Dopaminergic modulation of reward-guided decision making in alcohol-preferring AA rats

Ville Oinio[1,2], MSc (Pharm); Pia Bäckström[1], PhD; Johanna Uhari-Väänänen[1,2], MSc (Pharm); Atso Raasmaja[2], PhD; Petteri Piepponen[2], PhD; Kalervo Kiianmaa[1], PhD

[1]Department of Health, National Institute for Health and Welfare, Helsinki, Finland

[2]Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Corresponding author: Ville Oinio, Department of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, P.O. Box 56, 00014 University of Helsinki, Helsinki, Finland

Tel.: +358-(0)294 1911

Fax: +358-(0)2941 59138

E-mail: ville.oinio@helsinki.fi

Acknowledgements

We thank Ms. Leena Tanner-Väisänen for her technical assistance. This study was supported by the Finnish Foundation for Alcohol Studies. The authors declare that they do not have any conflicts of interest related to the data presented in this manuscript.

Abstract

Results from animal gambling models have highlighted the importance of dopaminergic neurotransmission in modulating decision making when large sucrose rewards are combined with uncertainty. The majority of these models use food restriction as a tool to motivate animals to accomplish operant behavioral tasks, in which sucrose is used as a reward. As enhanced motivation to obtain sucrose due to hunger may impact its reward-seeking effect, we wanted to examine the decision-making behavior of rats in a situation where rats were fed ad libitum. For this purpose, we chose alcohol-preferring AA (alko alcohol) rats, as these rats have been shown to have high preference for sweet agents. In the present study, AA rats were trained to self-administer sucrose pellet rewards in a two-lever choice task (one pellet vs. three pellets). Once rational choice behavior had been established, the probability of gaining three pellets was decreased over time (50%, 33%, 25% then 20%). The effect of D-amphetamine on decision making was studied at every probability level, as well as the effect of the dopamine D_1 receptor agonist SKF-81297 and D_2 agonist quinpirole at probability levels of 100% and 25%. D-Amphetamine increased unprofitable choices in a dose-dependent manner at the two lowest probability levels. Quinpirole increased the frequency of unprofitable decisions at the 25% probability level, and SKF-82197 did not affect choice behavior. These results mirror the findings of probabilistic discounting studies using food-restricted rats. Based on this, the use of AA rats provides a new approach for studies on reward-guided decision making.

1. Introduction

Impairments in risk/reward evaluations occurs in many psychiatric disorders. One of these is gambling disorder, also called pathological gambling, that has been reclassified as a behavioral addiction in the DSM-5 [1], [2], [3]. Gambling disorder combines reward reinforcement and addiction with cognitive processes like reward-guided decision making and other executive functions [4] in a fascinating and complex way, and is characterized by an overstated preference for high and uncertain rewards [5]. Numerous theories have been proposed concerning the etiology and regulation of neuronal processes responsible for the disadvantageous decision-making behavior that leads people to gamble, despite the negative consequences and awareness of the fact that monetary loss is inevitable in the long run ("the house always wins").

Mesolimbic dopamine neurons have been proposed to modulate the reinforcing effects of gambling, and these same neurons appear to act as neural substrates for the reinforcement produced by ethanol and other substances of abuse [6], [7]. It has previously been shown that reward unpredictability can recruit brain dopamine systems in a similar

way to chronic exposure to drugs of abuse, and several research models have proposed that alterations in dopamine signaling mediate the transition from recreational gambling to gambling disorder [3], [8]. Risky and unprofitable decision making has been shown to be modulated by dopaminergic neuronal activity [9], [10], [11], [12], [13]. Systemic activation of the dopaminergic system by D-amphetamine or dopamine D_1 or D_2 agonists has been shown to increase the choice of large and risky options in a probabilistic discounting model in rats [9], and irrational choices under uncertainty have been found to be correlated with dopamine $D_{2/3}$ receptor activity in a rat betting task [11].

Numerous animal models have been developed to study the decision-making deficit that occurs in gambling or in other clinical psychiatric disorders [9], [10], [14], [15]. Despite having different approaches toward decision making, the majority of these models share two common aspects: (1) sucrose pellets are used as a reinforcement, and (2) animals that are used in the experiments are kept under restricted feeding (usually 85% of daily caloric intake) during the experiments. Restricted feeding is a valid way to make animals motivated enough to work for the reward, and thus, complete all necessary steps during operant behavioral tasks to fulfill the requirements determined by the researcher. Additionally, it has been shown that rats kept under food restriction also perform better at decision-making tasks based on probabilistic discounting protocols [9]. According to this, extra motivation of the animal due to hunger may impact on decision-making processes. However, whether behavior during these tasks is modulated purely by the rewarding aspects of the reinforcement or other motivational factors (like the need for calories) cannot be determined. To specifically model reward-guided decision making related to gambling in animals, the observations need to target the rewarding aspects of decision making. Sucrose was used as a reinforcement in the current study, which could still be achieved when rats were fed ad libitum, and in doing this, we could assume that the rats sought to obtain sucrose due to the additional reward gained from this behavior. This approach creates a new kind of challenge, as it is more challenging to train rats to accomplish operant behavioral tasks without food restriction. The midbrain dopamine neurons also play an important role in classifying the reward of nutrients (e.g., sucrose and fat), and activation of these neurons is enhanced by fasting [16], [17]. Recent findings also suggest that dopamine neuronal activity is regulated by numerous messengers, which increase dopamine neuronal activity under conditions of nutrient deficit and decrease activity during satiety [18]. Considering that decision making and gambling studies emphasize the role of the dopaminergic system, the impact of food restriction in the decision guiding of rodents should be considered.

In our approach, the genetically-selected alcohol-preferring AA (alko alcohol) rat line provided us with a useful option. The AA rat line was produced by selecting for high voluntary ethanol consumption from a foundation stock that

included both Wistar and Sprague-Dawley strains [19]. AA rats quickly learn to self-administer ethanol without any initiation or shaping [20], and studies have reported a close association between the consumption of alcohol and sweet substances [21], [22]. In addition, findings that AA rats consume sucrose solutions beyond the limits of their normal fluid intake suggests that these rats can be used to model the clinical phenomenon known as loss of control [23], which might make them useful for use in studies of behavioral addictions like gambling. The sweet preference of AA rats is hypothesized to occur due their abnormal function of opioidergic mechanisms, which is in present knowledge closely related to the dopamine function. Furthermore, data from our preliminary experiments demonstrated that the counterpart of AA rats, the ANA (alko non-alcoholic) rats, do not show enough motivation for sucrose self-administration, even in a food-restricted state (Appendix I). As such, it was appropriate to use only AA rats in this study.

To study the effect of dopaminergic drugs on reward-guided decision making, we studied the decision-making behavior of ad libitum-fed AA rats in a probabilistic decision-making task. Rats were firstly trained to make rational choices based on two different sized sucrose rewards (i.e., choosing the most profitable option), before requiring them to adapt their learned behavior. This was achieved by gradually decreasing the probability of gaining a reward to the point where choosing the large but uncertain reward leads to an unprofitable outcome in long run, compared to choosing the smaller but certain option. The aim of this study was to test whether alcohol-preferring rats can be used as a complementary behavioral research method for probabilistic discounting studies. To achieve this, we demonstrated that systemic dopaminergic modulation (D-amphetamine, the dopamine D_1-agonist SKF-81297 and the $D_{2/3}$-agonist quinpirole) of decision making of ad libitum-fed AA rats is comparable with findings from other similar decision-making studies.

