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Group I metabotropic glutamate receptor antagonists impair discriminability of reinforcer magnitude, but not risky choice, in a probability-discounting task

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Abstract

The glutamatergic system has been identified as an important mediator of risky choice. However, previous studies have focused primarily on ionotropic glutamate receptors (e.g., NMDA receptors). Little research has examined the contribution of metabotropic glutamate receptors (mGluRs) on risky choice. The goal of the current experiment was to determine the effects of mGluR₁ and mGluR₅ antagonism on risky choice as assessed in probability discounting (PD). Male Sprague Dawley rats ($n = 24$) were trained in PD, in which consistently choosing a large, probabilistic reward (LR) reflects risky choice. For half of the rats, the odds against (OA) receiving the LR increased across blocks of trials, whereas the OA decreased across the session for half of the rats. Following training, rats received injections of the mGluR1 antagonist JNJ 16259685 (JNJ; 0, 0.1, 0.3, or 1.0 mg/kg; i.p.) and the mGluR5 antagonist MTEP (0, 1.0, 3.0, or 10.0 mg/kg; i.p.). Regardless of which schedule was used, JNJ and MTEP decreased preference for the LR when its delivery was guaranteed. In contrast to delay discounting, in which blocking the mGluR₁ has been shown to alter impulsive choice, these results show that the Group I mGluR family does not selectively alter risky choice. Instead, blocking these receptors appears to impair discriminability of reinforcers of varying magnitudes in PD.

Keywords

risky choice; probability discounting; sensitivity to probabilistic reinforcement; discriminability of reinforcer magnitude; metabotropic glutamate receptor; rat

Probability discounting (PD) is often used to measure risky choice in animals (see [1] for a recent review). Recent evidence has implicated the glutamatergic system as being an important mediator of PD. The N-methyl-D-aspartate (NMDA) receptor antagonists MK-801 and ketamine differentially alter performance in this task, as MK-801 decreases sensitivity to probabilistic reinforcement (i.e., increases risky choice), whereas ketamine

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impairs discriminability of reinforcer magnitude (i.e., decreases responding for the large magnitude reinforcer [LR], even when its delivery is guaranteed) [2–3]. Furthermore, the GluN2B-selective antagonists Ro 63–1908 and CP-101,606 increase risky choice when the probability of earning the LR decreases across the session [4].

In contrast to NMDA receptors, the contribution of metabotropic glutamate receptor (mGluR) ligands on risky choice has not been explored. Thus far, research has primarily examined the contribution of the Group I mGluR family (composed of the mGluR₁ and the mGluR₅) to impulsive choice, as assessed with delay discounting (DD). Overall, mGluR₅ antagonists do not alter impulsive choice [5–6]; however, previous work has reported some inconsistent effects of mGluR₁ antagonists on DD. One study found that blocking the mGluR₁ decreases impulsive choice [7], whereas we found that blocking these receptors *increases* impulsive choice [6]. Overall, the goal of the current experiment was to determine the effects of Group I mGluR antagonists on PD performance. Following behavioral training, rats received injections of JNJ 16259685 (selective mGluR1 antagonist; referred to as JNJ from here on) and MTEP (selective mGluR5 antagonist). Because our previous work has shown that the order in which probabilities are presented modulates drug effects in discounting procedures [3–4; 6], we tested two separate groups of rats: one in which the probability of earning the LR increased across the session and one in which the probability decreased across the session.

A total of 24 male, adult Sprague Dawley rats (200–224 g upon arrival to the laboratory; Envigo, Indianapolis, IN) were used in the current experiment. Rats were previously used in a conditioned place preference (CPP) experiment. Twelve rats were given four injections of methamphetamine (1.0 mg/kg) and one injection of the NMDA GluN2B subunit antagonist Ro 63–1908 (3.0 mg/kg for six rats; 10.0 mg/kg for six rats). Twelve rats were given four injections of Ro 63–1908 (3.0 mg/kg). The rats' drug history did not affect baseline discounting (data not shown). Rats were individually housed in clear polypropylene cages (51 cm long × 26.5 cm wide × 32 cm high) with metal tops containing food and a water bottle in a room maintained on a 12:12-h cycle. Rats were tested during the light phase and were restricted to 15 g of food each day but had *ad libitum* access to water. All experimental procedures were carried out according to the Current Guide for the Care and Use of Laboratory Animals (USPHS) under a protocol approved by the Northern Kentucky University Institutional Animal Care and Use Committee.

