5-HT depleted rats choose for the option with the highest punishments, i.e. a high frequency of quinine pellets' presentation and of reward omission, respectively. Apparently, 5-HT depletion leads to a lower sensitivity to punishments, as indicated above. Alternatively, or in addition to such punishment insensitivity, it is well known that prefrontal 5-HT mediates attention to environmental stimuli arriving as outcome of actions (i.e. reward, but also punishments, Branchi, 2011; Homberg, 2012). It may be proposed that 5-HT depleted rats have a lower capacity to notice the outcome of their actions in both tasks, and therefore do not adapt their behaviour. Finally, another way to explain the observed differences might be that depleted rats are inflexible or rigid in their responding. That is, while the behaviour of control rats shifts under the guide of a cognitive/motor loop, which allows them to develop and maintain a long-term strategy (Tanaka et al., 2004, 2007, 2009; see next section), depleted rats remain driven by the limbic loop, which is actually rendered less sensitive to changes in reward-punishment contingencies.

Chronic dietary TRP depletion led to extremely reduced levels of HVA, and impaired DA turnover in the PFC. An effect on DA and/or its metabolites after 5-HT manipulation was expected, because of the well known influence exerted by 5-HT upon central dopaminergic systems (see for review Di Giovanni et al., 2010). Indeed, it has been suggested that changes in the relative levels of these two neurotransmitters could be one of the crucial factors in ADHD (Oades, 2002). The present strong reduction of DA turnover may suggest a compromised COMT-mediated catabolism, which is the major metabolic fate of prefrontal DA (Morón et al., 2002). Adequate decision-making requires the integrity of the anterior cingulate cortex (ACC) and the OFC (Cohen et al., 2005), as well as associated subcortical circuitry including the basal ganglia (Krawczyk, 2002) and amygdala (Bechara et al., 2003). A deficit in the OFC may be particularly critical in decisions involving reward and punishment, including gambling (Rogers et al., 1999). Patients with OFC lesions, but not patients with other prefrontal lesions, showed impaired decision-making in a decision-gamble task. Therefore, altered prefrontal 5-HT together with compromised DA transmission may have contributed to the behavioural performance observed for depleted rats (for a review, see Rogers, 2011).

Chronic dietary TRP depletion also reduced the levels of NA in the PFC. Relatively little is known, so far, about the role of NA in probabilistic reinforcement (Cardinal, 2006). It has been suggested that NA neurons encode some aspects of uncertainty in the general process of making predictions in a given context (Yu and Dayan, 2005). Systemic NA blockade has been shown to affect decision-making under uncertainty in humans, by reducing the discrimination between magnitudes of different losses (Rogers et al., 2004).

Conversely, NA re-uptake inhibition had no effect in the human IGT (O'Carroll and Papps, 2003). In our hands, reduced NA in the PFC of T− rats may hence favour insensitivity to reward omission.

### 4.4. Relationship between rIGT and rPDT performance

Significant correlations between the performance parameters of the rIGT and rPDT were found within controls but not within the 5-HT depleted rats. The fact that we did not observe a correlation between these two traits in depleted rats may suggest that poor decision-making does not necessarily predict gambling proneness. That is, in control rats, lower choice values in the rIGT, identifying good decision-makers, were associated with lower choice values in the rPDT, identifying risk-averse subjects. When 5-HT systems were intact, these two traits did correlate, while this link disappeared when rats were depleted of 5-HT. This may indicate that a dissociation between decision-making and risk-proneness can only be unmasked by altered function of the 5-HT system. As such, poor decision-making emerges in some individuals whereas a risk-prone trait emerges in other individuals, only when experimental subjects suffer from 5-HT depletion.

There are three types of choosing under uncertainty, distinguished by the degree of awareness about probabilistic rules and choice outcome. Decisions "under ambiguity" are those with unknown rules, corresponding to the early (training) part of both the rIGT and rPDT (Stoltenberg and Vandever, 2010). When the contingencies have been learnt, decisions under risk imply a possible detrimental impact on outcome, corresponding to the last sessions of both tasks. We underline that actual details of task options can be designed to produce low *versus* high levels of risk, as we did presently by using rIGT and rPDT, respectively. Decision-making under low *versus* high risk may involve dissociable brain 5-HT sub-circuits: choosing under low risk may involve a cognitive loop (Brand et al., 2006), whilst we proposed that high-risk options may trigger a "temptation" (Adriani and Laviola, 2006) and therefore involve a limbic loop of motivational/affective processing.