2. Materials and methods

2.1. Animals

One group of 15 and two groups of 12 male alcohol-preferring AA rats (National Institute for Health and Welfare, Helsinki, Finland and University of Helsinki, Helsinki, Finland), weighing 250–300 g at the beginning of training, were used. On arrival, rats were given 1 week to acclimatize to the environment. Food (regular chow SDS RM1 [E] SQC; Witham, Essex, England) and water were available ad libitum in the home cage. Rats were housed three per cage in a temperature and humidity controlled room. The light/dark cycle was 12/12 h. All experiments were conducted in the dark phase of the light cycle. All testing was in accordance with the Animal Experiment Board of Finland.

2.2. Drugs

The dopamine releaser D-amphetamine sulphate (Sigma–Aldrich), the dopamine D_1-agonist SKF-81297 (Sigma–Aldrich) and the $D_{2/3}$-agonist quinpirole (Sigma–Aldrich) were used as test drugs. Doses of D-amphetamine were 0.1, 0.3 and 1.0 mg/kg, doses of SKF-81297 were 0.1, 0.3 and 0.5 mg/kg and doses of quinpirole were 0.003, 0.010 and 0.030 mg/kg. All drug doses were calculated as salt weights and dissolved in 0.9% saline which was also used as a vehicle control. Drugs and vehicle were administered in a Latin square design where all rats received all doses. Drug doses were administered as subcutaneous (s.c.) injections at a volume of 1 ml/kg 10 min prior to testing, which is comparative to other studies [9], [24]. Each drug/vehicle test day was preceded by at least 3 drug-free baseline days. Stable baseline in lever choice behavior was required for 3 consecutive days before the next injection was administered. The criterion for stable baseline was achieved when the standard error of the mean in LL lever choices (±SEM) of three previous baseline session averages was under 5.00. Each rat had their baseline values calculated separately and each rat also received drugs based on their individual stability in baseline values.

2.3. Apparatus

Behavioral testing was conducted in operant chambers (30.5 cm × 24 cm × 21 cm; Med-Associates, St Albans, VT, USA) enclosed in sound-attenuating wooden boxes. The boxes were equipped with a fan that provided ventilation and masked extraneous noise. Each chamber was fitted with two retractable levers, one located on each side of a central food tray where sucrose reinforcement (45 mg; Opend, Denmark) was delivered by a pellet dispenser. Above each lever was a cue light. The chambers were illuminated by a single 100 mA house light located in the top center of the wall opposite the levers.

2.4. Lever press training

Three days before the first lever press training session, rats were placed in the operant chambers for 15 min each day with the food tray in the chamber containing nine sucrose reward pellets. After this, the rats were returned to their home cage and approximately 30 sucrose pellets were given per cage. This procedure was done to habituate rats to the operant chamber environment and the taste of sucrose.

After the habituation days, the training period, which included three phases (A, B and C) was initiated. In phase A the rats were trained for 60 min so that only one lever was present (left or right) while another lever remained retracted

throughout the session. By pressing the lever rats received one sucrose pellet with a 3 s timeout during which the cue light was on. Phase A consisted of a total of six training sessions and the present lever was changed each session.

In phase B the rats were trained for 30 min so that only one lever was present (left or right) at the start of the session. Rats received one sucrose pellet for each press. After each press the pressed lever was retracted and the other lever was presented after a 3 s time out. During the time out, the cue light above the pressed lever was lit for 3 s. Phase B consisted of a total of six training sessions.

In the last training phase C, the rats were trained for 15 min so that both levers were presented at the same time. By pressing either lever the rat received one sucrose pellet. The cue light above the pressed lever was then lit for 15 s and both levers were retracted before being presented again after 15 s. Phase C consisted of a total of three training sessions.

All sessions were conducted in darkness. The house light was turned on before and after each session but was off during the sessions.

3. Experiment 1 – D-amphetamine

3.1. Rational choice behavior

The task was modified from procedures described by Adriani and Laviola [15] and St Onge et al. [12]. Rats received daily sessions consisting of up to 60 trials. One session included 15 min during which rats had free choice to press the levers without at will at 15 s intervals. At the beginning of the session rats were placed in the operant chambers where the house light was on and both levers were retracted. When the session started the house light was turned off and both levers were presented simultaneously. One lever was designated the SS-lever ("small/sure") and the other the LL-lever ("large/lucky"). Levers were randomized left/right and remained consistent throughout the sessions.

Choice of the SS-lever always delivered one pellet with 100% probability and choice of the LL-lever delivered three pellets with 100% probability. Multiple pellets were delivered 0.5 s apart. After pressing a lever, both levers retracted and the cue light above the pressed lever was lit and one (SS lever) or three (LL lever) sucrose pellets were delivered to the food tray. After sucrose was delivered, the cue light remained on for another 15 s, after which both levers were presented again.

Drug injections were started when the group average for LL lever choice % (calculated as the percentage of LL-lever choices out of the total lever responses) in the rational choice task was over 80% and rats expressed stable LL lever

choice baseline of three consecutive sessions. The first two injections were saline injections (s.c.) which were given to habituate the rats to injections.

3.2. Probabilistic discounting task

After drug injections were completed at the LL-lever 100% probability level, probabilistic discounting was initiated. The probabilistic discounting study was divided into five different probability levels where the probability of gaining three sucrose pellets by pressing the LL-lever was decreased over time while the SS-lever always delivered one pellet with 100% probability. Rats received one 15-min session per day in the same manner as previously described for the rational choice behavior task.

The following probability level after rational choice task was 50%. Rats received 10 sessions at this probability without any injections. After this, the rats were administered D-amphetamine or vehicle again in the previously described manner. Every time drug injections were started at the new probability level, stable baseline of LL lever choices were required. The same procedure was conducted for the remaining probability levels – 33%, 25% and 20%. A total of 139–170 sessions per animal were conducted.

3.3. Satiety control

During the probabilistic discounting task we discovered that after administration of the highest dose of D-amphetamine (1.0 mg/kg) some of the rats did not consume all the pellets they received. Because of these observations, the effect of D-amphetamine on sucrose pellet consumption was also examined after the probability discounting task was completed. In this test rats were placed in the operant chamber for 15 min (house light off) with an additional food cup placed in front of the central food tray. The food cup was filled with 10.0 g of sucrose pellets. After 15 min rats were removed and the remaining sucrose pellets were weighted in order to calculate pellet consumption. Sucrose consumption was calculated g/kg for each rat. The effects of different doses of D-amphetamine (0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg) on sucrose pellet consumption were examined and saline was given as a vehicle. Drugs were administered s.c. in a Latin square design with 3 days recovery time between each dose.

