Published in final edited form as:

Behav Brain Res. 2018 September 03; 349: 125–129. doi:10.1016/j.bbr.2018.03.039.

Effects of N-methyl-D-aspartate receptor (NMDAr) uncompetitive antagonists in a delay discounting paradigm using a concurrent-chains procedure

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Abstract

Impulsive choice is often assessed in rodents using a delay discounting (DD) paradigm in which the delay to a large reinforcer (LR) increases across the session. This procedure allows one to test the effects of pharmacological manipulations within a single session. Because discounting is influenced by sensitivity to reinforcer magnitude (SRM) and sensitivity to delayed reinforcement (SDR), applying quantitative analyses (e.g., fitting hyperbolic function) is important for determining the precise behavioral mechanisms being altered following drug administration. One caveat to this approach is that observing increases in SMR/SDR can be difficult (e.g., most rats choose the LR when its delivery is immediate, whereas some rats may show exclusive preference for the small reinforcer [SR] when a delay on the LR is imposed). We utilized a variant of a concurrent-chains procedure in which rats ($n = 8$) could not show exclusive preference for either reinforcer, thus allowing one to observe increases/decreases in responding at each delay. The NMDAr antagonists MK-801 (0, 0.003, 0.01, 0.03 mg/kg), ketamine (0, 1.0, 5.0, 10.0 mg/kg), and memantine (0, 2.5, 5.0, 7.5 mg/kg) were administered following baseline training because this receptor has recently been implicated in DD. MK-801 (0.03 mg/kg) decreased SRM and SDR. Memantine (7.5 mg/kg) decreased SRM only. These results show that this variant of the concurrent-chains procedure can be used to study the effects of pharmacological manipulations on distinct aspects of DD.

Keywords

Impulsive choice; concurrent-chains; delay discounting; NMDA receptor; rat

Impulsive choice is often measured using delay discounting (DD) procedures. The most common DD paradigm used in rats allows subjects to choose between a small, immediate reinforcer (SR) and a large, delayed reinforcer (LR), and the delay to the LR increases across the session [1]. The advantage of this procedure is that it allows one to determine how pharmacological manipulations alter DD within a single session. Specifically, a hyperbolic function can be used to describe discounting, which is defined by the equation $V = A/(1+kD)$, where V is the subjective value of the reinforcer, A is the intercept of the function

and indicates how much an animal responds for the LR when its delivery is immediate (for simplicity, we will use the term “sensitivity to reinforcer magnitude” [SRM]), k is the slope of the function and reflects sensitivity to delayed reinforcement (SDR; note: the term impulsive choice is often used interchangeably with SDR, but these terms are not always isomorphic; [2]), and D is delay.

Although this procedure allows one to dissociate the neurochemical basis of SRM/SDR, one limitation is that detecting increases in SRM is difficult, due to the finding that rats typically show exclusive preference for the LR when its delivery is immediate (i.e., ceiling effect for the A parameter). Related to this point, detecting increases in the slope can be difficult for some subjects as they show exclusive preference for the SR when a delay is imposed on the LR. One way to circumvent these limitations is to incorporate a procedure that prevents subjects from showing exclusive preference for one reinforcer across each delay. In a variant of a concurrent-chains procedure [3], the active lever is predetermined at the beginning of each trial. Thus, for half of the trials, the lever associated with the LR is active; for half of the trials, the lever associated with the SR is active. By using a concurrent-chains procedure, we can better determine if pharmacological manipulations alter SRM and/or SDR, thus allowing us to further understand the neurochemical basis of DD.

In the current experiment, we tested the effects of the NMDAr uncompetitive antagonists MK-801, ketamine, and memantine on DD. The NMDAr was chosen because recent evidence has identified it as an important mediator of DD [4–8]. MK-801 has been shown to decrease impulsive choice [6–7; but see 8] and to increase SRM [7; but see 8]. Ketamine and memantine have been shown to increase impulsive choice [4–5], but other evidence has suggested these ligands decrease SRM without altering SDR [8]. Overall, the main goal of the current study was to determine how NMDAr antagonists alter SRM/SDR in a concurrent-chains procedure. The hypotheses were as follows: (1) MK-801 would increase SRM without altering SDR, and (2) ketamine/memantine would decrease SRM without altering SDR.