3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(cis-4-methoxycyclohexyl)-methanone (JNJ 16259685) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine hydrochloride (MTEP) were purchased from Tocris Bioscience (Ellisville, MO). Both drugs were dissolved in saline (0.9% NaCl) mixed with 10% Tween 80. JNJ had to be heated and stirred to get into solution. All injections occurred at room temperature at a volume of 1 ml/kg.

Eight operant conditioning chambers (28 × 21 × 21 cm; ENV-008; MED Associates, St. Albans, VT) located inside sound attenuating chambers (ENV-018M; MED Associates) were used. A description of the operant chambers has been detailed previously [8]. Rats were tested in a PD task as previously described [3]. See Figure 1 for a schematic of the procedure. Briefly, each session consisted of five blocks of trials (8 forced-choice trials and

10 free-choice trials), in which the odds against (OA; $OA = [1/\text{probability}] - 1$) [9] receiving the LR either increased (0–31 OA for half of the subjects) or decreased (31–0 OA for half of the subjects) across the session. In contrast to our previous work [2–4], we used a 2:1 magnitude ratio as opposed to a 4:1 ratio. We used these reinforcer magnitudes in an effort to attenuate baseline differences that have been observed in subjects trained on ascending and descending schedules in our laboratory [3–4]. Following behavioral training (47 sessions), rats received injections of the mGluR₁ antagonist JNJ (0, 0.1, 0.3 or 1.0 mg/kg, i.p.) and the mGluR5 antagonist MTEP (0, 1.0, 3.0, or 10.0 mg/kg; i.p.). Half of the rats received JNJ injections first, whereas the other half received MTEP injections first. Each dose was administered in a randomized order. Rats received each dose of a particular drug before receiving injections of the other drug. JNJ injections occurred 40 min prior to task performance, and MTEP injections occurred 15 min prior to each session. The doses and pretreatment times were chosen based on previous work [5–6; 10].

The exponential discounting function was fit to each subject's data and is defined by the equation $V = Ae^{-h\theta}$, where V is the subjective value of the LR, A denotes preference for the LR when its delivery is guaranteed, h is the rate of discounting (i.e., risky choice), and θ is the OA delivery of the LR. The exponential function was fit to the data via nonlinear mixed effects modeling (NLME) using the NLME tool in the *R* statistical software package [11], with A and h as free parameters. To determine if baseline A and h parameter estimates differed across rats trained on the ascending and the descending schedule, the NLME models defined schedule as a fixed, nominal between-subjects factors, OA as a fixed, continuous within-subject factor, and subject as a random factor. Specifically, both A and h parameters were allowed to vary across subjects. To determine if JNJ or MTEP altered parameter estimates, similar NLME models were used, except that dose was defined as a fixed, nominal within-subjects factor. Separate NLME models were used to analyze each drug (JNJ and MTEP) treatment. Because this analysis did not reveal a significant main effect of schedule or a significant schedule $\times$ dose interaction for either drug, a second analysis was conducted in which the ascending and descending schedules were combined. A significant main effect of dose was probed using contrasts in *R*. Statistical significance was defined as $p < .05$.

The number of completed trials was analyzed with the non-parametric Friedman test. Because omissions did not differ between rats trained on the ascending and the descending schedules, a single Friedman test was used for each drug. A main effect was probed with Wilcoxon signed-rank tests. Statistical significance was defined as $p < .05$ for the Friedman test; however, significance was defined as $p < .017$ for the Wilcoxon signed-rank tests.

Figure 2a shows the raw proportion of responses for the LR option as a function of OA in rats trained on the ascending schedule and on the descending schedule. The exponential discounting function was fit to individual subject data *via* NLME, and the A and h parameter estimates are depicted in Figures 2b and 2c, respectively. Rats trained on the descending schedule had significantly higher A parameter estimates, $F(1, 93) = 4.220$, $p = .043$ (Fig. 2b), and lower h parameter estimates, $F(1, 93) = 4.219$, $p = .043$ (Fig. 2c), relative to rats trained on the ascending schedule.

Figure 3a shows the raw proportion of responses for the LR option as a function of OA following injections of JNJ. Because the original NLME analysis showed no main effect of schedule or a significant schedule $\times$ dose interaction on each parameter, F 's = 1.101, .349, the NLME analyses only included the factor dose in subsequent analyses. Figure 3b shows A parameter and h parameter estimates following JNJ administration. JNJ (each dose) significantly decreased A parameter estimates (i.e., decreased preference for the LR when its delivery was guaranteed), $F(3, 371) = 12.968$, $p < .0001$, and the highest dose (1.0 mg/kg) decreased h parameter estimates (i.e., decreased sensitivity to probabilistic reinforcement), $F(3, 371) = 5.467$, $p = .001$, (Fig. 3b). Figure 3c shows the number of completed trials following JNJ administration. Each dose of JNJ significantly decreased the number of completed trials, $\chi^2(3, N = 24) = 22.197$, $p < .0001$.