4. Experiment 2 – SKF-81297 and quinpirole

For SKF-81297 and quinpirole groups only probability levels 100% and 25% were chosen. The probability level 100% was chosen to show any drug effects to the rational decision making and the probability level 25% was chosen as a level were effects of drugs to the unprofitable decision making could be observed.

4.1. Rational choice behavior

In experiment 2 the rational choice task was modified based on the results obtained from experiment 1, which showed innate lever side preferences toward the right or left side lever during training phase C. Because of this, SS- and LL-levers were not randomized but the LL-lever was designated as the lever that the rat did not spontaneously prefer. The rational choice task was carried out in the same manner as with the D-amphetamine group. Drug injections were started when the group average for LL lever choice % (calculated as the percentage of LL-lever choices out of the total lever responses) in the rational choice task was over 80% and rats expressed stable LL lever choice baseline of three consecutive sessions. The first two injections were saline injections (s.c.) which were given to habituate the rats to injections.

4.2. Probabilistic discounting task

Drug administration started after rats showed rational decision making behavior. The first two injections were saline injections (s.c.) which were given to habituate rats to injections. After all these injections were given rats received five sessions at a 50% probability level without any injections followed by five sessions at a 33% probability level without any injections. After this, rats proceeded to the 25% probability level and continued this for 10 sessions. After rats displayed stable baseline LL lever choices, drugs and vehicle were administered again in the previously described manner. In total, 77–97 sessions were conducted.

4.3. Data analysis

Data was analyzed with SPSS version 22.0. Statistical analysis of the data from the rational choice behavior test were conducted using a repeated measures two-way ANOVA [session, group and session × group interaction]. Statistical analysis of the data from the probabilistic discounting task, number of lever responses and satiety control were conducted using a repeated measures ANOVA in a within subject manner. If a significant main effect was found, a paired two-tailed t-test with Holm–Bonferroni method was used to detect differences between vehicle and drug

treatments. The criterion for significance was set at $p < 0.05$. Data from every probability level (100%, 50%, 33%, 25% and 20% for D-amphetamine group; 100% and 25% for SKF-81297 and quinpirole groups) was analyzed separately to show the effects of drugs at each probability level.

5. Results

5.1. Training

All rats learned to self-administer sucrose pellets during training phases A and B. During training phase C, rats showed a lever preference toward the right or left side lever despite the fact that both levers delivered one sucrose pellet at the 100% probability (Fig. 1).

5.2. Group sizes

In the D-amphetamine group, three rats were disqualified due to the absence of rational behavior or discounting behavior, making the total n = 12 for this group. Based on the data from the D-amphetamine group, the criterion for sufficient discounting for SKF-81297 and quinpirole groups was set to LL-lever choice of 50% or less after 10 sessions at the 25% probability level. Four rats from SKF-81297 and five rats from the quinpirole group did not achieve this criterion, making n = 8 for the SKF-81297 group and n = 7 for the quinpirole group.

6. Experiment 1

6.1. Rational choice behavior

Rats received one daily session starting at a LL-lever probability of 100% (Fig. 1). During the rational choice behavior task, the average of the LL-lever choice in the D-amphetamine group (n = 12) increased from 49.63% ± 7.43 (Session 1) to 81.27% ± 4.90 (Session 20). The averages in the last three sessions were 80.38% ± 5.66, 81.45% ± 4.70 and 81.27% ± 4.90, respectively. When the data was observed we noticed a different pattern of behavior between rats that had the LL-lever designated right versus left. For this reason, we analyzed the data from the LL-lever right and LL-lever left group separately (Fig. 2). Two-way ANOVA revealed a significant main effect of sessions $[F(1,19) = 7.120, p < 0.001]$ and group $[F(1,10) = 4.99, p < 0.05]$. No session × group interaction was detected $[F(1,19) = 0.997, p = 0.467]$.

6.2. Effect of D-amphetamine at different probability levels

At the 100% probability level, D-amphetamine revealed a trend in lever choices shifting from the LL-lever toward the SS-lever in a dose dependent manner [$F(3,33) = 3.782$, $p = 0.0516$, Fig. 3]. At the 50% and 33% probability levels, D-amphetamine had no effect on lever choices [$F(3,33) = 2.519$, $p = 0.0991$, $F(3,33) = 0.09276$, $p = 0.9197$, respectively]. At the 25% probability level, D-amphetamine caused a dose dependent shift from the SS-lever toward the LL-lever [$F(3,33) = 6.022$, $p = 0.0111$]. Post hoc analysis revealed significant differences in LL-lever choices between vehicle and 1.0 mg/kg D-amphetamine ($p = 0.0173$). At the 20% probability level, D-amphetamine caused a dose-dependent shift from the SS-lever toward the LL-lever [$F(3,33) = 12.01$, $p = 0.0004$]. Post hoc analysis revealed significant differences in LL-lever choices between vehicle and 0.3 mg/kg D-amphetamine ($p = 0.001$) and vehicle and 1.0 mg/kg D-amphetamine ($p = 0.009$).

6.3. Effect of D-amphetamine on lever responses

The number of lever responses rats made during sessions were measured at each probability level after drug and vehicle administration (Table 1). A statistically significant decrease in lever responses (compared to vehicle) were observed between the 1.0 mg/kg D-amphetamine and vehicle groups at all but the 100% probability level
[50%: $F(3,33) = 11.11$, $p < 0.0001$, post hoc $p = 0.0006$ between vehicle and dose 1.0 mg/kg,
33%: $F(3,33) = 29.12$, $p < 0.0001$, post hoc $p = 0.042$ between vehicle and dose 0.3 mg/kg; $p < 0.001$,
25%: $F(3,33) = 33.33$, $p < 0.0001$, post hoc $p < 0.001$ between vehicle and dose 1.0 mg/kg,
20%: $F(3,33) = 15.56$, $p = 0.01$, post hoc $p < 0.001$ between vehicle and dose 1.0 mg/kg].

The effects of D-amphetamine examined in the 15 min satiety control revealed a significant reduction in sucrose pellet consumption (compared to vehicle) after administration of 0.3 mg/kg and 1.0 mg/kg D-amphetamine (Table 2) [$F(3,33) = 56.36$, $p < 0.001$]. Post hoc analysis revealed significant differences between the vehicle and 0.3 mg/kg D-amphetamine ($p < 0.001$) and 1.0 mg/kg D-amphetamine groups ($p < 0.001$).

7. Experiment 2

7.1. Rational choice behavior

Rats received one daily session starting at an LL-lever probability of 100% (Fig. 4). During the rational choice behavior task the average LL-lever choice of the SKF-81297 group increased from 31.69% ± 6.09 (Session 1) to 76.30% ± 3.39 (Session 20) to 83.42% ± 5.41 (Session 30) and the average LL-lever choice of the quinpirole group increased from 41.28% ± 5.89 (Session 1) to 79.85% ± 4.60 (Session 20) to 87.97% ± 2.68 (Session 30). Two-way ANOVA revealed a significant main effect of sessions [$F(1,29) = 22,787$, $p < 0.001$]. No group [$F(1,13) = 0.004$, $p = 0.949$] or session × group interaction was detected [$F(1,29) = 1.137$, $p = 0.289$].

