Home Cage Testing of Decision-Making

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ABSTRACT

Testing rodents in their home cages has become increasingly popular. A new low-cost computer-controlled operant panel was designed, which can be placed inside the home cage. A pilot study was carried out, using a decision-making protocol, which was adapted from the original maze rodent Iowa Gambling Task (r-IGT). Male adult rats were tested in their home cages, containing the operant panel provided with nose-poking holes. Nose-poking was associated with rewards of different value and probability. A tryptophan-free diet was fed to investigate the effect of lowering central serotonin concentration on performance in the r-IGT. The data suggested that control rats behave in a way similar to rats tested in the original r-IGT; that is, they tend to choose the option with the best long-term payoff more often as the test progresses. Tryptophan depleted rats showed a weaker improvement across trials than controls.

Author Keywords

Rat, Home cage Testing, Iowa Gambling Task, Serotonin

INTRODUCTION

Recently, much effort has been devoted to developing new methods for behavioral phenotyping. Especially testing animals in their home cages has become increasingly popular. Behavior can be recorded undisturbed and continuously for prolonged periods of time, while human intervention is minimized [5], [7]. A new low-cost computer-controlled home cage operant panel (HOP) was developed by joint effort of the Istituto Superiore di Sanità, Rome, and PRS Italia, Rome. This apparatus is designed to be placed inside a standard-size (Macrolon IV) cage enabling the rodent to operate it 24 h/day. The HOP provides an alternative for yet existing home cage systems, which are either too complex or not able yet to carry out operant-learning tests. In order to test the functionality of this panel, a pilot study was carried out focusing on decision-making.

Decision-making plays an important role in everyday life of both human and non-human animals. The Iowa Gambling Task (IGT) is the most frequently used task to assess decision-making performance under uncertainty [2],[3], in which a conflict between the immediate and the long-term payoff options is represented. Decision-making is often disturbed in psychiatric conditions affected by the common human serotonin transporter promoter length polymorphism (5-HTTLPR). This fact raises the hypothesis that decision-making is indeed modulated by the serotonergic system [6], possibly by maintaining a choice option once established [10]. Furthermore, stress affects decision-making in a cortisol-dependent fashion [12]. The availability of a rodent version of the IGT (r-IGT) makes it possible to study the underlying mechanisms [11]. Testing rodents in their home cage could provide a less stressful environment compared to other methods, due to a reduction in human intervention, handling, and transport. Here, we report the results of an experiment in which the HOP was used in a modified r-IGT protocol. In addition, to study the effect of serotonin on IGT

performance, rats received a tryptophan (TRP) deficient (or control) diet to reduce brain serotonin synthesis.

MATERIALS AND METHODS

All experimental procedures were approved by Institutional Animal Survey Board on the behalf of Italian Ministry of Health, and 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 use alternatives to in vivo testing.

Subjects

Twelve male adult Wistar rats (Charles River, Italy; mean bodyweight 365.5gr) were kept in an air-conditioned room (temperature 21 ± 1°C) on a 12-hr reversed light-dark cycle (lights on at 7.00 pm). Prior to the experiments animals were housed in pairs in Macrolon III cages, but from the start of the training/test protocol animals were singly housed. 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 protocol. After four weeks of acclimation, rats were randomly assigned to one of two experimental groups: one group received a TRP-free diet (T-), while the other group received a control diet. The TRP-free diet (DP/1069 mod., A. Rieper S.p.A., Vandoies, Italy) was a standard diet, but with the complete lack of TRP. The control group (T+) was fed the same diet, except that TRP (2,8 gr/kg diet) was added.

Apparatus

The operant-testing apparatus, consisting of four prototype computer-controlled panels (HOP; PRS Italia, Rome), one for each of the subjects, was placed in a Macrolon IV cage with sawdust bedding. The panel contained two nose-poking holes, hole lights, a chamber light, two feeder devices, a food magazine where pellets (sugar pellets F0042 and quinine [4.44 g/kg diet] pellets F06498, Dustless Precision Pellet 45 mg; Bio-Serv, Frenchtown, NJ) 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 (Sca020; PRS Italia, Rome) controlled and recorded all events.

