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THE ROLE OF OPIOIDERGIC SYSTEM IN MODULATING COST/BENEFIT DECISION-MAKING IN ALCOHOL-PREFERRING AA RATS AND WISTAR RATS

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ABSTRACT

Research has highlighted the association of a positive family history of alcoholism with a positive treatment response to opioid antagonists in those with a gambling disorder. However, the role of the opioidergic system in gambling behavior is not well understood, and preclinical studies are needed to clarify this. In this study, Alko Alcohol (AA) and Wistar rats went through operant lever pressing training where the task was to choose the more profitable of two options. Different sized sucrose rewards guided the lever choices, and the probability of gaining rewards changed slowly to a level where choosing the smaller reward was the most profitable option. After training, rats were administered subcutaneously with opioid agonist morphine or opioid antagonist naltrexone to study the impact of opioidergic mechanisms on cost/benefit decisions. No difference was found in the decision-making between AA rats or Wistar rats after the morphine administration, but control data revealed a minor decision enhancing effect in AA rats. Naltrexone had no impact on the decisions in AA rats but promoted unprofitable decisions in Wistar rats. Supporting behavioral data showed that in both rat strains morphine increased, and naltrexone decreased, sucrose consumption. Naltrexone also increased the time to accomplish the operant task. The results suggest that opioid agonists could improve decision-making in cost-benefit settings in rats that are naturally prone to high alcohol drinking. The naltrexone results are ambiguous but may partly explain why opioid antagonists lack a positive pharmacotherapeutic effect in some subgroups of gamblers.

Keywords: Gambling Disorder, Decision-Making, Opioids, Animal Model

INTRODUCTION

Gambling disorder (GD, DSM-5) is considered a behavioral addiction and has no approved pharmacotherapy available, although opioid antagonists naltrexone and nalmefene have shown varying efficacy in recent years (Grant et al., 2006; Grant et al., 2008; Potenza, 2008). One of the reasons for different treatment outcomes with opioid antagonists is likely due to the diverse neurobiological background of GD (Clark, 2010; Singer et al., 2014). The literature hypothesizes that there are many subgroups or "gambling genotypes," although the "gambling phenotype" is mostly similar and can be viewed as excessive gambling without the ability to control one's actions. Treatment outcomes have shown an association of a positive family history of alcoholism with a positive treatment response to opiate antagonists in GD

(O'Brien, 2005; Monterosso et al., 2001; Krishnan-Sarin et al., 2007), indicating that one GD subgroup could be gamblers who have a genetic vulnerability to alcohol use disorders (AUD) (Grant et al., 2008). Comorbidity with GD and AUD is also well-documented (Slutske et al., 2000; Lobo and Kennedy, 2009; Mann et al., 2017), and twin and adoption studies provide strong evidence of shared genetic vulnerability in these disorders (Slutske et al., 2000; Slutske et al., 2013).

A critical aspect of GD is poorly functioning decision-making processes that may bias decisions that are made by the evaluation of the cost vs. benefit of probabilistic outcomes. These biases may occur for various reasons, e.g., overweighting the probabilities of winning, the individual tendencies towards risk aversiveness/proneness, or impulsiveness in decision-making. Mainstream findings in the neurobiology of decision-making underlie the relevance that dopamine may be controlling these biases (St Onge and Floresco, 2009; Winstanley and Floresco, 2016). Opioidergic mechanisms have not been studied as broadly, although opioidergic functions are closely linked to the dopamine system through their ability to modulate the activity of GABAergic spiny neurons that regulate dopamine release in the mesolimbic areas (Wise, 2002; Kemppainen et al., 2012). Opioids have also shown to have independent mechanisms of modulating "liking" of rewards, whereas dopamine seems to modulate more the "wanting" of rewards (Berridge 2003; Peciña, 2008; Berridge and Kringelbach, 2015). Thus, it is hypothesized that the dopaminergic mechanisms control the decisions that are made based on expected results, and additionally, opioids have an impact on the hedonic experience of the outcome of the results (Berridge, 2007; Petrovic et al. 2008). Based on the theory of reward prediction error (RPE) by Schultz et al. (1997) and the fMRI findings of Huang et al. (2014), it can be hypothesized that these neurobiological changes have an impact on the upcoming decision via RPE. Therefore, since opioidergic mechanisms have an impact on dopaminergic functions, it could be possible to modulate decision-making by opioidergic drugs. Preclinical research of decision-making and opioids is very much limited to research of impulsive decision-making in which opioidergic drugs have shown to have an impact on controlling this behavior (Kieres et al., 2004; Pattij et al., 2009; Harvey-Lewis et al., 2015). Further studies are warranted since the contribution of the opioidergic system on other aspects of GD is not very well understood (Victorri-Vigneau et al., 2018).

