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Gambling proneness in rats during the transition from adolescence to young adulthood: A home-cage method

Francesca Zoratto, Giovanni Laviola, Walter Adriani*

Section of Behavioural Neuroscience, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Viale Regina Elena 299, I-00161 Rome, Italy

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ABSTRACT

Pathological gambling is widespread among adolescents (3–8%). Gambling proneness can be evaluated in animals using the Probabilistic Delivery (PD) task. In this operant protocol, rats learn to choose for large over small reward. Subsequently, the probability of large reward-delivery decreases progressively to very low levels. Using a home-cage version of the PD task, we studied (Exp. 1–3) the development of preference for the largest reward in middle (pnd 34–35) and late (pnd 48–49) adolescent rats, using the standard paradigm (Zoratto et al., 2012) and then modifying: (*i*) probability "*p*" initially associated with the largest reward; (*ii*) size difference between rewards; (*iii*) "removable" or "fixed" partitions (allowing to house animals in couples, separating them only during testing). The standard paradigm ($p = 50\%$, 2 *vs* 6 pellets; "removable" partitions) does not allow the establishment of preference for the largest reward, at neither adolescent age. Conversely, the modified paradigm ($p = 66\%$; 1 *vs* 5 pellets; "fixed" partitions) allows the development of such preference, already at pnd 34–35. By using the best combination of these factors, we then investigated (Exp. 4) the characteristics of gambling behaviour in middle adolescent (pnd 36–49) and young adult (pnd 67–80) rats. Gambling proneness appears slightly increased during adolescence when compared to adulthood. Notably, inadequate responses (expressed during post-choice timeout, 30 s) appear markedly reduced, suggesting developing animals to be insensitive to reward-delivery omission. In conclusion, methodological refinement is essential to allow the study of risk-prone behaviour during rat adolescence, thus contributing to a better understanding of psychobiological determinants of gambling.

1. Introduction

The rapid worldwide growth of legalised gambling, and the recent legalization of Internet gambling, has further increased availability and accessibility of betting opportunities, raising concern about its impact and its consequences on public health. While gambling may represent a leisure activity for the majority of people, it may become a serious behavioural disorder for others (Petry et al., 2005). Problem and pathological gambling (PG) indeed affect 0.2–5.3% of adults worldwide and are highly comorbid with a range of other mental disorders and with substance abuse (Hodgins et al., 2011). Consequently gambling represents a public concern being both a social and a health issue.

Far from being an adult concern, gambling is becoming a serious behavioural problem among adolescents (Cunningham-Williams and Cottler, 2001; Dickson et al., 2002), whose involvement has increased substantially over the past 20 years (Huang and Boyer, 2007). Epidemiological studies show that the prevalence of PG is two to four times higher among adolescents than among adults, with 3.5–8.0% of adolescents that meet the criteria for such pathology (Caillon et al., 2012; Ellenbogen et al., 2007; Felsher et al., 2004; Hodgins et al., 2011). Adolescence and young adulthood may represent periods of especially heightened vulnerability for the development of gambling disorders, which are therefore receiving increasing attention by clinicians and preclinical researchers (Jazaeri and Habil, 2012).

The transition from early adolescence to adulthood is not exclusive to humans. On the contrary, many psychobiological aspects of adolescence can be identified in most mammalian species, including rodents (Adriani and Laviola, 2003, 2004; Kelley et al., 2004; Laviola et al., 1999; Laviola and Marco, 2011; Spear, 2000). The developmental period of adolescence in humans, generally considered as the period between 12 and 18 years old, has been compared, in a broad sense, with the age window between weaning, usually at postnatal day (pnd) 21, to young adulthood, set conventionally at pnd 60. According to the literature, the

* Corresponding author. Tel.: +39 06 4990 3171; fax: +39 06 495 7821.
E-mail address: walter.adriani@iss.it (W. Adriani).

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adolescent period can be subdivided into three phases, namely early (approx. pnd 24–34), middle (approx. pnd 35–48) and late (approx. pnd 49–59) adolescence (Adriani et al., 2002, 2012a; Laviola et al., 2003; Marco et al., 2011).

In animal models, in order to study (in)tolerance to uncertainty and/or risk proneness, many operant paradigms have been developed (*e.g.* Adriani et al., 2006; Cardinal and Howes, 2005; Mobini et al., 2000; Wilhelm and Mitchell, 2008). The Probabilistic Delivery (PD) task is based on the choice between either certain, small amounts of food or larger amounts of food delivered (or not) depending on a given (and progressively decreasing) probability (Adriani and Laviola, 2006; Adriani et al., 2006). In this operant task, rats initially learn to prefer the large over the small food reward. Subsequently, the probability of occurrence of large reward-delivery decreases progressively to very low levels. Final sessions with very high uncertainty levels (in which large-reward choice is mathematically suboptimal, *i.e.* risky) represent the real "gambling" part of the experiment (Adriani and Laviola, 2006). Indeed, while risk averse rats usually change their preference towards a "Small & Sure" (SS) reward, which is a "safer" option, risk prone rats show sustained attraction for a "Large & Luck-Linked" (LLL) reward, despite high uncertainty and low payoff in the long term (Adriani et al., 2009; Adriani and Laviola, 2006).

An emerging issue in the animal model literature is the consideration that testing animals directly in their home-cages would allow a considerable reduction of the impact of different factors that may potentially affect subjects' performance, thus representing a bias. These factors include: (*i*) extensive human handling, (*ii*) removal and transport from the home-cage and (*iii*) exposure to a novel test apparatus (de Visser, 2008; de Visser et al., 2006; Winter and Schaefers, 2011). In this perspective, we adopted here a home-cage version of the PD task, implementing a validated protocol (Adriani et al., 2009; Koot et al., 2012), and using a modified Skinner box-like panel, which was directly available to rats in their home-cages (Adriani et al., 2012b; Zoratto et al., 2012).