Nose poking in the holes of the panel resulted in the delivery of sugar or quinine pellets (see Protocol for ratio and amount). After nose-poking (adequate nose-poking) and before food delivery, the hole light was turned on for 1sec. Following food delivery, the magazine light was turned on for 15sec, during which nose-poking (inadequate nose-poking) was recorded but was without any scheduled consequence (time-out, TO). The trapdoor was opened 2sec before the end of the TO. The magazine light was then turned off, the chamber light was turned on, and the system was ready for the next trial.

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. For adequate nose poking, the dependent variable was the choice preference (calculated as the percentage of adequate nose-pokes at the "bad" hole over total amount of all adequate nose-pokes). For inadequate nose poking, the dependent variable was the raw frequency of inadequate nose-pokes per trial.

Protocol

One week before the start of the training/testing protocol, rats were handled for 2min daily, their bodyweight was taken, and they were familiarized with the sugar pellets in the home cage (two pellets per animal per day). Five days before the start of the training/testing protocol the normal food was removed and animals received ad libitum the T- or control diet (animals were familiarized with the diet by receiving approximately 4gr per rat per day in the home cage, two days before the change of diet).

On the morning of the first day of training, rats were placed individually in the test cages with water available ad lib (no food), where they were left undisturbed for an hour before the first session started. Two sessions were run per day, which took place around 9.00AM and 5.00PM respectively (for arguments, see [9]). The training phase consisted of three sessions of magazine training and one session of nose-poke training, i.e. two days in total. During the magazine training, two sugar pellets were dropped automatically in the magazine with an interval of 60sec. After a variable time window, the trapdoor was opened to remove the uneaten pellets; this window was kept fixed for each session and was progressively decreased across subsequent sessions (time to eat, TTE, 20-15-10sec). Once all rats were reliably consuming the pellets, they were trained to nose-poke on each nose-poking hole (right/left alternating each trial, 10 trials per hole): they learned to collect two sugar pellets per adequate nose-poke from both holes, in an attempt to prevent a biased preference for either hole. In the magazine training phase, nose-poking holes were closed by covering Plexiglas plates, while the food magazine was always covered by an aluminium plate (to protect the underlying mechanics against sawdust entering the magazine) which was only removed during sessions.

Once the rats were nose-poking reliably and eating the sugar pellets within 10sec, the test phase started. The IGT was based on the r-IGT performed in a maze as described previously [6], [11], modified to be adapted in the home cage operant panels. Rats received 40 trials per session for a total of 240 trials; thus this phase lasted 3 days. Each session started with turning on the chamber light accompanied by the free delivery of two sugar pellets. The TO was 15sec, including a TTE of 13sec 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 "bad" hole presented occasionally big rewards (four sugar pellets,

probability 30%) among series of quinine pellets (4, probability 70%), i.e. 12 sugar pellets per 10 trials. The "good" hole presented regularly small rewards (two sugar pellets, probability 80%) among quinine pellets (2, probability 20%), i.e. 16 pellets per 10 trials. The total number of nose-pokes for "good" and "bad" holes was calculated for each block of 40 trials to obtain the IGT score. This choice parameter (see Figure 1) was the percentage of adequate nose-pokes at the "bad" hole over total amount of all adequate nose-pokes.

Before each session, rats received 0.5gr of T- or control diet respectively, and after each session they received the rest of their diet needed to maintain them on 95-98% of free feeding bodyweight. Daily after the AM-session, animals' bodyweight was taken. Rats were food restricted from the first day of training throughout the entire protocol in order to increase their motivation to work for food delivery. The entire training/test protocol lasted 5 days.