Figure 4a shows the raw proportion of responses for the LR option as a function of OA following injections of MTEP. Because the original NLME analysis showed no main effect of schedule or a significant schedule $\times$ dose interaction on each parameter, F 's = 1.959, .120, the NLME analyses only included the factor dose in subsequent analyses. Figure 4b shows A parameter and h parameter estimates following MTEP administration. MTEP (each dose) significantly decreased A parameter estimates (i.e., decreased preference for the LR when its delivery was guaranteed), $F(3, 363) = 57.854$, $p < .0001$, and the highest dose (10.0 mg/kg) decreased h parameter estimates (i.e., decreased sensitivity to probabilistic reinforcement), $F(3, 363) = 3.731$, $p = .012$, (Fig. 4b). Figure 4c shows the number of completed trials following MTEP administration. The two higher doses of MTEP significantly decreased the number of completed trials, $\chi^2(3, N = 24) = 41.240$, $p < .0001$.

Concerning baseline differences, rats trained on the descending schedule had lower h parameter estimates (i.e., decreased sensitivity to probabilistic reinforcement), which is consistent with previous research conducted in our laboratory using a 4-pellet LR [3–4]. However, rats in the ascending schedule also showed decreased preference for the LR when its delivery was guaranteed (A parameter estimates) compared to rats trained on the descending schedule. This finding may appear to suggest that the A and h parameters are not independent of one another (e.g., as one decreases, the other increases); however, this is not necessarily the case. Our previous work [3–4] has shown baseline differences in the h parameter estimate without corresponding changes in the A parameter estimate.

Interestingly, even though baseline differences were observed between rats trained on the ascending and the descending schedules, probability presentation order did not modulate the effects of JNJ or MTEP on A parameter or h parameter estimates. These results somewhat contrast with our previous work showing that JNJ differentially alters A parameter estimates in rats trained in a DD procedure [6]. In that study, rats trained on the descending schedule were less likely to respond on a manipulandum associated with a 4-pellet reinforcer when its delivery was immediate following JNJ (1.0 mg/kg) administration, whereas JNJ did not significantly alter A parameter estimates in rats trained on an ascending schedule. However, it is important to note that rats trained on the ascending schedule showed a decrease in responding for the LR at the 0-s delay block (94% following vehicle vs. 82% following 1.0 mg/kg JNJ). The discrepant results between the current study and our previous work using DD [8] may be the result of using a 2:1 magnitude ratio instead of a 4:1 ratio. Theoretically,

if we had used a 4:1 magnitude ratio in the current experiment, we may have potentially observed differential drug effects in rats trained on the ascending and the descending schedules. This hypothesis will need to be directly tested in a future study.

Overall, the current results may suggest that blocking Group I mGluRs elicits satiation, accounting for the decreased responding for the LR when its delivery is guaranteed. Although Group I mGluR antagonists can attenuate mGluR agonist-induced feeding behavior, these drugs, when administered alone, do not suppress feeding behavior in food-deprived rats [12]. Thus, satiation does not appear to account for the decreased *A* parameter estimates observed following JNJ/MTEP administration.

Instead of satiation, these results suggest that blocking Group I mGluRs impairs an animal's ability to discriminate reinforcers of varying magnitude. Somewhat similar to this point, Cie lak et al. [13] found that mGluR5 knockdown mice make more random choices in a probabilistic reinforcement learning task. In this task, subjects can respond on one of two manipulandum. Responses on either manipulandum result in delivery of a liquid reinforcer (10 μ l water). For one manipulandum, the probability of receiving this reinforcer is 0.8, whereas the probability of receiving the reinforcer is 0.2 for the other manipulandum. Mice that had decreased mGluR5 expression in dopaminergic neurons were more likely to randomly respond on each manipulandum, suggesting they no longer understood the contingencies of reinforcement. In the current experiment, administration of JNJ/MTEP may have prevented subjects from identifying which lever is associated with the LR alternative. However, it is important to note that Cie lak et al. [13] did not find that a reduction in mGluR5 expression impaired performance in a PD procedure, in which mice chose between a 10- μ l reinforcer and a 20- μ l reinforcer (2:1 ratio; the same as in the current experiment). Directly comparing the current results to those obtained by Cie lak et al. [13] is difficult due to differences in the species used (outbred rat vs. mGluR5 knockdown mouse), the probability range (100, 25, 12.5, 6.25, 3.125% vs. 100, 75, 50, 25%), the way in which probabilities are altered (within a session vs. across sessions), and the reinforcer type (sucrose-based pellets vs. water).