Tanaka et al. (2004, 2007) suggested a functional shift in cortico-basal ganglia loops, from predicting immediate outcomes towards predicting long-term or future outcomes, in subjects performing a decision-making task: activity of the lateral OFC - ventral striatum ("limbic") loop was correlated with reward prediction in shorter time scales, mainly at low 5-HT levels, while activity of the dorsolateral prefrontal cortex (dlPFC) – dorsal striatum ("cognitive/motor") loop correlated with longer time scale predictions, being stronger at high 5-HT levels (Tanaka et al., 2007). This was recently confirmed in a study using immediate and delayed punishments after TRP depletion (Tanaka et al., 2009). Low levels of 5-HT would be associated with loss of prefrontal top-down control from OFC (and dlPFC) over ventral (and dorsal) striata (Homberg, 2012). These two independent sub-sets (i.e. "limbic" *versus* "cognitive" loop) have recently been imaged (Canese et al., 2011), by the use of serotonergic compounds. Accordingly, in the rIGT, 5-HT depleted animals would remain focused on immediate wins and losses; they would not develop a choice for the long-term advantageous option since this would require both: a) integrating the wins and the losses into combinations differing in values (as normally done by the OFC), and b) shifting towards the above-mentioned control by the cognitive loop (de Visser et al., 2010, 2011a,b; Homberg et al., 2008; van den Bos et al., 2006b, 2007). In the rPDT, however, a lack of affective feedback within the limbic loop would lead, in the 5-HT depleted animals, to a lower ability to detect changes in contingencies: these rats would thus continuously choose for the high-magnitude reward, although coming only rarely. These cognitive *versus* limbic loops would be both affected, though independently, and would impede a shift of behaviour towards a "safer" option, represented in both cases by the small reward. In other words, the 5-HT depleted rats were stuck on a short-term focus and unable to recruit cognitive self-control (over the limbic system) effectively; the latter conversely allowed control subjects to change their strategy towards a long-term advantageous behaviour.

As far as present data are concerned, we may hypothesize that, under normal conditions, there is a crosstalk and reciprocal balance between these two looping systems, mediated by 5-HT. Thus, good decision-making and risk aversion are both associated with a correct function of the limbic and cognitive loops. Conversely, when 5-HT is depleted, both sub-sets of 5-HT system (for images, see Canese et al., 2011) become actually impaired and possibly uncoupled, so that the observed phenotype may depend on which system loop is affected most. Given the relatively low number of individual animals in the present correlation analysis, further research would be necessary to substantiate these conclusions.

### 4.5. Potential experimental caveats

The final remark about our present TRP depletion approach is to rule out a potential "bias" of the study, due to the fact that the TRP-free diet (in combination with the food-restriction schedules) not only decreased 5-HT synthesis in the brain (thus affecting cognition and risk aversion), but also led to whole-body metabolic adaptation processes, leading to weight loss. A diminished food intake, and consequently a weight loss, is observed in animals when fed with a diet lacking only one amino acid (Leung and Rogers, 1973), as in the case of tryptophan (Walters et al., 1979). It is however unlikely that reduced body weight alone may account for the different choice patterns shown presently. In our hands, covariate and correlation approaches confirm this notion. In agreement with our result, authors who also assessed the overall consequences of a TRP-free diet, concluded that decreased food intake, and consequently weight loss in T− animals, could not account for their observed behavioural profiles (Tanke et al., 2008). Such a conclusion is, at least for the rIGT, in line with recent data (Rivalan et al., 2009) showing that rats' performance in a closely-related task was independent of deprivation levels (0–20% loss of body weight).

Also, we assume that the metabolic impact of the TRP-free diet was similar throughout the entire one-month deprivation period, and likely did not differ between the first and second operant tests. It is well known that the 5-HT system tends to reach very quickly, and to maintain thereafter, a "stable minimum" until sacrifice. Indeed, Fadda et al. (2000) reported that, already after four days, a TRP-free diet induced an almost total disappearance of 5-HT extracellular levels in the frontal cortex. Similarly, Tanke et al. (2008) observed a marked decrease in blood 5-HT levels and neuronal 5-HT content on day 6 already. Accordingly, in our present experiment, we decided to wait for five days (after providing diets), thus starting operant protocols on the sixth day.

### 4.6. Conclusion

The present data show that L-tryptophan depletion leads to changes in behavioural responses within a decision-making as well as a gambling-proneness task. Future studies should investigate in more detail the 5-HT mediated changes, occurring within forebrain loops involved in cognitive control and motivation/affective processes.

**Conflict of interest**

The authors declare no conflict of interest.

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