7.2. Effect of SKF-81297 at probability levels 100% and 25%

Repeated measured ANOVA did not reveal any significant effects of SKF-81297 in the LL-lever choices of AA rats at the 100% probability level [$F(3,21) = 2.714$, $p = 0.1015$] or at the 25% probability level [$F(3,21) = 0.3319$, $p = 0.7936$] (Fig. 5).

7.3. Effect of quinpirole at probability levels 100% and 25%

Quinpirole did not reveal any significant effects on LL-lever choices of AA rats at the 100% probability level [$F(3,18) = 0.6505$, $p = 0.5564$] (Fig. 6). At the 25% probability level, repeated measures ANOVA revealed a significant main effect of treatments [$F(3,18) = 3.689$, $p < 0.0465$] and post hoc analysis revealed a significant difference between the vehicle and 0.010 mg/kg quinpirole groups ($p = 0.0479$) were quinpirole increased LL-lever choices compared to vehicle.

7.4. Effects of SKF-81297 and quinpirole on lever responses

The number of lever responses rats made during sessions were observed at probability levels of 100% and 25% with each drug dose and vehicle (Table 3). A statistically significant decrease in lever responses (compared to vehicle) was observed at the highest doses of both drugs and also with the 0.1 mg/kg dose of SKF-81297 at the 25% probability level. At probability level of 100% significant decrease in lever responses was observed with SKF-81297 [$F(3,21) = 5.156$, $p = 0.0341$] and post hoc analysis revealed a difference between the vehicle and SKF-81297 dose of 0.1 mg/kg ($p = 0.0152$) and the vehicle and SKF-81297 dose of 0.5 mg/kg ($p = 0.0352$). At the probability level of 25% significant decrease in lever responses was observed [$F(3,21) = 13.13$, $p = 0.0054$] and post hoc analysis revealed a

difference between the vehicle and SKF-81297 dose of 0.1 mg/kg ($p < 0.0152$) and the vehicle and SKF-81297 dose of 0.5 mg/kg ($p < 0.001$).

At probability level of 100% significant decrease in lever responses was observed with quinpirole [$F(3,18) = 12.78$, $p = 0.0022$] and post hoc analysis revealed a difference between the vehicle and quinpirole dose of 0.030 mg/kg ($p = 0.0069$). At the probability level of 25% significant decrease in lever responses was observed [$F(3,18) = 8.686$, $p = 0.0029$] and post hoc analysis revealed a difference between the vehicle and quinpirole dose of 0.030 mg/kg ($p < 0.001$).

8. Discussion

Here, we have demonstrated the effects of dopaminergic drugs on decision making in alcohol-preferring AA rats using a probabilistic discounting task with an ad libitum approach. To the best of our knowledge, this is the first study to examine the decision making of alcohol-preferring rats in this manner. Our study provides evidence that increasing dopaminergic activity with D-amphetamine acts to promote irrational and unprofitable decision making when the decisions are guided by rewards of two different values. Experiments with SKF-82197 and quinpirole revealed that this irrational decision-making pattern was likely due to activation of the dopamine $D_{2/3}$ receptors, but not activation of dopamine D_1 receptors.

Rational choice behavior reliably demonstrated that AA rats could learn to make rational choices based on a reward of one versus three sucrose pellets. This likely occurred because the three sucrose pellet reward provided a higher reward value than one sucrose pellet. Because rats were fed ad libitum throughout the study, we can conclude that this choice behavior was guided by the reward obtained from sucrose, and not by hunger. On the other hand, a decrease in LL-lever choice was observed after the probability of receiving a three pellet reward dropped from 100% to 50%, and this continued to decline throughout all probability levels. We believe this reflects the risk-averse behavior of AA rats. Similar behavior is also observed in most humans, in terms of avoiding risky options when a guaranteed reward is simultaneously available [25], [26]. This kind of behavior has also been observed in various rodent models of decision making in different rat strains [9], [15]. Based on the rational choice behavior and discounted lever choice, AA rats express similar decision-making patterns to humans, and therefore, are suitable for studying the effects of pharmacological challenges on decision-making behavior.

Increased dopaminergic activity has been shown to play a critical role in biasing choices toward riskier options associated with larger rewards, and this risky decision making has been shown to be mediated by D_1 and D_2 receptors [9], [13], [22]. In our study, administration of D-amphetamine increased LL-lever choice at the two lowest probability levels, and promoted unprofitable decisions in AA rats. Our findings with AA rats are consistent with those that demonstrate the significance of D-amphetamine as a promoter of risky decision making in rat strains that represent "normal" rat populations [9], [10], [11]. After administration of D-amphetamine, the greatest change in LL-lever choice was observed when the probability of gaining three pellets was lowest (25% and 20%). This observation supports the role of increased dopaminergic activity as a promoter of risky decision-making choices, because at these probability levels, rats are losing pellets in the long run by choosing the LL-lever compared to a situation where they always choose the SS-lever. This indicates that excessive dopaminergic activity biases decisions away from the more rational and risk-averse behavior shown by AA rats at their baseline level.

Although the highest D-amphetamine dose (1.0 mg/kg) increased the LL-lever choice most dramatically, it also suppressed the number of lever responses that rats made per session. Because changes in the response rate can bias the calculation of the relative proportion of LL-lever responses, the most reliable evidence of unprofitable decision promotion was observed at the 20% probability level when rats were administered the 0.3 mg/kg dose of D-amphetamine, which increased the LL-lever choice without having a significant effect on the number of lever responses. Similar behavioral effects of D-amphetamine on decision making have also been reported in a previous study that used a traditional probabilistic discounting protocol, in which D-amphetamine was found to increase the choice of large but uncertain sucrose rewards at doses of 0.25, 0.5 and 1.0 mg/kg, with the latter dose having the most dramatic effect when the probability was as low as 12.5% [9]. Increased dopaminergic activity has also been reported to impair optimal decision making in a rodent gambling task, where rats had to choose among four different value options in order to earn as many sugar pellets as possible during a 30 min session [10].

An important observation regarding control was evident at the 33% probability level, where D-amphetamine had no effect on the decision making of AA rats. In this experiment, the 33% probability level acted as the "indifference point", described by Adriani and Laviola [15]. At this probability level, choosing either the SS- or LL-levers makes no difference to pellet gain in the long run, because the odds ratio is 1 for both levers. The lack of effect of D-amphetamine at this level emphasizes the role of dopamine in decision making between rewards of different value, rather than just as

a promoter of impulsive and risky behavior. The role of D-amphetamine in biasing reward expectancy, rather than simply increasing risk-taking in general, is also supported by findings that D-amphetamine reduces risky choices in rats in experiments where a sucrose reward is paired with a probabilistic foot shock [27], [28]. In probabilistic discounting models, where the probability of gaining a large reward is discounted during the session, rather than between sessions, D-amphetamine also seems to promote large reward choices at the indifference point, or even before [12], [13]. This indicates that D-amphetamine can also promote a shift between choices that are not completely based on the value of the reward. This was not the case in our study, which leaves an open question of the differences between traditional and long-term discounting models. A previous study showed that rats express individual sensitivity to wagers, and the effect of D-amphetamine differs between rats of different sensitivities by increasing the preference for uncertain options by wager-sensitive, but not wager-insensitive, rats [11]. In our study, AA rats expressed stable sensitivity to the probability of gaining the large reward, and decreased their LL-lever choice as the probability of gaining the large reward decreased. This risk-averse behavior of AA rats was diminished by the action of D-amphetamine, which might reflect similar behavior as wager-sensitive animals described in the study by Cocker et al. [11].