Eight male Sprague Dawley rats (Envigo, Indianapolis, IN) were used in the current experiment. Three of the rats (200–224 g upon arrival; approximately 49–52 days of age) had previous training in the Evenden and Ryan [1] procedure and received four injections of amphetamine (0.5 mg/kg) during a conditioned place preference paradigm. The other five rats (240–260 g upon arrival; approximately 56–60 days of age) were housed in the colony for approximately three months before testing began (note, these rats were experimentally naïve). The rats with prior operant training and amphetamine exposure had similar discounting functions at the end of baseline training relative to the experimentally naïve rats (A parameter estimates: 0.722 vs. 0.701; k parameter estimates: 0.003 vs. 0.003). All rats were held in a housing room that has been described previously [10]. Rats were tested in the light phase (approximately 1400–1600 h). Rats were individually housed in cages previously described [8]. Rats were restricted to approximately 10 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.

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. The chambers have been described in detail elsewhere [8].

All drugs were purchased from Sigma Aldrich (St. Louis, MO). (+)-MK-801 hydrogen maleate, (±)-ketamine hydrochloride, and memantine hydrochloride were prepared in sterile 0.9% NaCl (saline). Each drug was injected at room temperature in a volume of 1 ml/kg. The doses were calculated based on salt weight.

Rats received two 10-min sessions of magazine training and five sessions of lever-press training. These procedure have been described in detail elsewhere [8]. Rats received five sessions of magnitude discrimination training. This procedure was similar to those described in [8], with a couple of exceptions. First, responses were reinforced on a variable interval (VI) 2 s schedule of reinforcement. The response requirement was increased between sessions (VI 2 s, VI 4 s, VI 8 s, VI 16 s, VI 30 s; only one VI s schedule was used in a single session). The Fleshler and Hoffman [9] method was used to generate each VI schedule (for the VI 30 s schedule of reinforcement, the intervals were 1.55, 4.89, 8.65, 12.95, 17.98, 24.02, 31.60, 41.79, 57.49, 99.08 s). Second, there was no limited hold in place (i.e., rats did not have to respond within a certain amount of time).

A concurrent-chains procedure was used to measure DD. Each session consisted of five blocks of 10 trials and began with illumination of the house light. On each trial, rats had to initiate the extension of both levers by breaking a photo beam in the food tray. The lever associated with reinforcement was pseudo-randomized (no more than two consecutive trials) throughout the session. During the initial link, responses (VI 30 s) on the “active” lever resulted in (a) the house light turning off, (b) the retraction of both levers, and (c) the initiation of a 2-s dark delay to the terminal link. During the terminal link, both levers were extended into the operant chamber, but only responses (FR 3) on the active lever (the same lever as designated during the initial link) led to reinforcement. The stimulus light above the active lever was illuminated during the terminal link. Both levers were made available during the terminal link because this allowed us to determine if NMDAr blockade altered perseverative responses during this component. Averaged across all trials in a block, the proportion of responses on this lever should be 0.5 during the terminal link. Values above 0.5 indicate perseverative responding on the lever associated with the LR, whereas values below 0.5 indicate perseverative responding on the lever associated with the SR. None of the NMDAr antagonists altered terminal link responses (data not shown).

One lever was associated with delivery of a SR (1 45-mg dustless precision pellet; F0021; Bio Serv, Frenchtown, NJ), and one lever was associated with delivery of a LR (4 pellets). The delay to delivery of the LR increased across blocks of trials (0, 10, 30, 60, 100 s). Following completion of the terminal link, the stimulus light above the active lever was extinguished. Following delivery of the reinforcer, a 30-s intertrial interval (ITI) occurred. The next trial began upon completion of the ITI.

After 28 sessions, rats received treatments of MK-801 (0, 0.003, 0.01, 0.03 mg/kg; s.c.), ketamine (0, 1.0, 5.0, 10.0 mg/kg; i.p.), and memantine (0, 2.5, 5.0, 7.5 mg/kg; i.p.) either 15

min (MK-801/ketamine) or 30 min (memantine) prior to the session. The doses and pre-session treatment times were chosen based on previous research [4–5; 7–8]. The highest dose of memantine (7.5 mg/kg) was chosen because our previous work has shown that a higher dose (10.0 mg/kg) completely suppressed behavior, as evidenced by a near maximum number of omissions [8]. Each drug and dose were administered in a counterbalanced order; rats received each dose of a drug before receiving injections of another drug. Treatments occurred once every four days, and rats received each dose once. Rats were tested in the concurrent-chains procedure as normal in between each injection.

