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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