To clarify the role of dopamine receptors in the decision making of AA rats, we also studied the effects of selective D_1 and $D_{2/3}$ agonists on LL-lever choice at the 100% and 25% probability levels. The $D_{2/3}$ agonist quinpirole significantly increased LL-lever choice at the second highest dose (0.010 mg/kg). This dose had no significant effect on the number of lever responses. On the other hand, the highest dose of quinpirole (0.030 mg/kg) did not significantly increase LL-lever choice, but significantly decreased the overall number of lever responses compared to rats administered the vehicle. Thus, it is difficult to evaluate whether the absence of an effect on LL-lever response results from the biphasic effect of quinpirole on behavioral responses to sucrose [29], or the result of a decrease in the number of lever presses. A decrease in the number of lever responses, also observed with SKF-81297, makes it difficult to evaluate the impact of the drug on decision making. This is because when the number of lever presses decreases, the relative proportion of single lever responses is emphasized, which may bias reliable evaluation. Thus, it is difficult to make reliable conclusions about the role of D_1 receptors in this study. In the probabilistic discounting task SKF-81297 was found to have a biphasic effect at a dose of 1.0 mg/kg, which made rats more risk-averse when the probability of the large reward was 50%, but more risk-prone when the probability decreased to 25%. However, similar to that observed in our experiment, this dose also significantly increased trial omissions compared to those given a vehicle [9].

The finding that D-amphetamine and quinpirole promoted unprofitable decision making is in line with previous studies. D-Amphetamine and the dopamine $D_{2/3}$ receptor agonist quinpirole, but not the D_1 agonist SKF-81297, have been shown to facilitate the modulation of reward expectancy during a rat slot machine task, prompting rats to make erroneous decisions [24]. In addition, systemic treatment with the D_2 agonist bromocriptine has been shown to increase the frequency of choosing large but uncertain rewards in a probabilistic discounting task [9], and administration of quinpirole to the medial prefrontal cortex impairs optimal decision making by flattening the discounting curve [30]. On the contrary, in a four-choice rat gambling task, quinpirole and bromocriptine failed to show any effects [10]. It might also be possible that D_2 receptors play an important role in probabilistic discounting, where rats have to adapt their behavior to changes during the game, with increased D_2 activation shown to interrupt this adaptation process. In our task, the probability of receiving the large reward during the session did not change in the same way as in traditional probabilistic discounting. This resembles a situation where rats must make choices mainly based on the reward value and the stable probability of gaining the reward. Despite this, quinpirole increased the frequency of choosing the unprofitable option, which indicates a role for the dopamine $D_{2/3}$ receptors in biasing the choice between options that are kept constant during the game.

All dopaminergic drugs tested in this study affected the number of lever responses, and the free pellet-eating test showed a dramatic effect of D-amphetamine on sucrose pellet consumption. This raises an important question concerning the effect of dopaminergic neurotransmission on decision making in studies using rats. In many decision-making tasks, rats are taught to respond to levers within a certain time period or the reward is omitted [9], [10], [31]. Some of these studies have reported that D-amphetamine and other dopaminergic drugs caused only few or no trial omissions in tasks where all rats completed a limited number of trials per session [9], [10]. Administration of D-amphetamine has also been shown to increase premature responses in a rat gambling task, resulting from the ability of the amphetamine to increase impulsive behavior [31]. In our study, these omissions or premature responses were not observed, as rats had an unlimited choice to press the lever every 15 s during the 15-min task. The theoretical maximum in the task was 60 lever presses per session, but none of the rats achieved this rate. Because the lever maximum was so high, we could easily observe the effect of the drugs on overall motivation for wanting the pellets.

Given that most decision-making studies with rats [9], [10], [24] use a restricted feeding method (85% of free feeding), it might also be possible that, despite the appetite-suppressant effect of D-amphetamine, the rats are hungry enough to complete all the trials in these studies. The completion of trials, however, does not exclude the possibility that restricted

feeding might mask the anorexic effects of dopaminergic drugs, which could in turn bias behavioral outcomes. It is known that food restriction affects dopaminergic systems by reducing the reuptake of dopamine in striatal brain regions [32]. Animals in a food-restricted state are more motivated toward appetitive rewards [33], and even a modest restriction in feeding can profoundly affect dopamine transporter activity and sensitivity to the behavioral effects of dopaminergic drugs. Based on the results of this study, ad libitum-fed AA rats express similar risky decision-making patterns after administration of D-amphetamine when compared to other rat strains that are food deprived. We consider this an important finding, as these results establish a role for excessive dopaminergic neurotransmission as a modulator of unprofitable decision making in situations where decisions are guided purely by a reward of two different values. In conclusion, AA rats offer a new possibility for studying harmful decision-making behaviors that are present in gambling disorder, and in the future, these rats could be used to determine the role of a genetic susceptibility to alcohol dependence in modulating this behavior.

References

[1] American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (5th ed.), American Psychiatric Publishing, Arlington, VA, USA (2013)

[2] M.N. Potenza The neural bases of cognitive processes in gambling disorder Trends Cogn. Sci., 18 (2014), 429-438

[3] B.F. Singer, P. Anselme, M.J.F. Robinson, P. VezinaNeuronal and psychological underpinnings of pathological gambling Front. Behav. Neurosci., 8 (230) (2014), 1-2

[4] L. Clark Decision-making during gambling: an integration of cognitive and psychobiological approaches Philos. Trans. R. Soc. B, 365 (2010), 319-330

[5] D. Brevers, G. Koritzky, A. Bechara, X. Noël Cognitive processes underlying impaired decision-making under uncertainty in gambling disorder Addict. Behav., 39 (2014), 1533-1536

[6] G.F. Koob, A.J. Roberts, G. Schulteis Neurocircuitry targets in ethanol reward and dependence Alcohol Clin. Exp. Res., 22 (1998), 3-9

[7] R.A. Wise Drug-activation of brain reward pathways Drug Alcohol Depend., 51 (1998), 13-22

[8] M. Zack, R.E. Featherstone, S. Mathewson, P.J. Fletcher Chronic exposure to a gambling-like schedule of reward predictive stimuli can promote sensitization to amphetamine in rats Front. Behav. Neurosci., 8 (36) (2014), 1-15

[9] J.R. Onge St, S.B. Floresco Dopaminergic modulation of risk-based decision making Neuropsychopharmacology, 34 (2009), 681-697