This study aimed to examine the impact of opioidergic drugs on the cost/benefit decision-making of rats in a task where rats had to make choices based on different probabilities in reward outcomes. Options for outcomes were small and sure or large but uncertain sucrose rewards. We conducted a study using two different rat strains, where one was the standard laboratory rat (Wistar) and the other was the alcohol-preferring AA (Alko Alcohol) rat. The AA rat line is produced by

selective breeding based on high voluntary alcohol consumption, and therefore, these rats are naturally prone to high alcohol consumption, which is hypothesized to occur due to abnormal function of opioidergic mechanisms (Eriksson, 1968; Hyytiä and Sinclair, 1989; Koistinen et al., 2001; Sommer et al., 2006). AA rats, thus, provide a potent tool for studying decision-making deficits of the animals whose brain is neurobiologically wired to favor alcohol. To investigate how opioidergic mechanisms alter cost/benefit decision-making in these two rat strains, we used a modulated probabilistic discounting task (Cardinal and Howes, 2005; Adriana and Laviola, 2006; St Onge and Floresco, 2009). We examined the effects of the opioid agonist morphine and the opioid antagonist naltrexone via systemic administration.

MATERIAL AND METHODS

Animals

Two groups of 12 male alcohol-preferring AA (Alko Alcohol) rats (University of Helsinki) and two groups of 12 male Wistar rats were used in the study. One group of AA rats and one group of Wistar rats were chosen randomly for morphine treatments, and the other two groups were chosen for naltrexone treatments. For At the beginning of the experiment, rats were three to four months old. On arrival, rats were given 1 week to acclimate to the environment. Food (regular chow SDS RM1 [E] SQC; Witham, Essex, England) and water was available ad libitum in the home cage. Rats were housed three per cage in a temperature and humidity controlled room, with lights controlled on a reversed light/dark cycle of 12/12 hours. All experiments were conducted in the dark phase of the light cycle. All testing was in accordance with the Animal Experiment Board of Finland.

Drugs

The opioid agonist morphine (Yliopiston Apteekki) and opioid antagonist naltrexone (Sigma-Aldrich) were used as the test drugs. Doses of both drugs were 0.3 and 1.0 mg/kg. Drug doses were calculated as salt weights and dissolved in 0.9% saline. 0.9 % saline was used as a vehicle for injection. Drugs and vehicle were given in a Latin square design. Drug doses were administered as injections (s.c.) at a volume of 1 ml/kg 20 min before testing. Each drug/vehicle test day was preceded by at least three drug-free days. A stable baseline of operant behavior was required for three consecutive days before the next injection was administered. Each rat had their baseline values calculated separately, and each rat also received drugs based on individual stability in baseline values. The criterion for stable baseline was

achieved when the standard error of the mean in LL ("large-lucky," defined later) lever choices (± SEM) of three previous baseline session averages was under 5.00.

Apparatus

Behavioral testing was conducted in operant chambers (30.5 X 24 X 21cm; 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.

Lever Press Training

Three days before the first lever press training session, rats were placed in the operant chambers for 15 minutes 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 habituation days, the training period, which included three phases (A, B, and C), was initiated. In phase A the rats were trained in forced-choice for 60 minutes so that only one lever was always present (left or right). By pressing the lever, rats received one sucrose pellet with a three-second time-out during which the cue light was on. Phase A consisted of a total of six training sessions, and the presented lever was changed each session.