The number of completed trials was analyzed with Friedman tests because this variable was not normally distributed (due to a ceiling effect). Significant effects were probed with Wilcoxon signed-rank tests. Statistical significance was defined as $p < .05$ for the Friedman tests and $p < .017$ for the Wilcoxon signed-rank tests.

The hyperbolic function (see above for equation) was fit to the raw proportion of responses for the LR. The hyperbolic model was fit to each individual subject via nonlinear mixed effects modeling (NLME) using the NLME package in *R* [10]. NLME is an extension of ANOVA that it is applied to nonlinear data and accounts for partially missing data [11]. Because one rat treated with ketamine (10.0 mg/kg) and three rats treated with memantine (7.5 mg/kg) did not respond during one or more blocks of trials, using ANOVA (the typical analysis of DD) would have resulted in listwise deletion of the entire dataset for those subjects. The NLME models defined delay as a fixed, continuous within-subjects factor, dose as a fixed, nominal within-subjects factor, and subject as a random factor. Statistical significance was defined as $p < .05$.

MK-801 did not significantly alter the number of completed trials, but ketamine, $\chi^2(3, N = 8) = 13.909, p = .003$, and memantine (7.5 mg/kg), $\chi^2(3, N = 8) = 19.800, p < .001$, significantly decreased the number of completed trials. Post hoc tests did not reveal significant differences between vehicle and any individual dose of ketamine (due to using a Bonferroni adjusted alpha level of .017; lowest p value reported was for 10.0 mg/kg [.043]). On average, rats completed approximately 43 trials and 38 trials following ketamine (10.0 mg/kg) and memantine (7.5 mg/kg) administration, respectively.

Figure 1a shows the raw proportion of responses for the LR following MK-801 administration. NLME analyses showed that MK-801 (0.03 mg/kg) significantly decreased A parameter estimates, $F(3, 145) = 3.634, p = .015$ (Fig. 1b), and k parameter estimates, $F(3, 145) = 6.568, p < .001$ (Fig 1c). Figure 2a shows the raw proportion of responses for the LR following ketamine administration. Ketamine did not significantly alter A or k parameter estimates (Figs. 2b and 2c, respectively). Figure 3a shows the raw proportion of responses for the LR following memantine administration. NLME analyses showed that memantine significantly decreased A parameter estimates, $F(3, 139) = 18.223, p < .001$ (Fig 3b), without altering k parameter estimates (Fig. 3c).

We originally hypothesized that MK-801 would increase SRM (A parameter estimates) without altering SDR (k parameter estimates); however, MK-801 *decreased* SRM and decreased SDR. Furthermore, we predicted that ketamine would decrease SRM, but this did

not occur. The only hypothesis that was supported was the finding that memantine selectively decreased SRM. Before discussing these findings in more detail, one caveat that needs to be addressed is the low discounting rates (k values < 0.0065) observed in the current experiment. The shallow discounting is most likely an artifact of the number of sessions we used (28); typically, discounting rates increase across sessions [e.g., 3; 8]. As such, we compared the final 3 sessions of training before receiving the first injection (sessions 26–28) to the final 3 sessions before receiving the final injection (sessions 70–72). Even though we found a statistically significant difference in k parameter estimates (0.004 ± 0.001 vs. 0.007 ± 0.001 ; $p = 0.012$), we need to note that the k values observed after 72 sessions were shallower than those reported previously using a similar procedure [3]. Whereas Aparicio et al. [3] used a random interval 12 s schedule of reinforcement, we used a VI 30 s schedule of reinforcement. The differential schedules of reinforcement may explain the shallower discounting observed in our study, as previous research has shown that leaner schedules of reinforcement produce greater responding for the LR [12].