[10] F.D. Zeeb, T.W. Robbins, C.A. Winstanley Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task Neuropsychopharmacology, 34 (2009), 2329-2343

[11] P.J. Cocker, K. Dinelle, R. Kornelson, V. Sossi, C.A. Winstanley Irrational choice under uncertainty correlates with lower striatal $D_{2/3}$ receptor binding in rats J. Neurosci., 32 (44) (2012), 15450-15457

[12] J.R. Onge St, S. Ahn, A.G. Phillips, S.B. Floresco Dynamic fluctuations in dopamine efflux in the prefrontal cortex and nucleus accumbens during risk-based decision making J. Neurosci., 32 (47) (2012), 16880-16891

[13] B. Mai, S. Sommer, W. Hauber Dopamine D_1/D_2 receptor activity in the nucleus accumbens core but not in the nucleus accumbens shell and obitofrontal cortex modulates risk-based decision making Int. J. Neuropsychopharmacol. (2015), 1-9

[14] R.N. Cardinal, N.J. Howes Effects of lesions of the nucleus accumbens core on choice between small certain rewards and large uncertain rewards in rats BMC Neurosci., 6 (37) (2005), 1-19

[15] W. Adriani, G. Laviola Delay aversion but preference for large and rare rewards in two choice tasks: implications for the measurement of self-control parameters BMC Neurosci., 7 (52) (2006), 1-11

[16] A. Laque, S. Yu, E. Qualls-Creekmore, S. Gettys, C. Schwartzenburg, K. Bui, C. Rhodes, H.R. Berthoud, C.D. Morrison, B.K. Richards, H. Münzberg Leptin modulates nutrient reward via inhibitory galanin action on orexin neurons Mol. Metab., 16 (4(10)) (2015), 706-717

[17] L. Puga, V. Alcántara-Alonso, U. Coffeen, O. Jaimes, P. de Gortari TRH injected into the nucleus accumbens shell releases dopamine and reduces feeding motivation in rats Behav. Brain Res., 1 (306) (2016), 128-136

[18] J. du Hoffmann, S.M. Nicola Activation of dopamine receptors in the nucleus accumbens promotes sucrose-reinforced cued approach Behav. Front. Behav. Neurosci., 10 (2016), 144

[19] W. Sommer, P. Hyytiä, K. Kiianmaa The alcohol-preferring AA and alcohol-avoiding ANA rats: neurobiology of the regulation of alcohol drinking Addict. Biol., 11 (2006), 289-309

[20] P. Hyytiä, J.D. Sinclair Demonstration of lever pressing for oral ethanol by rats with no prior training or ethanol experience Alcohol, 6 (1989), 161-164

[21] J.D. Sinclair, A. Kampov-Polevoy, R. Stewart, T.K. Li Taste preferences in rat lines selected for low and high alcohol consumption Alcohol, 9 (1992), 155-160

[22] A.B. Kampov-Polevoy, O.P. Kasheffskaya, D.H. Overstreet Pain sensitivity and saccharin intake in alcohol-preferring and -nonpreferring rat strains Physiol. Behav., 59 (1996), 683-688

[23] A.B. Kampov-Polevoy, J.C. Garbutt, D.S. Janowsky Association between preference for sweets and excessive alcohol intake: a review of animal and human studies Alcohol Alcohol., 34 (1999), 386-395

[24] C.A. Winstanley Gambling rats: insight into impulsive and addictive behavior Neuropsychopharmacology, 36 (2011), 359

[25] D. Kahneman, A. Tversky Prospect theory: an analysis of decision under risk Econometrica, 47 (1979), 263-292

[26] D. Kahneman A perspective on judgment and choice: mapping bounded rationality
Am. Psychol., 58 (2003), 697-720

[27] N.W. Simon, R.J. Gilbert, J.D. Mayse, J.L. Bizon, B. Setlow Balancing risk and reward: a rat model of risky decision making Neuropsychopharmacology, 34 (10) (2009), 2208-2217

[28] M.R. Mitchell, C.M. Vokes, A.L. Blankenship, N.W. Simon, B. Setlow Effects of acute administration of nicotine, amphetamine, diazepam, morphine, and ethanol on risky decision-making in rats Psychopharmacology (Berl), 218 (4) (2011), 703-712

[29] K.M. Serafine, T.A. Bentley, D.J. Kilborn Drinking sucrose or saccharin enhances sensitivity of rats to quinpirole-induced yawning Eur. J. Pharmacol., 764 (2015), 529-536

[30] J.R. Onge St, H. Abhari, S.B. Floresco Dissociable contributions by prefrontal D_1 and D_2 receptors to risk-based decision making J. Neurosci., 31 (23) (2011), 8625-8633

[31] F.D. Zeeb, A.C. Wong, C.A. Winstanley Differential effects of environmental enrichment, social-housing, and isolation-rearing on a rat gambling task: dissociations between impulsive action and risky decision-making Psychopharmacology, 225 (2013), 381-395

[32] R.J. Sevak, W. Koek, W.A. Owens, A. Galli, L.C. Daws, C.P. France Feeding conditions differentially affect the neurochemical and behavioral effects of dopaminergic drugs in male rats
Eur. J. Pharmacol., 11 (592 (1–3)) (2008), 109-115

[33] J.M. Kirk, A.W. Logue Effects of deprivation level on humans' self-control for food reinforcers
Appetite, 28 (3) (1997), 215-226

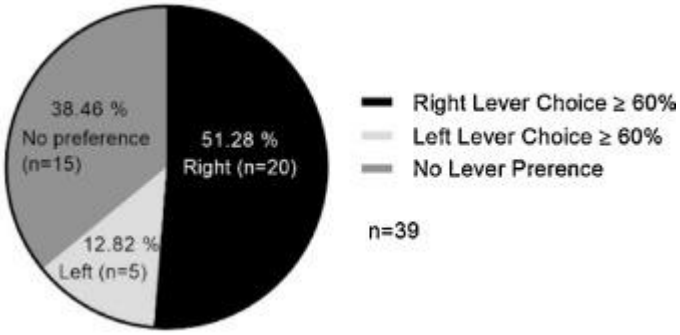

Fig. 1. Some rats showed innate preferences toward the right or left side lever in training phase C. [Lever choice (%) = percentage of right or left lever choices out of the total lever responses.]

