Endocannabinoids Promote Cocaine-Induced Impulsivity and Its Rapid Dopaminergic Correlates

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Background: Impaired decision making, a hallmark of addiction, is hypothesized to arise from maladaptive plasticity in the mesolimbic dopamine pathway. The endocannabinoid system modulates dopamine activity through activation of cannabinoid type 1 receptors (CB1Rs). Here, we investigated whether impulsive behavior observed following cocaine exposure requires CB1R activation.

Methods: We trained rats in a delay-discounting task. Following acquisition of stable performance, rats were exposed to cocaine (10 mg/kg, intraperitoneal) every other day for 14 days and locomotor activity was measured. Two days later, delay-discounting performance was re-evaluated. To assess reversal of impulsivity, injections of a CB1R antagonist (1.5 mg/kg, intraperitoneal) or vehicle were given 30 minutes before the task. During the second experiment, aimed at preventing impulsivity rather than reversing it, CB1Rs were antagonized before each cocaine injection. In this experiment, subsecond dopamine release was measured in the nucleus accumbens during delay-discounting sessions before and after cocaine treatment.

Results: Blockade of CB1Rs reversed and prevented cocaine-induced impulsivity. Electrochemical results showed that during baseline and following disruption of endocannabinoid signaling, there was a robust increase in dopamine for immediate large rewards compared with immediate small rewards, but this effect reversed when the delay for the large reward was 10 seconds. In contrast, dopamine release always increased for one-pellet options at minimal or moderate delays in vehicle-treated rats.

Conclusions: Endocannabinoids play a critical role in changes associated with cocaine exposure. Cannabinoid type 1 receptor blockade may thus counteract maladaptive alterations in afferents to dopamine neurons, thereby preventing changes in dopaminergic activity underlying a loss of self-control.

Key Words: CB1 receptors, cocaine, decision-making, dopamine, fast-scan cyclic voltammetry, self-control

Human and nonhuman species discount delayed rewarding outcomes; the subjective value of a rewarding outcome depends on how distant in the future it is. In general, when questioned about future options, large distant future rewards are chosen over smaller less distant ones. However, as time elapses and depending on the value of the delayed reward, this preference switches (1); this occurs because immediate rewarding outcomes have a greater subjective value than delayed ones. When the larger delayed reward is chosen over the smaller immediate reward, self-control has been exerted. When the opposite occurs, impulsivity has taken place (2,3).

Drug addiction can be seen as an impairment of decision-making processes, in which the weight of the delayed reward has very little repercussion on the preference of the individual. Therefore, immediate rewards and the instant gratification that drugs of abuse produce are disproportionally chosen over the long-term gratification of a healthy lifestyle (4,5). When tested under laboratory settings, individuals with substance abuse problems (4,6–8), as well as nonhuman subjects that had been sensitized to the effects of drugs of abuse, perform impulsively in tasks that assess self-control (3,9–11). A key neural substrate involved in decision making and an important mediator of rewarding stimuli is the mesolimbic dopaminergic system (12–14). Dopamine (DA) neurons in the ventral tegmental area (VTA) encode the subjective value of reward (15–17). When measured during a delay-discounting task (DDt), in which the size and delay of reward delivery are varied, putative DA neurons fire at a higher rate for cues that predict larger rewards and for rewards with smaller delays (18). When phasic accumbal DA release is measured in a task that manipulates effort and delay to obtain a reward, release is higher for the cue that predicts less effort, as well as for the cue that predicts an immediate reward (19). However, it is presently unclear whether different aspects of a DDt are encoded by phasic DA release in the nucleus accumbens (NAc) and whether they change following a sensitizing cocaine exposure.

Most drugs of abuse, directly or indirectly, alter DA neurotransmission (20) and chronic exposure to them produces plastic changes in multiple brain areas that are believed to underlie addiction (21). Rodents sensitized to the effects of stimulant drugs —and in particular cocaine— show increased DA availability in the NAc (22–24). This increase has been hypothesized to arise from different neural adaptations. Some researchers have emphasized changes in transduction at D1 and/or D2 receptors (13, 25–28), whereas others stress the interaction between DA neurons and inhibitory (29–35) and excitatory neurotransmitters (36–44).