In phase B, the rats were trained for 30 minutes 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 lever that was pressed retracted, and the other lever was presented after three seconds time out. During this, the cue light lit above the lever that had been pressed and stayed on for three seconds. Phase B consisted of a total of six training sessions.

In the last training phase, C, the rats were trained in a free-choice task for 15 minutes so that both levers were presented

at the same time and by pressing either one of the levers the rat received one sucrose pellet, the cue light above the lever pressed lit for 15 seconds, both levers were retracted, and they were presented again after 15 seconds. Phase C consisted of a total of six training sessions.

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

Rational Decision-Making Task

The rational decision-making task used here was modified from its original form based on the literature (Cardinal and Howes, 2005; Adriani and Laviola, 2006; St Onge and Floresco, 2009) and our previous experiments (Oinio et al., 2017; Oinio et al., 2018). The task consisted of a total of 15 sessions, one session per day for five days a week. One lever was designated the SS-lever ("small/sure"), the other the LL-lever ("large/lucky"). The choice of the SS-lever always delivered one pellet with a probability of 100%, and the choice of the LL-lever delivered three pellets with a probability of 100%. When the session started, the house light went off, and both levers were presented simultaneously. After pressing a lever, both levers retracted, and the cue light above the lever that had been pressed was lit, and one (SS lever) or three (LL lever) sucrose pellets were delivered to the food tray. Multiple pellets were delivered 0.5 s apart. After sucrose was delivered, the cue light remained on for another 15 seconds, after which both levers were presented again.

In one session, rats had free choice to press the levers at 15-second intervals, and the duration of one session was set to 24 lever presses or 30 minutes. Session durations were registered from all sessions. Based on the literature and our previous findings (Floresco et al., 2008; Haluk and Floresco, 2009; Stopper et al., 2013; Oinio et al., 2017; Oinio et al., 2018), levers were not randomized but were counterbalanced so that for each rat the LL-lever was designated to be the side that the rat did not spontaneously prefer during training phase C. Designated levers remained consistent throughout sessions for each rat.

The criterion for rational choice behavior (Rational Choice Criterion, RCC) was set to LL-lever choice of ≥75% or three times the starting level (session 1). Either one of these criteria had to be achieved at least until the 15th session. Rats that achieved either one of these criteria were considered to behave rationally and proceeded to the probabilistic discounting task.

The Probabilistic Discounting Task

This task was divided into three different probability levels where the probability of gaining three sucrose pellets by pressing the LL-lever was decreased over time (50%, 33%, 25%) while the SS-lever always delivered one pellet with 100% probability. Rats received five consecutive sessions with LL-lever probability of 50%, after that five consecutive sessions with LL-lever probability of 33% and finally ten consecutive sessions with LL-lever probability of 25%, respectively. After the tenth session at the probability level of 25%, rats were given two saline injections (s.c.) to habituate the rats to the upcoming drug challenges.

Initiation of Drug Challenges

The criterion to initiate the drug challenges (Risk Aversion Criterion, RAC) was set so that the LL-lever choice of rats had to be ≤ 50% at least after the 10th session at the probability level of 25%. In addition, completion of all the 24 lever presses for 30 minutes was required. During the drug challenges, rats went through one session each day at the probability level of 25%, and rats were given injections in the previously described manner.

Satiety control

After drug challenges, the effect of morphine and naltrexone on the sucrose pellet eating was studied in a 30-minute free sucrose pellet-eating test. In this test, rats were placed in the operant chamber for 30 minutes (house light off) with the additional food cup placed in front of the central food tray. The food cup was filled with 10.0 g of sucrose pellets. After 30 minutes, rats were removed, and the remaining sucrose pellets were weighed in order to calculate sucrose pellet consumption. Sucrose consumption was calculated in g/kg for each rat. The effects of both drugs were examined with two doses (0.3 mg/kg and 1.0 mg/kg), and saline was given as a vehicle. Drugs and vehicle were given s.c. in a Latin square design 20 minutes prior to satiety test with three days injection-free time between each dose.