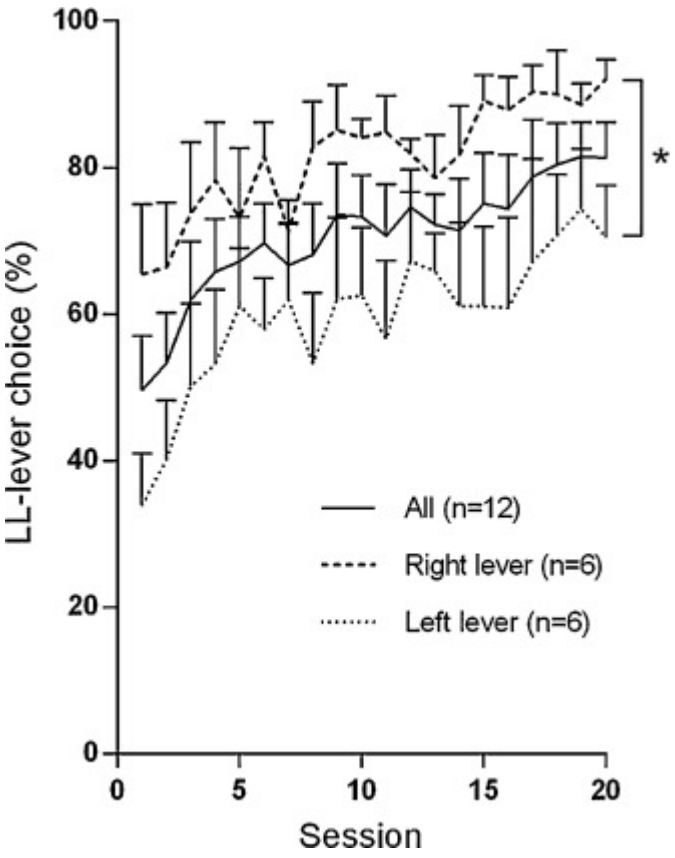

Fig. 2. AA rats show rational choice behavior based on a one (SS) versus three (LL) sucrose pellet rewards. A clear difference is evident between rats that had the LL-lever designated on the right versus rats that had the LL-lever designated on the left. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 6 for both groups, n = 12 for all rats.]

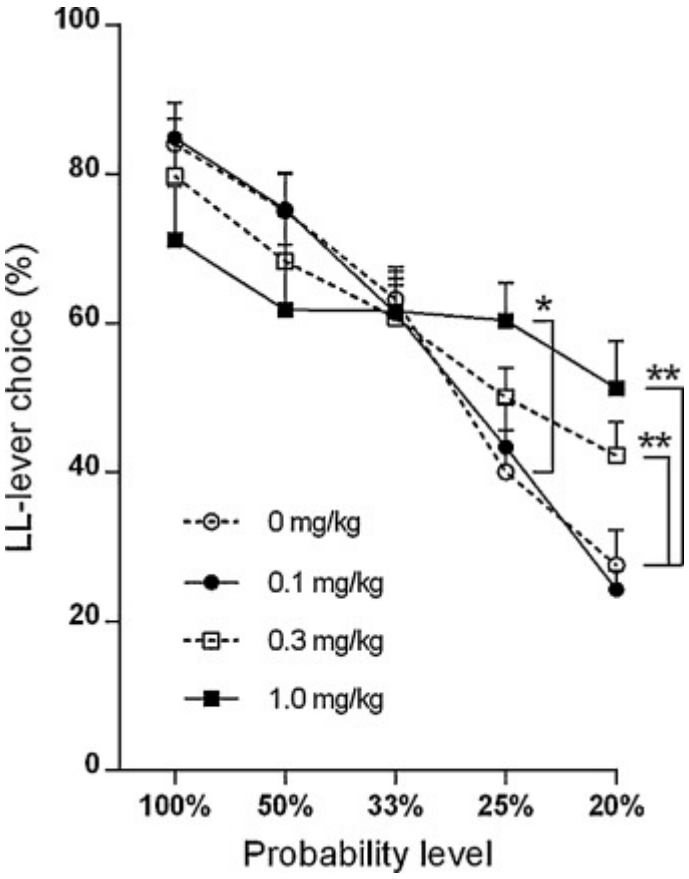

Fig. 3. Effect of D-amphetamine on the LL-lever choices of AA rats at different LL-lever probability levels. Data were analyzed separately with repeated measures ANOVA. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 12.]

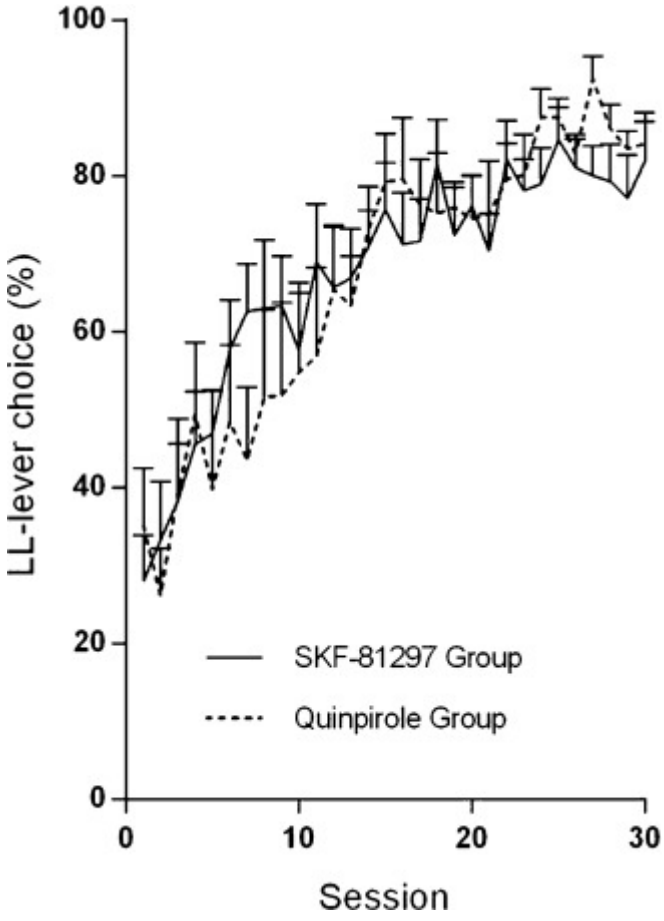

Fig. 4. Both AA rat groups in experiment 2 showed rational choice behavior based on one (SS) versus three (LL) sucrose pellet rewards. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 7 for quinpirole group and n = 8 for SKF-81297 group.]

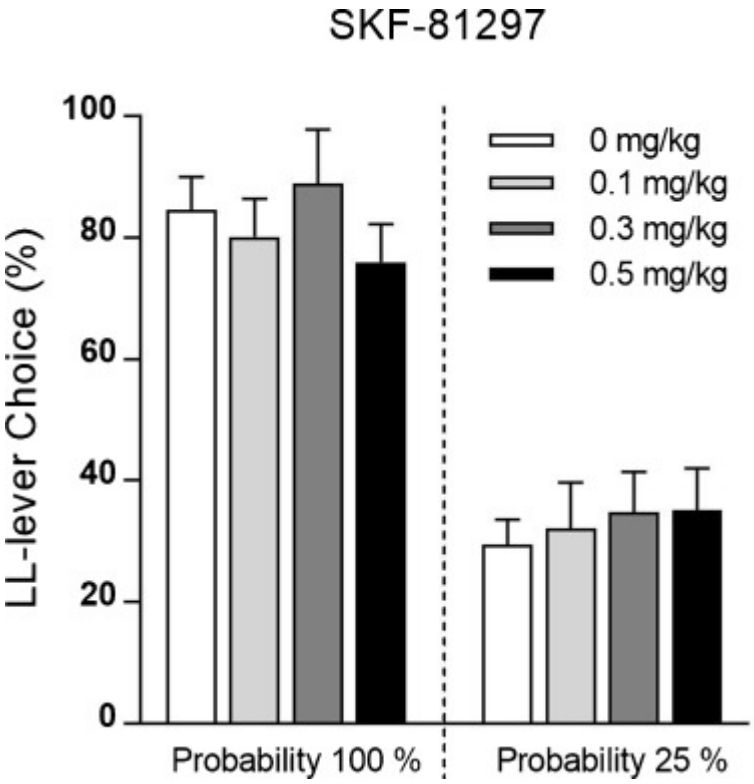

Fig. 5. Effect of SKF-81297 on the LL-lever choices of AA rats at probability levels 100% and 25%. Both probability levels were analyzed separately with repeated measures ANOVA. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 8.]