The endocannabinoid (eCB) system is an important component of the reward circuitry. Endocannabinoids are retrograde lipid messengers that bind to cannabinoid type 1 receptors (CB1Rs) localized presynaptically, mainly on excitatory (glutamatergic) and inhibitory (gamma-aminobutyric acidergic) synapses (45,46) where their main effect is to decrease the probability of neurotransmitter release (47,48). These molecules participate in

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synaptic plasticity in the VTA (49,50), where they modulate DA neuron excitability (48,51) and they are also necessary for drug- and reward-predictive cues to evoke DA release (52,53). Endocannabinoid involvement in different forms of plasticity and regulation of DA release makes this system critical in fine-tuning goal-directed behavior (54), particularly when it is compromised, as is the case in drug addiction. Endocannabinoid signaling is involved in conditioned drug seeking and relapse (51,55,56), as well as in cue-induced reinstatement (56–58). Importantly, eCBs play a key role in cocaine sensitization [(59–62) but see (59)], by inducing the cocaine psychomotor response and increasing the reliability of sensitization (62).

Given the pivotal role eCBs play in neural plasticity at the circuit level and their involvement in modulating psychostimulant-induced changes in DA release, the present study investigates the role of CB1R activation in the reversal and blockade of impulsive behavior in a DDt following repeated cocaine administration. In addition, we provide the first demonstration that the eCB system modulates phasic DA release during the development of cocaine-induced impulsivity.

Methods and Materials

Subjects and Surgery

Thirty-six male Long-Evans (Charles-River, St. Constant, Quebec, Canada, or Wilmington, Massachusetts) rats weighing 300 to 350 g at the time of arrival, served as subjects. Rats were individually housed in a temperature- and humidity-controlled room with a 12-hour light-dark cycle (lights on at 07:00 hours). Animals were divided as follows: 18 rats were used in the reversal experiment; 12 of those received cocaine and 6 received saline. The remaining 18 were used in the blockade experiment. Of those rats, 12 were anesthetized with isoflurane and stereotaxically implanted with chronic carbon fiber electrodes (63) aimed at the NAc shell (+1.7 anterior-posterior, +.8 medial-lateral, −7.0 dorsal-ventral), ipsilateral bipolar stimulating electrodes (Plastics One, Roanoke, Virginia) aimed at the VTA (−5.4 anterior-posterior, +.5 medial-lateral, −8.7 dorsal-ventral), and contralateral silver/silver chloride reference electrodes. A triangular input waveform (initial ramp, −.4 to 1.3 V, 400 V/sec) (64) was applied to the recording electrode at 60 Hz for 30 minutes and then reduced to 10 Hz. At this point, electrically evoked DA release was monitored. If needed, the recording electrode was lowered by .1 mm from the initial coordinates until release was observed. Our electrode placement in the dorsal-ventral direction was guided by the kinetics of uptake, which are slower in the shell compared with the core of the NAc. For a detailed description, see Oleson *et al.* (65). Electrodes were secured with dental acrylic and skull-screw anchors. Rats were allowed to recover for at least 10 days, during which time they received food and water ad libitum. Before starting behavioral sessions, rats were food restricted at 85% ± 5% of their free-feeding weight and maintained around this weight throughout the experiment. The final recordings were carried out in eight rats due to poor electrode sensitivity. The remaining six rats received a combination of cannabinoid type 1 receptor (CB1) antagonist and saline. All procedures were carried out in accordance with established practices as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals, as well as by the Canadian Council on Animal Care. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of University of Maryland School of Medicine and by the Animal Research Ethics Committee of Concordia University.