Data Analysis

Data were analyzed with SPSS version 25.0. Data were collected on all rational choice behavior, probabilistic discounting, drug challenges, lever pressing activity, session duration, and satiety control. To detect effects on drug,

strain, or drug x strain interaction, all data were analyzed by two-way repeated measures ANOVA. If the significant main effect was detected, additional statistical testing was conducted by one-way ANOVA with repeated measures to observe any effects within each group. Bonferroni's test was used as a post hoc test. LL-lever choices in the drug challenges task were compared to the vehicle (LL-lever choice (%) = percentage of LL-lever choices of total lever responses. A criterion for significance in all tests was set at $p < 0.05$.

RESULTS

Rational Choice and Probabilistic Discounting

All four groups showed similar decision-making behavior during the rational choice and probabilistic discounting tasks (Fig 1). A two-way repeated measures ANOVA revealed significant main effects of sessions [$F(4, 156) = 121.57$ $p < 0.001$], but no group × session interaction was detected [$F(12, 156) = 0.87$, $p = 0.58$]. Post hoc test with Bonferroni revealed significant effects between session 1 and 15 ($p < 0.001$), session 15 and 20 ($p < 0.001$), session 20 and 25 ($p < 0.001$) and session 25 and 35 ($p < 0.001$). Data presented in the Fig. 1 represent only the behavior of those rats that fulfilled both criteria (RCC and RAC) that were demanded to proceed to the drug challenges.

Fig 1. Session 1 represents lever choices at the beginning of the study, and session 15 represents the rational choice behavior of Wistar and AA rats that fulfilled the criteria set for proceeding to drug challenges. Session 20 represent lever choices at the last session at LL-lever probability level 50%, session 25 at level 33%, and session 35 at the level 25%. [LL-lever choice (%) = percentage of LL-lever choices of total lever responses, RCC = Rational Choice Criterion, RAC = Risk Aversion Criterion, ± SEM].

Drug Challenges

No statistically significant main effect on drug challenge [$F(2,40)=1.06$, $p=0.36$] or strain x drug challenge interaction [$F(2,40)=2.44$, $p=0.1$] was detected between AA and Wistar rats after administration of morphine.

Fig 2. There was no difference in the LL-lever choices between AA or Wistar rats after the administration of morphine [LL-lever choice (%) = percentage of LL-lever choices of total lever responses, ± SEM].

No statistically significant main effect on drug challenges was observed [F(2,64)=2.64, p=0.84] after administration of naltrexone. However, significant strain x drug challenge interaction was detected [F(2,38)=7.07, p=0.002] and post hoc test with Bonferroni reveleaded significant effect between vehicle and naltexone dose of 0.3 mg/kg (p=0.043). One-way ANOVA within the strains showed that the naltrexone significantly increased LL-responding in the Wistar rats [(2,20)=11.05, p=0.001] and post hoc test with Bonferroni revealed significant effect between vehicle and naltrexone dose of 0.3 mg/kg (p=0.009) and vehicle and naltrexone dose of 1.0 mg/kg (p=0.001). No significant effects in AA rats were detected [(F2,18)=0.93, p=0.41].

Fig 3. Naltrexone increased LL-lever choices in Wistar rats with doses of 0.3 mg/kg and 1.0 mg/kg. In AA rats, naltrexone did not affect the lever choices [LL-lever choice (%) = percentage of LL-lever choices of total lever responses, ± SEM].

Learning effect

After all drug administrations were completed, we wanted to observe any strain-specific differences at the baseline lever choosing behavior due learning effect. We conducted this by comparing the mean of three-day baselines preceding the first drug/vehicle administration to the mean of three-day baselines before the last drug/vehicle administration. The mean baseline LL-lever choice in groups before the first dose were AA (n=10) 29.17 ± 4.02, AA (n12) 30.79 ± 3.07, Wistar (n10) 28.89 ± 3.69 , Wistar (n=11) 34.68 ± 4.11 and the mean baseline LL-lever choice in groups before the last dose were AA (n=10) 23.19 ± 4.27, AA (n=12) 25.46 ± 2.18, Wistar (n=10) 25.32 ± 3.42 and Wistar (n11) 22.22 ± 3.06. Two-way repeated measures ANOVA revealed significant main effect of time [(F1,39)=23.35, p<0.001)], but no group x time interaction was detected [(F3,39)=1.91, p=0.14)]. This result indicates that there occurred learning throughout the study. However, the Latin Square design was used to balance this learning effect, and the LL-lever choices after drug administrations were also compared to the three-day baseline preceding the drug administration to ensure the effects of drugs to the lever choosing behavior (SUPPLEMENT).