Data Analysis

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 ate quinine pellets. Throughout the r-IGT, repeated measures ANOVAs were performed on the preference for the "bad" hole (within subjects factor: block; between-subjects factor: treatment). Other data were analysed by ANOVAs (between-subjects factor: treatment) to detect group differences in the number of inadequate nose-pokes per trial, as well as in time needed per trial and in bodyweight (% of ad libitum bodyweight). Differences were considered significant when $p = 0.05$; NS = non-significant. All statistics are two-tailed.

RESULTS

Six T- and five control rats were tested in a modified version of the rodent Iowa Gambling Task [11], adapted to measure the animals' behavior in their home cage. One control subject was excluded from further testing as he did not reliably nose-poke for pellet delivery. Figure 1 shows that, independent of treatment, all subjects improved their performance over blocks (trial block: $F(5,9) = 7.576$, $p<0.001$; trial block x treatment: $F(5,45) = 0.958$, NS). Visual inspection of the data suggests that T- rats do show a weaker improvement across trials than control rats. This is confirmed by separate repeated measures per group: control $F(5,20)=7.63$, $p<0.001$, vs T- $F(5,25)=2.339$, $p<0.07$. T- subjects showed significantly more inadequate nose-pokes per trial (mean ± SEM = 3.18 ± 0.26) than controls (1.80 ± 0.19; treatment $F(1,9) = 7.071$, $p = 0.026$). T- rats needed less time to complete their trials (minutes needed per trial, mean ± SEM = 0.34 ± 0.01) than controls (0.55 ± 0.04) as the test progressed (trial block x treatment $F(5,45) = 4.244$, $p = 0.003$; treatment $F(1,9) = 49.557$, $p<0.001$). No difference was found in bodyweight between the groups (treatment $F(1,9) = 0.079$, NS).

DISCUSSION

The present study was a first attempt to translate the maze r-IGT [11] to a home cage setting using an operant panel. The data thus far indicate that rats, like humans and rodents in the original r-IGT, do seem to choose the best long-term option more often as the test progresses. However, the learning curves for present rats (see Figure 1) and rats tested in the original maze r-IGT [11] with respect to this decision-making task are not exactly similar: the present rats seem to show a slower improvement across trials than rats tested in the maze r-IGT. This may be partly due to the fact that we used a slightly different ratio of sugar and quinine pellets for the two different options, as we noted that the original ratio used in the maze was too easy in the HOP.

It has been reported in both humans and rats that serotonin transporter dosage affects long-term decision-making in the IGT, thereby substantiating an important modulatory role of the serotonergic system in decision-making [6]. In line with this, T- rats (with diet-induced TRP depletion reducing brain serotonin synthesis) seemed to show a weaker improvement across trials than controls. It is clear that more rats need to be tested to substantiate this observation. Still in line, T- rats showed more inadequate nose-pokes, which provides an index of inability to wait [1], and were faster in completing their trials than control rats. Both findings are signs of increased motor impulsivity, caused by an altered function of the serotonergic system which modulates premature and impulsive responding [4], [8].

The present findings show that in principle it is possible to run the r-IGT in a home cage setting. Future experiments are directed at further validation of this approach and refinement of the protocol.

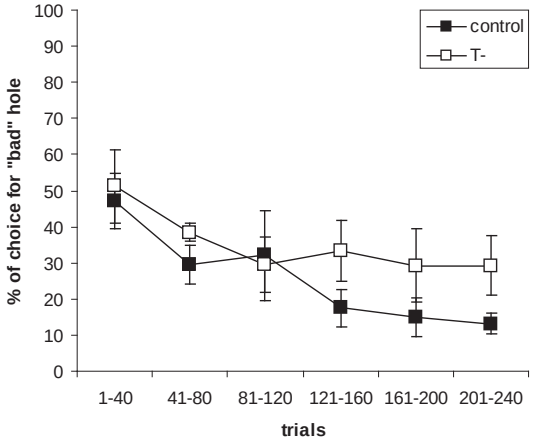

Figure 1. Performance of T- (n = 6) and control (n = 5) rats tested in the home cage r-IGT. Shown are the mean (± SEM) proportions of choices for the "bad" hole per block of 40 trials.

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