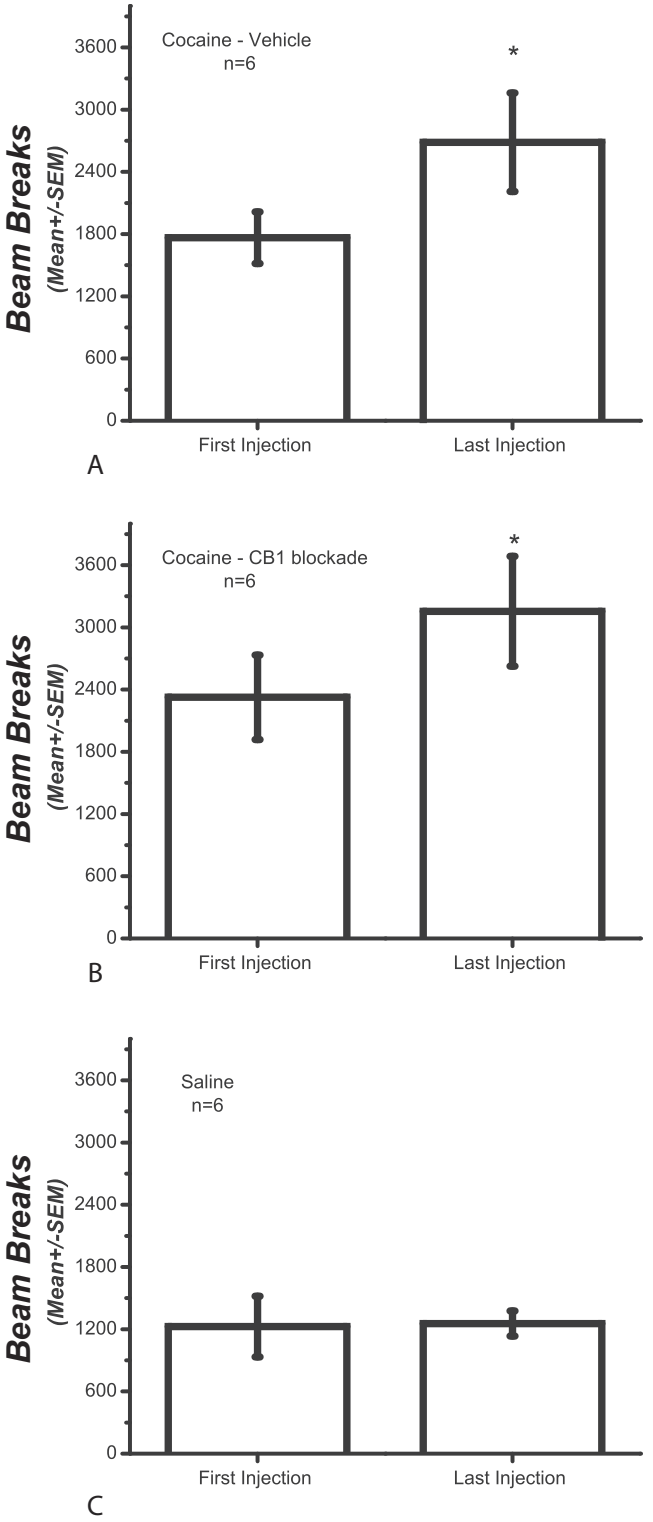
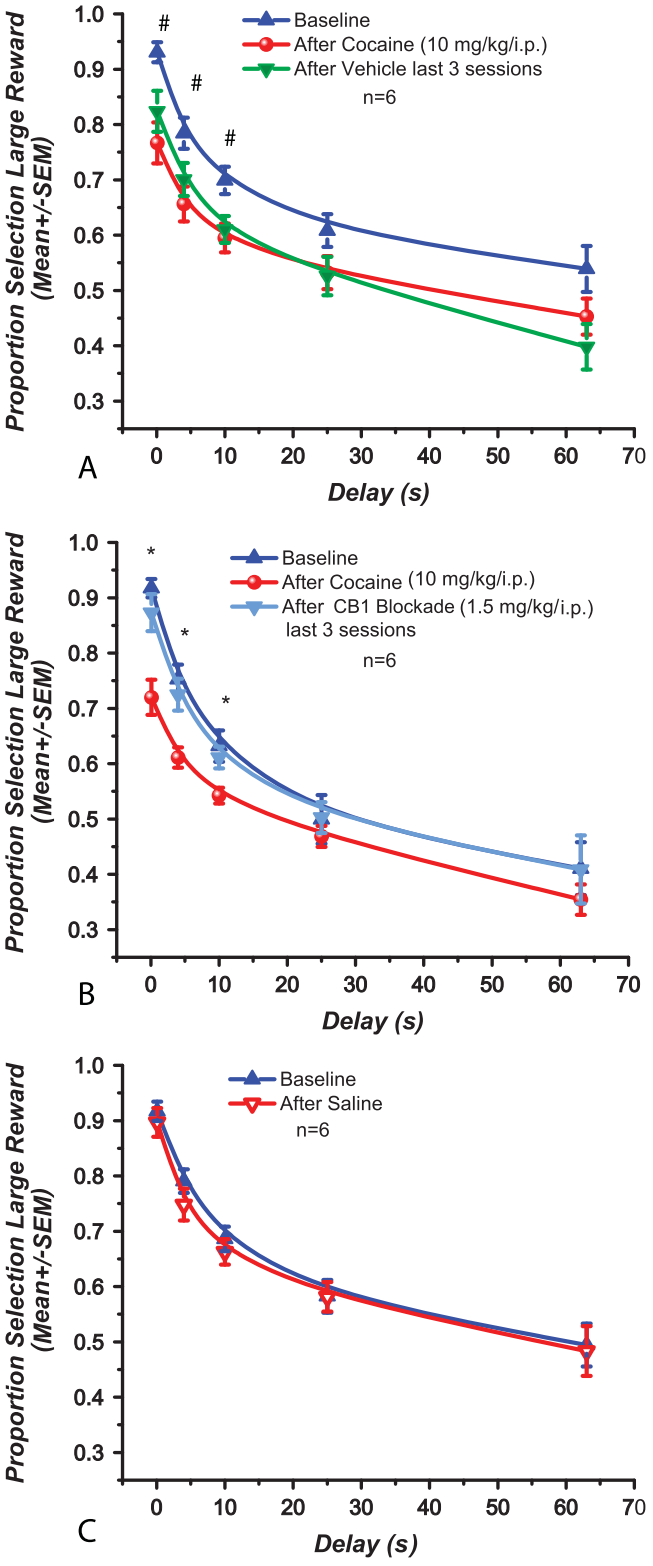