Effect of morphine and naltrexone on the session duration and lever pressing

The secondary effects of morphine and naltrexone were observed in the sessions. With morphine there was no effect detected on the session duration [F(2,40)=2.70, p=0.079] or strain x session duration interaction [F(2,40)=0.11, p=0.89].

Naltrexone dose-dependently increased the of session duration in all groups [$F(2,38)=22.06$, $p<0.001$], but no strain x session duration interaction was found [$F(2,38)=0.47$, $p=0.63$]. Post hoc test with Bonferroni revealed significant effects on session durations between vehicle and naltrexone dose of 0.3 mg/kg ($p=0.002$) and vehicle and naltrexone dose of 1.0 mg/kg ($p<0.001$).

Morphine had no effect on the lever responses during the sessions (Table 2). Naltrexone reduced the lever responses of AA rats, but only in few animals, so we were not able to execute any appropriate statistical testing.

Effect of morphine and naltrexone on sucrose pellet consumption

In the sucrose eating test, rats had free access to an unlimited amount of sucrose pellets in operant chambers for 30 minutes. The amount of sucrose consumed was converted to g/kg. In morphine treated rats two-way repeated measures ANOVA revealed significant main effect on sucrose eating [$(F2,40)=10.16$, $p<0.001$], but no strain x sucrose eating interaction was detected [$(F2,40)=0.65$, $p<0=0.53$].

In naltrexone treated rats two-way repeated measures ANOVA revealed significant main effect on sucrose eating [$(F2,36)=29.82$, $p<0.001$], but no strain x sucrose eating interaction was detected [$(F2,36)=0.98$, $p<0=0.39$. One Wistar rat was out of this study due to dying before entering to this study.

DISCUSSION

Here, we studied the role of opioid agonist morphine and opioid antagonist naltrexone on the cost/benefit decision making in AA and Wistar rats. As expected, naltrexone decreased the motivation to pursue rewards in AA and Wistar, but surprisingly resulted in Wistar rats executing significantly more non-beneficial choices compared to vehicle. This irrational response to naltrexone is challenging to explain because of the lack of reliable support from the literature. After morphine administration we found no difference in the decision-making between AA and Wistar groups, but after exploring the control data, we found decision enhancing effect in AA rats. Collectively, these data indicate that brain functions that mediate cost/benefit decisions respond differently to opioidergic drugs in AA and Wistar rats.

Overall, our model used in this study captures the essence of "normal" decision-making by showing the similar decision-making behavior in both rat groups, seen similarly in humans considering the theory published by Kahneman and Tversky in 1979. Both rat groups display a rational decision-making pattern in the first 15 sessions, but right after the LL-lever probability changed to 50%, both groups immediately start to shift the lever choosing towards the SS lever. However, it would be beneficial to choose the LL-lever, showing rats acting as risk-aversive in the situation where uncertainty is involved. At the probability level of 33%, lever choosing of rats is approximately indifferent, as it should be, because at this level choices make no difference in sucrose gain. After moving to the probability level of 25%, choices shift towards the SS-lever, indicating that rats are capable of making choices that optimize reward gain. For the nature of the model used in this study, we must note that the rats are continually learning to choose the better option, and because of this the LL-lever choices at the probability level 25% are slowly decreasing throughout the study. Although it is a definite con of the study, it is also a major pro because instead of fixating to specific choice behavior, rats are continually weighing the costs vs. benefits of the lever choice. We controlled this slowly changing baseline level by using the Latin square design and also comparing drug effects to the three-day baseline preceding the drug testing session (SUPPLEMENT) during the drug administrations. We must note that although baseline differences between AA and Wistar rat groups (selected based on RCC and RAC) showed no significant differences at any session time point of the study, this does not exclude the possibility that there is a small strain-specific difference in the baseline decision-making between these strains. This is an issue that should be studied using a higher number of animals.