Although the decreased SRM observed following MK-801 administration was unexpected, the decreased SDR observed in the current experiment is similar to past findings [6–7]. Because MK-801 flattened the discounting curve (due to the decreased responding at the 0-s delay), these effects may be interpreted as an example of impaired stimulus control [13–14] as opposed to a change in impulsive choice. This interpretation is plausible, as MK-801 is a known psychotomimetic [15] that alters response perseveration in a discounting procedure [16]. However, if the effects observed with MK-801 were due to impaired stimulus control alone, one would expect MK-801 (0.03 mg/kg) to alter the proportion of responses for the LR during the terminal link, which was not the case. However, one potential limitation needs to be discussed. During the initial link, rats had to break a photobeam in the food tray to extend the levers in the chamber, thus ensuring they were between each lever at the beginning of each trial. During the terminal link, the levers extended into the chamber, regardless of where the rat was located. This is somewhat problematic because the rat may have stayed in the same location following completion of the initial link and then responded on the manipulandum directly in front of them during the terminal link. This may partially explain the altered responses observed during the initial link but not during the terminal link.

Unlike previous research [7–8], ketamine did not significantly decrease SRM in the present study, although ketamine somewhat reduced responding for the LR at the 0-s delay. The null effect reported for SRM could be due to the fact that, following vehicle administration, rats responded for the LR ~75% of the time, whereas they responded 66% of the time following ketamine (10.0 mg/kg) administration, thus observing further decreases in responding at this delay difficult. However, this did not appear to be the case as memantine (7.5 mg/kg) significantly decreased SRM (~77% vs. ~62% responses for the LR at the 0-s delay following vehicle and memantine, respectively). Instead, the schedule of reinforcement used in the concurrent-chains procedure may have modulated the effects of ketamine in discounting. Some evidence, albeit indirect, has suggested that the schedule of reinforcement can alter drug effects in discounting paradigms. Specifically, MK-801 decreased impulsive choice when an FR 1 schedule of reinforcement was used [6–7], but had no effect on discounting when an FR 10 was used [8]. Furthermore, ketamine decreased SRM in a probability discounting procedure when an FR 1 schedule was used [7], but not when an FR

10 schedule was used [16]. Thus, using leaner schedules of reinforcement (FR 10, VI 30 s) seems to negate the effects of ketamine on SRM. Future studies are needed to directly compare how various schedules of reinforcement can modulate drug effects in discounting procedures.

Previous studies have argued that memantine and ketamine increase impulsive choice [4–5]. Our previous work has shown that these ligands decrease SRM without altering impulsive choice [7–8], which was consistent with the current results for memantine, but not for ketamine. Cottone et al. [4] used a modified adjusting delay procedure, which produces a single data point (mean adjusting delay [MAD] scores), thus confounding SRM and SDR. Because these parameters were confounded, Cottone et al. [4] could not determine if changes in MAD scores were due to alterations in SRM, SDR, or a combination of both parameters. Floresco et al. [5] used a procedure similar to those reported by Yates et al. [7–8]; however, the delays were shorter than those used by Yates et al. [7–8] (0.4–6.5 s vs. 0–50 s and 0–100 s). The decreased responding for the LR observed following ketamine administration in the Floresco et al. [5] study may have merely reflected decreased SRM, not SDR. Therefore, using a short range of delays may have precluded Floresco et al. [5] from dissociating the effects of ketamine on SRM and SDR.

Although the concurrent-chains procedure has been used to compare DD across strains of rats [e.g., 3], this procedure has rarely been used to assess pharmacological manipulations on choice for the LR [but see 14, 17]. Considering the variant used in the current procedure does not allow subjects to show exclusive preference for one alternative relative to the other, one is able to observe increases/decreases in the proportion of responses for the LR at each delay. We recommend using this procedure in future studies to further examine the neural basis of impulsive choice.

Acknowledgments

The current study was supported by NIGMS grant 8P20GM103436–14, as well as a Northern Kentucky University Faculty Project Grant.