Both JNJ and MTEP significantly decreased the number of completed trials (i.e., increased omissions) in the current experiment. Group I mGluR antagonists do not affect omissions in DD [5–6]. Explaining why JNJ and MTEP significantly alter the number of completed trials in PD but not in DD is somewhat difficult. Although the PD task we use in our laboratory contains more free-choice trials (50) relative to DD (25), the length of each DD session (52.5 min) is longer than PD (45 min). Therefore, it is unlikely the number of free-choice trials can account for the discrepancy observed across PD and DD. One study found that mGluR5 knockdown mice took longer to respond during free-choice trials in a PD experiment, but this effect was only observed when the LR reinforcer was guaranteed [13]. In the current experiment, the decreased number of completed trials was not isolated to one trial block as opposed to what was observed in the Cie lak et al. study [13]. The observed effects following JNJ/MTEP administration may be the result of an interaction between the low probabilities of receiving the LR when its delivery is probabilistic (3.125–25%) and the magnitude of the LR (2 pellets). Cie lak et al. [13] used larger probabilities (25–75%); thus, mGluR5 knockdown mice may have still been motivated to respond for the LR. Conversely,

rats in the current study may not have been as motivated to respond on either lever following JNJ/MTEP administration due to the low probabilities of receiving reinforcement and/or the smaller magnitude of the LR. To test this hypothesis, a follow-up study can be conducted in which (a) rats receive a 4:1 ratio of reinforcement and/or (b) rats have a higher chance of receiving the LR.

Overall, the current study shows that blockade of Group I mGluRs causes a general impairment in PD performance. Specifically, these receptors appear to impair discriminability of reinforcer magnitude, but more work is needed to understand why this disruption is observed in a task using probabilistic reinforcement but is not observed in a task using delayed reinforcement. Future work should test how Group I mGluR ligands affect sensitivity to reinforcer magnitude (i.e., use different reinforcer magnitude ratios, such as 2:1, 4:1, etc.), and future studies can test the effects of mGluR_{1/5} ligands on a probabilistic reinforcement learning task [i.e., 13]. These additional studies can further our understanding of how mGluRs mediate decision making between certain and uncertain reinforcers that differ in magnitude.

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Figure 1. Schematic of the PD task. **(a)** Forced-choice trial. **(b)** Free-choice trial. Abbreviations: FR = fixed ratio; HL = house light; LL = left lever; LR = large magnitude reinforcer; LSL = left stimulus light; OA = odds against; RL = right lever; RSL = right stimulus light.

Figure 2.

(a) Mean ($\pm$ SEM) raw proportion of responses for the LR as a function of the OA receiving reinforcement. The closed symbols represent rats trained on the ascending schedule, and open symbols represent rats trained on the descending schedule. (b) Mean ($\pm$ SEM) A parameter estimates (preference for LR when its delivery is guaranteed) for rats trained on the ascending schedule (white bars) and on the descending schedule (black bars). (c) Mean ($\pm$ SEM) h parameter estimates (sensitivity to probabilistic reinforcement) for rats trained on the ascending schedule (white bars) and on the descending schedule (black bars). * $p < .05$, relative to rats trained on the ascending schedule.

Figure 3.

(a) Mean ($\pm$ SEM) raw proportion of responses for the LR as a function of the OA receiving reinforcement following injections of JNJ 16259685. (b) Mean ($\pm$ SEM) *A* parameter estimates (preference for LR when its delivery is guaranteed; left y-axis; closed symbols) and *h* parameter estimates (sensitivity to probabilistic reinforcement; right y-axis; open symbols). (c) Mean ($\pm$ SEM) number of completed trials. * $p < .05$, relative to vehicle. Note, data from the ascending and descending schedules were averaged together.

Figure 4.

(a) Mean ($\pm$ SEM) raw proportion of responses for the LR as a function of the OA receiving reinforcement following injections of MTEP. (b) Mean ($\pm$ SEM) A parameter estimates (preference for LR when its delivery is guaranteed; left y -axis; closed symbols) and h parameter estimates (sensitivity to probabilistic reinforcement; right y -axis; open symbols). (c) Mean ($\pm$ SEM) number of completed trials. * $p < .05$, relative to vehicle. Note, data from the ascending and descending schedules were averaged together.