These home-cage operant panels (HOPs) were used so far on adult rats, housed individually throughout the entire protocol (Adriani et al., 2012b; Zoratto et al., 2012). Due to the increasingly higher prevalence of PG among youths, this innovative gambling task would be particularly interesting to be performed in adolescent rodents. Nevertheless, the study of rats during this age may be difficult for two main reasons: (1) social deprivation during this ontogenetic period is known to induce changes in reward sensitivity (Van den Berg et al., 1999), as well as psychotic-like symptoms (Leussis and Andersen, 2008); (2) PD tasks generally require a considerable number of sessions and last up to 5 months, depending on the paradigm (Mobini et al., 2000, 2002). Thus, it could be even impossible to test adolescent rats at all. Here we report a considerable methodological improvement in the home-cage PD task which allowed us to test adolescent rats' performance in such operant task while socially living and within the limited span of this developmental phase.

2. Materials and methods

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

2.1. Subjects

Male Wistar rats (Charles River, Italy) belonging to different age groups (middle adolescence, late adolescence and adulthood; see below for details) were housed in couples within Makrolon® Type III cages with sawdust bedding, kept in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%), on a 12 h reversed light–dark cycle (lights off at 8.00 am). Prior to the start of the protocol, rats were moved in couples to novel Makrolon® Type III cages containing the operant panel (Adriani et al., 2012b; Koot et al., 2009, 2012; Zoratto et al., 2012). Water was available *ad libitum*, whereas food (Altromin-R, A. Rieper S.p.A., Vandoies, Italy) was available *ad libitum* until the start of the experimental protocol (see below for details). Rats had no previous experience in any behavioural task, and were left undisturbed for at least 1 week prior to the present experiment.

Rats were tested in their own home-cage with a home-cage version of the PD task for gambling proneness (Adriani et al., 2012b; Zoratto et al., 2012). Subjects had to obtain all their daily meal from the operant panels and no extra-food was given at the end of each experimental session. This was aimed at avoiding a potential recovery from the consequences of food loss (occurring because of its probabilistic delivery). A moderate food restriction was applied to increase the animal's motivation to work for food delivery. To this purpose, a session length of 90 min per day was chosen, based on a previous experiment (Zoratto et al., 2012), showing that such length was optimal to allow animals eating enough food and to prevent them from being fully satiated.

2.2. Apparatus

The testing apparatus consisted of home-cage operant panels (HOPs, PRS Italia, Rome, Italy; Adriani et al., 2012b; Koot et al., 2009, 2012; Zoratto et al., 2012), placed in the Makrolon® Type III home-cage with sawdust bedding. Each panel occupied one fourth of the total living area, which was further divided into two parts through a removable or fixed partition (see below for details). This partition was used to temporarily separate the two members of the couple for the duration of the daily testing sessions.

The operant panels were provided with two nose-poking holes, two hole lights, a single house light placed in the top middle of the panel, a feeder device, a central food-magazine where precision pellets (45 mg, F0021, BioServ, Frenchtown, NJ, USA) were delivered, and a magazine light. The panels were connected through an interface to a computer, where a software (Sk020, PRS Italia, Rome, Italy; Adriani et al., 2012b; Koot et al., 2009, 2012; Zoratto et al., 2012) controlled and recorded all events. Animals were alerted of the start of a session by the house light being turned on. Nose-poking in either hole resulted in the differential delivery of pellets (see below for details). After nose-poking, the house light was turned off and the nose-poked-hole light was turned on for 1 s before food delivery. Following food delivery, the magazine light was turned on for 30 s (timeout, TO), during which further hole visits were recorded but were without any scheduled consequence (*i.e.* inadequate nose-poking). Then, the magazine light was turned off, the house light was turned on, 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 session.

2.3. Pre-training and training protocol

On the evening before the first experimental day, rats were weighed and they were placed in couples in the new home-cages containing the operant panel, where they were left undisturbed for 12 h (one light phase of the cycle). During this adaptation period, the operant panel was off and subjects had *ad libitum* access to standard food and water.

The following morning, a continuous pre-training phase of 36 h started, during which nose-poking in either hole resulted in the delivery of two precision (BioServ) pellets. During the initial 12 h of pre-training, animals had also access to standard (Altromin-R) food; during the remaining 24 h of pre-training, standard (Altromin-R) food was removed and animals only had access to precision (BioServ) pellets upon delivery from the operant panel.

The pre-training phase was followed by 12 h of food deprivation (with operant panel and its house light switched off) in order (*i*) to increase rats' motivation to work for food delivery and (*ii*) to let them learn that nose-poking holes are inactive when the house light is off.

Subsequently, a continuous training phase of 36 h started, during which nose-poking in one of the two holes resulted in the delivery of the small reward (one or two pellets), whereas nose-poking in the other hole resulted in the delivery of the large reward (five or six pellets), whose delivery was randomly released or omitted, according to a set level of probability ("*p*" = percentage of actual food delivery over total demands). Specifically, the contrast between the small and the large reward could be less (two *vs* six pellets) or more (one *vs* five pellets; see below for further details) marked. During the training phase, the probability level ("*p*") was set at either 50% or 66% (see below for further details). Hence, animals had a choice between a "Large & Luck-Linked" (LLL) or a "Small & Sure" (SS) reward. When the delivery of the large reward was omitted, the magazine light was still turned on for a 30 s TO. The small reward-delivery was always unchanged ("*p*" = 100%).