References

- [1]. Evenden JL, Ryan CN, The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology* 128 (1996) 161–170. [PubMed: 8956377]
- [2]. Yates JR, Dissecting drug effects in preclinical models of impulsive choice: emphasis on glutamatergic compounds. *Psychopharmacology* 235 (2018) 607–626. [PubMed: 29305628]
- [3]. Aparicio CF, Elcoro M, Alonso-Alvarez B, A long-term study of the impulsive choices of Lewis and Fischer 344 rats. *Learn. Behav* 43 (2015) 251–271. [PubMed: 25862317]
- [4]. Cottone P, Iemolo A, Narayan AR, Kwak J, Momany D, Sabino V, The uncompetitive NMDA receptor antagonists ketamine and memantine preferentially increase the choice for a small, immediate reward in low-impulsive rats. *Psychopharmacology* 226 (2013) 127–138. [PubMed: 23104264]
- [5]. Floresco SB, Tse MT, Ghods-Sharifi S, Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. *Neuropsychopharmacology* 33 (2008) 1966–1979. [PubMed: 17805307]

- [6]. Higgins GA, Silenieks LB, MacMillan C, Sevo J, Zeeb FD, Thevarkunnel S, Enhanced attention and impulsive action following NMDA receptor GluN2B-selective antagonist pretreatment. *Behav. Brain Res* 311 (2016) 1–14. [PubMed: 27180168]
- [7]. Yates JR, Batten SR, Bardo MT, Beckmann JS, Role of ionotropic glutamate receptors in delay and probability discounting in the rat. *Psychopharmacology* 232 (2015) 1187–1196. [PubMed: 25270726]
- [8]. Yates JR, Gunkel BT, Rogers KK, Hughes MN, Prior NA, Effects of N-methyl-D-aspartate receptor ligands on sensitivity to reinforcer magnitude and delayed reinforcement in a delay-discounting procedure. *Psychopharmacology* 234 (2017) 461–473. [PubMed: 27837332]
- [9]. Fleshler M, Hoffman HS, A progression for generating variable-interval schedules. *J. Exp. Anal. Behav* 5 (1962) 529–530. [PubMed: 13945507]
- [10]. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team, nlme: Linear and Nonlinear Mixed Effects Models. R package 3.1–128. R Foundation for Statistical Computing, Vienna, Austria, 2016, <http://CRAN.R-project.org/web/packages/nlme/index.html>.
- [11]. Young ME, Clark MH, Goffus A, Hoane MR, Mixed effects modeling of Morris water maze data: advantages and cautionary notes. *Learn. Motiv* 40 (2009) 160–177.
- [12]. Huskinson SL, Anderson KG, Effects of different fixed-ratio requirements on delay discounting in rats. *Behav. Process* 100 (2013) 18–22.
- [13]. Slezak JM, Anderson KG, Effects of acute and chronic methylphenidate on delay discounting. *Pharmacol. Biochem. Behav* 99 (2011) 545–551.
- [14]. Johnson PS, Stein JS, Smits RR, Madden GJ, Pramipexole-induced disruption of behavioral processes fundamental to intertemporal choice. *J. Exp. Anal. Behav* 99 (2013) 290–317. [PubMed: 23436721]
- [15]. Harder JA, Aboobaker AA, Hodgetts TC, Ridley RM, Learning impairments induced by glutamate blockade using dizocilpine (MK-801) in monkeys. *Br. J. Pharmacol* 125 (1998) 1013–1018. [PubMed: 9846639]
- [16]. Yates JR, Breitenstein KA, Gunkel BT, Hughes MN, Johnson AB, Rogers KK, Sharpe SM, Effects of NMDA receptor antagonists on probability discounting depend on the order of probability presentation. *Pharmacol. Biochem. Behav* 150–151 (2016) 31–38.
- [17]. Pitts RC, Febbo SM, Quantitative analyses of methamphetamine's effects on self-control choices: implications for elucidating behavioral mechanisms of drug action. *Behav. Process* 66 (2004) 213–233.

Figure 1. Effects of MK-801 on discounting. Mean ($\pm$ SEM) raw proportion of responses for the LR (a). Mean ($\pm$ SEM) A parameter estimates (b) and k parameter estimates (c). * $p < .05$, relative to vehicle.

Figure 2. Effects of ketamine on discounting. Mean ($\pm$ SEM) raw proportion of responses for the LR (a). Mean ($\pm$ SEM) A parameter estimates (b) and k parameter estimates (c).

Figure 3.

Effects of memantine on discounting. Mean ($\pm$ SEM) raw proportion of responses for the LR (a). Mean ($\pm$ SEM) A parameter estimates (b) and k parameter estimates (c). * $p < .05$, relative to vehicle.