The results with morphine did not show difference in LL-lever choices between the AA and Wistar rats. Although after investigating the three-day baseline comparisons to the drug doses (SUPPLEMENT), we found a modest effect with morphine dose-dependently increasing the choosing the more beneficial option, but only in AA rats. Even though the design of the study (the number of animals) lacks statistical power to show the effect between AA and Wistar, the finding with AA rats can reveal some possible effects of opioidergic modulation in the decision-making. Unfortunately, animal studies on this subject are few. Therefore it is challenging to find preclinical data to strengthen this result. However, there is some clinical evidence that small to moderate doses of morphine could enhance cognitive performance in humans (Van Steenberg et al., 2019). Morphine was found to enhance the performance of cognitive functions such as improving accuracy on the choice reaction time task (Hanks et al., 1995; O'Neill et al., 2000) and increasing prepulse inhibition (Quednow et al., 2008). In a study by Syal et al. in 2015, the partial μ-opioid agonist buprenorphine was found to improve memory for reward ques. A study by Eikemo et al. (2017) reported that morphine

shifted choices towards high-value rewards in a two-choice paradigm conducted with human participants. Although in their study, the high-value rewards were not associated with an opportunity to win large uncertain rewards, it shows that opioid agonism could bias choices towards the most rewarding outcome, which is comparable to our results with AA rats.

The opioidergic system is also associated with impulsivity (Harvey-Lewis et al., 2014), and morphine is shown to discount reward value of delayed rewards in rats (Kieres et al., 2004; Pattij et al., 2019). A similar effect could also explain our results, although on this task, the reward is probabilistic and uncertain, not delayed. How rats perceive uncertain rewards are, however, not fully understood, and rats may perceive these as a delayed reward since choosing the LL and not receiving any pellets forces the rat to wait without any reward. In this case, increased impulsivity caused by morphine would shift choices towards the SS-lever. Mitchell et al. 2011 reported that morphine had a trend towards increasing risky choices in rats in decision-making when the large reward was accompanied by the risk of delivery of a mild footshock punishment with different probabilities. Interestingly, they also found that amphetamine decreased risky choices, although in cost/benefit paradigms (St Onge and Floresco 2009, Oinio et al. 2017), amphetamines have been robustly shown to increase choices of larger but more uncertain options. This indicates that decisions that are guided purely by the hedonic value of the reward are modulated differently than choices accompanied with an element of physical risk. However, opioid modulation could still alter the judgments of probability-based decisions.

The data of the satiety control shows that the morphine increased the sucrose consumption of AA and Wistar rats. The doses of morphine used in this study had no significant effects on the lever responses or session duration. Opioid agonism has been shown to increase the rewarding value of palatable food (Berridge, 1996; Eikemo et al., 2016; Nummenmaa et al., 2018), and the literature show's AA rats' heightened response to opioidergic drugs (Honkanen et al., 1999; Soini et al., 2002; Ojanen et al., 2003). However, in this study, we did not observe any difference between AA and Wistar. Even though the morphine would increase the palatability of sucrose rewards in AA rats, as could be expected by the literature, it would not explain why AA rats chose the SS-lever over the LL-lever because increased palatability should also affect large rewards. One considerable explanation is that instead of making AA rats more risk aversive, the morphine made AA rats more loss aversive, referring to a behavior where the risk of not receiving the reward is less acceptable than a smaller but guaranteed reward (Kahneman and Trersky, 1979; Cocker and Winstanley, 2015; Sokol-Hessner and Rutledge, 2019). Altogether, these findings cautiously suggest that morphine receptor activation could enhance the decision-making functions of AA rats. The same effect could also occur in Wistar rats if

the doses of morphine were higher, but there is also a possibility that in higher doses morphine could mask the decision-making behaviors due to indirect effects on motor behaviors or sedative effects.