Figure 1. Locomotor activity for the different groups in the reversal experiment. When the activity between the first cocaine injection and the last cocaine injection are contrasted, repeated cocaine administration produces an increase in locomotor activity ($p < .05$) in the groups that received AM251 **(A)** or vehicle **(B)** before the delay discounting task. In contrast, the locomotor activity of rats injected with saline **(C)** remained unaltered ($p > .05$). CB1, cannabinoid type 1 receptor.

Drugs

Two structurally similar CB1R inverse agonists SR141716A (Rimonabant) (National Institute on Drug Abuse Drug Supply Program, Raleigh, North Carolina) or AM251 (Tocris Bioscience, Bristol, United Kingdom) that produce comparable effects in several behavioral paradigms [see (66) for a review], as well as molecular changes (61) were used. They were dissolved in a solution of (1:1:18) ethanol, Emulphor (Rhodia, Cranbury, New Jersey), and saline and injected intraperitoneally at 0 and 1.5 mg/kg, a dose selected on its inability to alter locomotor activity (67). Cocaine (National Institute on Drug Abuse Drug Supply Program, Raleigh, North Carolina or Medisca Pharmaceutique Montreal, Quebec, Canada) was dissolved in saline and injected at 10 mg/kg. All drugs were given in an injection volume of 1 ml/kg.



Behavioral Training

Experiments were conducted in rat operant conditioning chambers (12.5′ length × 13.5′ width × 13.5 height; Med Associates, Georgia, Vermont) located within ventilated sound attenuation chambers. The operant boxes were equipped with a white noise amplifier, a house light located at the rear of the chamber, three cue lights above two retractable levers (Coulbourn Instruments, Whitehall, Pennsylvania), and a pellet receptacle and modular pellet dispenser. The pellet receptacle was centrally located between the two levers and was fitted with infrared photobeams located horizontally across the entrance, which allowed for detecting head entrances into the receptacle.