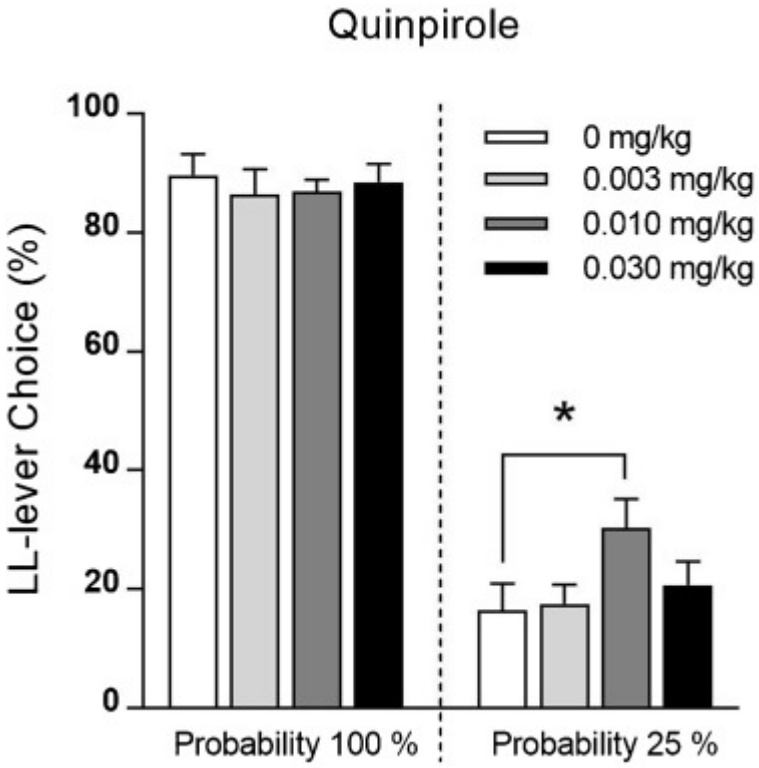

Fig. 6. Effect of quinpirole on the LL-lever choices of AA rats at probability levels 100% and 25%. Both probability levels were analyzed separately with repeated measures ANOVA. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ± SEM, n = 7.]

Table 1. Effect of d-amphetamine on the lever responses at different probability levels.

Probability levels					
d-Amphetamine dose	**100%**	**50%**	**33%**	**25%**	**20%**
0 mg/kg	25.67 (±2.34)	37.17 (±2.12)	41.00 (±2.95)	43.67 (±2.79)	40.08 (±3.06)
0.1 mg/kg	25.58 (±3.02)	36.83 (±2.78)	41.83 (±2.46)	44.08 (±2.95)	41.58 (±3.12)
0.3 mg/kg	25.42 (±2.76)	31.17 (±2.74)	31.83 (±2.41)*	36.92 (±3.33)	36.58 (±3.11)
1.0 mg/kg	21.83 (±2.62)	18.67 (±3.44)***	19.83 (±2.77)***	18.83 (±2.12)***	19.58 (±2.30)***

Number of lever responses at different probability levels after injections, n = 12, ±SEM.

* $p < 0.05$ versus vehicle.

*** $p < 0.001$ versus vehicle.

Table 2. Effect of d-amphetamine to the sucrose eating of AA rats.

d-Amphetamine dose	**Sucrose pellet eaten g/kg**
0 mg/kg	9.56 (±0.81)
0.1 mg/kg	7.81 (±0.73)
0.3 mg/kg	4.59 (±0.41)***
1.0 mg/kg	0.58 (±0.17)***

n = 12, ±SEM.

***

p < 0.001 versus vehicle.

Table 3. Effect of SKF-81297 and quinpirole on the lever responses at different probability levels.

Probability level			
		100%	**25%**
SKF-81297 dose (n = 8)	0 mg/kg	20.25 (±2.34)	31.50 (±2.14)
	0.1 mg/kg	17.63 (±2.31)*	24.38 (±1.46)*
	0.3 mg/kg	14.38 (±2.27)	18.25 (±4.59)
	0.5 mg/kg	11.75 (±2.45)*	10.75 (±2.58)***
Quinpirole dose (n = 7)	0 mg/kg	21.57 (±3.80)	33.86 (±4.56)
	0.003 mg/kg	19.86 (±3.14)	30.86 (±5.17)
	0.010 mg/kg	17.57 (±2.79)	27.76 (±5.58)
	0.030 mg/kg	10.00 (±1.23)**	20.71 (±2.88)***

Number of lever responses at different probability levels after injections, ±SEM.

*p < 0.05 versus vehicle.

**p < 0.01 versus vehicle.

***p < 0.001 versus vehicle.

Appendix I

Data presented here is to show that ANA (Alko Non-Alcohol) rats are not suitable rat strain for studying reward guided decision making in operant conditions without food restriction (Fig. A.1). One-way ANOVA $F(2,16) = 13.09$, $p = 0.001$. Post hoc test with Bonferroni revealed significant difference between AA and ANA rats ($p < 0.001$) and between AA and food restricted ANA rats ($p < 0.05$).

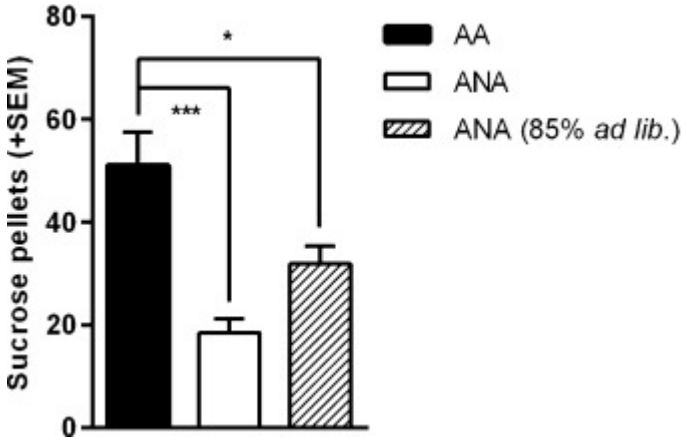

Fig. A.1. Ad libitum fed AA rats consume sucrose pellets (45 mg) in 60 min fixed ratio (one pellet received from either lever in 1 s intervals) training session significantly higher amounts when compared to ANA rats after ad libitum feeding or after food restriction of 85% of free feeding (n = 16).