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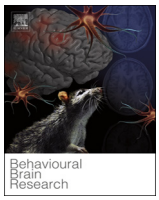

Short communication

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.

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].

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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.

Fig. 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). Fig. 2a shows the raw proportion of responses for the LR following ketamine administration. Ketamine did not significantly alter *A* or *k* parameter estimates (Fig. 2b and c, respectively). Fig. 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.