Rats were initially trained under a fixed-ratio 1 reinforcement schedule to lever press for a 45 mg chocolate flavored sucrose pellet (Bio-Serv, Frenchtown, New Jersey). Only one lever was presented at random on any of the two lever locations. Once animals pressed for a minimum of 100 times in an hour, they were trained to nose-poke in the pellet receptacle to trigger the presentation of the levers. This ensured that at the beginning of each trial, rats were positioned centrally between the levers. These trials began with the house and cue light in the pellet receptacle turned on; once the rat nose-poked, the pellet receptacle light went off and one cue light selected at random was turned on for 2 seconds before the presentation of its associated lever. When the rat pressed the lever, the house light and cue light extinguished, the lever retracted, and the light of the pellet receptacle went on. After the rat harvested the pellet, all lights in the box were off for the duration of the 15-second intertrial interval. After two consecutive sessions, rats started the delayed reward task.

The DDt was modified from Evenden and Ryan (68). Each session consisted of 6 blocks of 14 trials each (Figure S1 in Supplement 1). The first block was considered a warm-up phase.

Figure 2. AM251 (1.5 mg/kg/intraperitoneal [i.p.]) reverses effects of cocaine pre-exposure on choice of the delayed reward. **(A)** Shows how cocaine pre-exposure (red circles) consistently reduces the selection of the delayed large reward. Vehicle administration (green triangles) before the delay discounting task does not have any effect on the cocaine-induced decrease. Both performances are statistically similar ($p > .05$). The reductions in the selection of the large delayed reward observed after cocaine and vehicle are statistically significant when contrasted against the baseline performance ($p < .05$ denoted by #) during the first three delays (0, 4, 10 sec). **(B)** As before, cocaine pre-exposure (red circles) consistently reduces the selection of the delayed large reward. However, such reduction is reversed by AM251 administration (light blue triangles) before the delay discounting task. When performance is contrasted against baseline, only cocaine's effects are statistically different; this difference is particularly salient during the first three delays (0, 4, 10 sec) ($p < .05$ denoted by *). **(C)** Pre-exposure to saline instead of cocaine has no effect on the selection of the delayed large reward ($p > .05$). CB1, cannabinoid type 1 receptor.