Naltrexone, on the other hand, had no effects on the decision-making of AA rats, but surprisingly had a significant effect of promoting the Wistar rats to execute more choices towards large and uncertain rewards. To our knowledge, there is only one study where naltrexone's effect is studied in a rat gambling task (Di Ciano and Le Foll, 2016). In this study, naltrexone improved decision-making in a subset of animals that favored the disadvantageous choice at the baseline. The dose of naltrexone used was 3 mg/kg, which we could not use because our preliminary data showed that doses higher than 1 mg/kg reduced the lever-pressing activity of AA rats too much (presumably due to ad libitum feeding). Another study in mice found that naltrexone did not affect the decision-making in the mouse gambling task, although it decreased the impulsive behavior in the five-choice serial reaction time task (Sanchez-Roige et al., 2015). Because of the limited number of preclinical studies, we must mirror the results to clinical findings. Naltrexone has shown to reduce, e.g., the craving to gamble, gambling-related thoughts, and gambling frequency (Victorri-Vigneau,

2018). The mechanism of action of naltrexone on the control of decision-making during gambling, however, is unclear. One study conducted on recreational gamblers found that naltrexone modulated the responsiveness to wins, but in the opposite direction that was predicted (Porchet et al., 2013). Instead of blunted responsiveness to wins, the participants treated with naltrexone displayed higher electrodermal activity and heart rate following wins – indicating heightened responsiveness to wins. They reported that naltrexone made test subjects more confident in the decision-making setting. In terms of risk aversion, this could mean that naltrexone made test subjects less risk aversive by modulating those neuronal systems that make people avoid risks (fear of losing), which might be the case found in the Wistar rats in this study.

Naltrexone reduced sucrose consumption very effectively in both rat strains, as we predicted because the opioidergic system has a major role in the control of appetitive factors (Majuri et al., 2017; Nummenmaa et al., 2018). In particular, the "liking" and "wanting" of sucrose is linked to opioidergic mechanisms of the nucleus accumbens (Berridge, 1996; Berridge and Kringelbach, 2015). Naltrexone also increased session time in both groups, and in AA rats it also reduced the number of lever presses rats made during the sessions. These results are in line with other studies where naltrexone was found to decrease lever responding in operant conditions (Sanchez-Roige et al., 2015). Altogether these results indicate that naltrexone could reduce the experienced value of the rewards and thus decrease the motivation to pursue

these which is also in line with the findings that naltrexone can reduce the, e.g., alcohol drinking or urge to gamble (Grant et al., 2006; Grant et al., 2008; Potenza, 2008). However, the clinical findings have shown that naltrexone is effective in some individuals (especially if they have a family history of AUD), but not in all (Grant et al., 2006; Grant et al., 2008; Potenza, 2008), and the reason for this remains unexplained. Our results cautiously suggest a theory that although naltrexone can decrease the experienced value of the rewards, it could at the same time, increase the cognitive biases in some individuals and reduce risk aversiveness. In a clinical setting, this could mean that albeit decreasing value of experienced rewards, naltrexone could also make some individuals more confident in decision-making resulting in no observed changes in the overall gambling behavior.

As in all studies in behavioral pharmacology, we must note some limitations. First, this study examines the role of the opioidergic mechanisms in the "whole brain" and thus makes it impossible to interpret exact mechanisms of how the opioidergic system is modulating decision-making. The findings emphasize the need for more specific preclinical studies focusing on the neurobiological factors. Also, one issue is that the effects of drugs were only examined at the baseline decision-making of the rats and only in rats that fulfilled the criteria for "normal" decision-making. The outcome could be different in a setting where the dopaminergic activity is already disturbed as it is supposed to be in individuals with GD or where a larger cohort of rats were used without selection based on RCC and RAC.

In conclusion, this study shows that activating or inactivating the opioidergic system modulates the strategy for choosing options that are made by cost/benefit judgments, and in AA rats, opioid receptor activation could enhance decision-making behavior. The results also create a logical assumption that individual differences in the opioidergic system alter cost/benefit decisions.

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