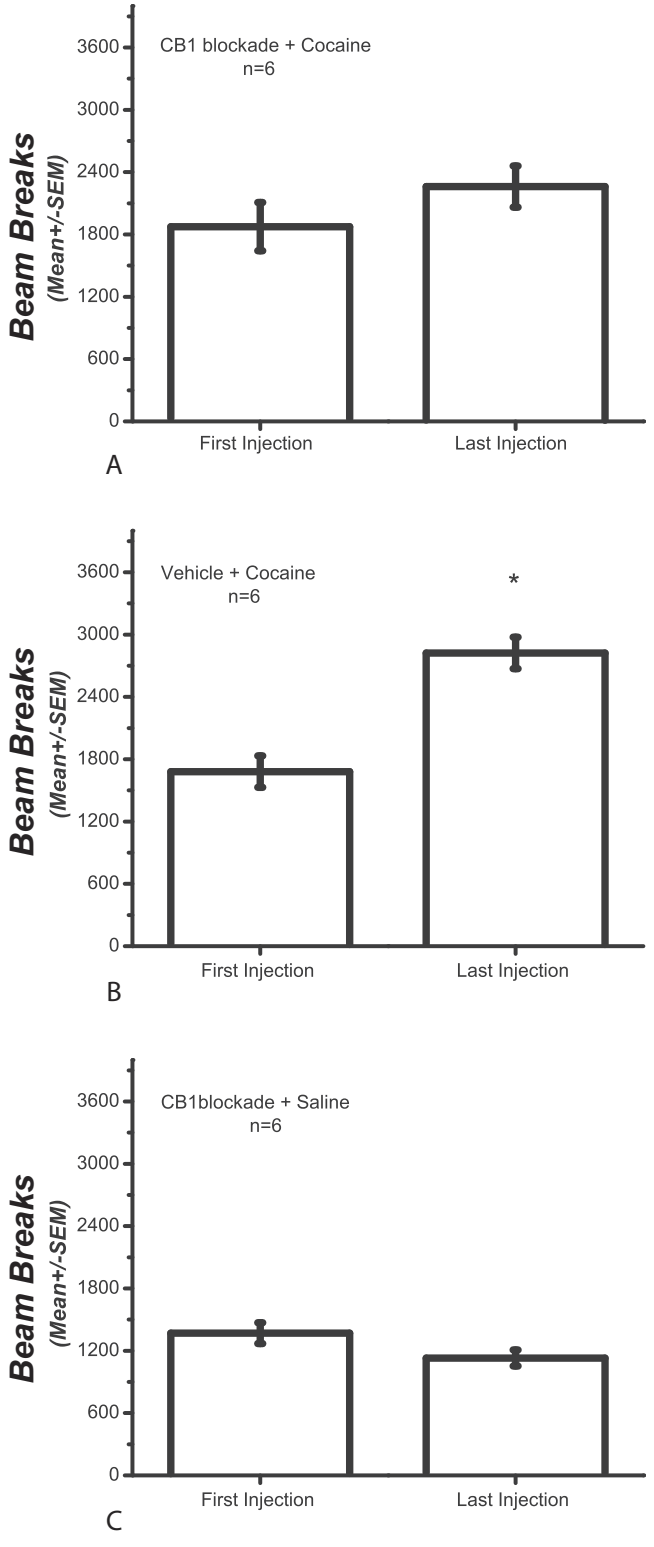


Figure 3. Locomotor activity for the different groups in the blockade experiment. There is an increase in the average locomotor counts between the first and the last cocaine injection in the cannabinoid type 1 (CB1) blockade + cocaine group **(A),** as well as in the vehicle + cocaine group **(B)**. However, the increase in locomotor activity between the first cocaine injection and the last cocaine injection is only statistically reliable ($p < .05$) for the latter. Locomotor activity of the CB1 blockade + saline group **(C)** is similar for the first and last saline injection ($p > .05$).

Each block began with a pair of forced trials in which the left or the right cue was turned on and 2 seconds later its associated lever was presented. These trials were followed by 12 free-choice trials in which both cues and levers were presented. Between blocks, the assignment of the lever associated with the reward delivery delay was changed randomly to minimize potential selection bias. The cue on top of the lever assigned with the immediate delivery of a single pellet was always nonflashing, whereas the cue on top of the lever assigned with the delivery of four pellets after a delay was flashing; the frequency at which the cue flashed changed with each delay and the selection of flashing frequencies for each delay were counterbalanced between subjects. The reward delay of the four pellets option was increased between blocks in a sequential manner from 0 seconds in blocks 1 and 2 to 4, 10, 25, and 63 seconds in each of the remaining blocks. The intertrial interval during this part of the training was of variable length depending on the choice made, so that the time between trials was always 75 seconds. The latency to press the levers and response omissions were registered. Rats were trained under this schedule until performance was stable, e.g., a nonsignificant difference across 5 consecutive days as per repeated-measures analysis of variance. Rats received at least 21 days of training before moving to the other phases of the experiment.

Locomotion Measurements. Locomotor activity was assessed in individual activity boxes containing infrared sensors located at the bottom of the longitudinal axis (Med Associates, Inc., St. Albans, Vermont). Locomotor activity was defined as the consecutive interruption of two photocell beams. Activity for both experiments was monitored over 1 hour at approximately the same time each day. On the first 2 days, rats were injected with saline and habituated to the locomotor boxes. Following habituation, rats used for the reversal experiment were injected with cocaine on alternate days for 14 days and locomotor activity was assessed. For the blockade experiment, either rimonabant or vehicle was injected 30 minutes before the injection of cocaine or saline and subsequent measurement of locomotor activity. Two days after the last injection, rats returned to the delay-discounting task.

For the reversal and blockade experiment, cocaine preexposure effects on the delay-discounting task were assessed for the next 5 consecutive days, but for the reversal experiment, performance was evaluated for another 5 days following a daily injection of AM251 or vehicle 30 minutes before the beginning of the DDt.

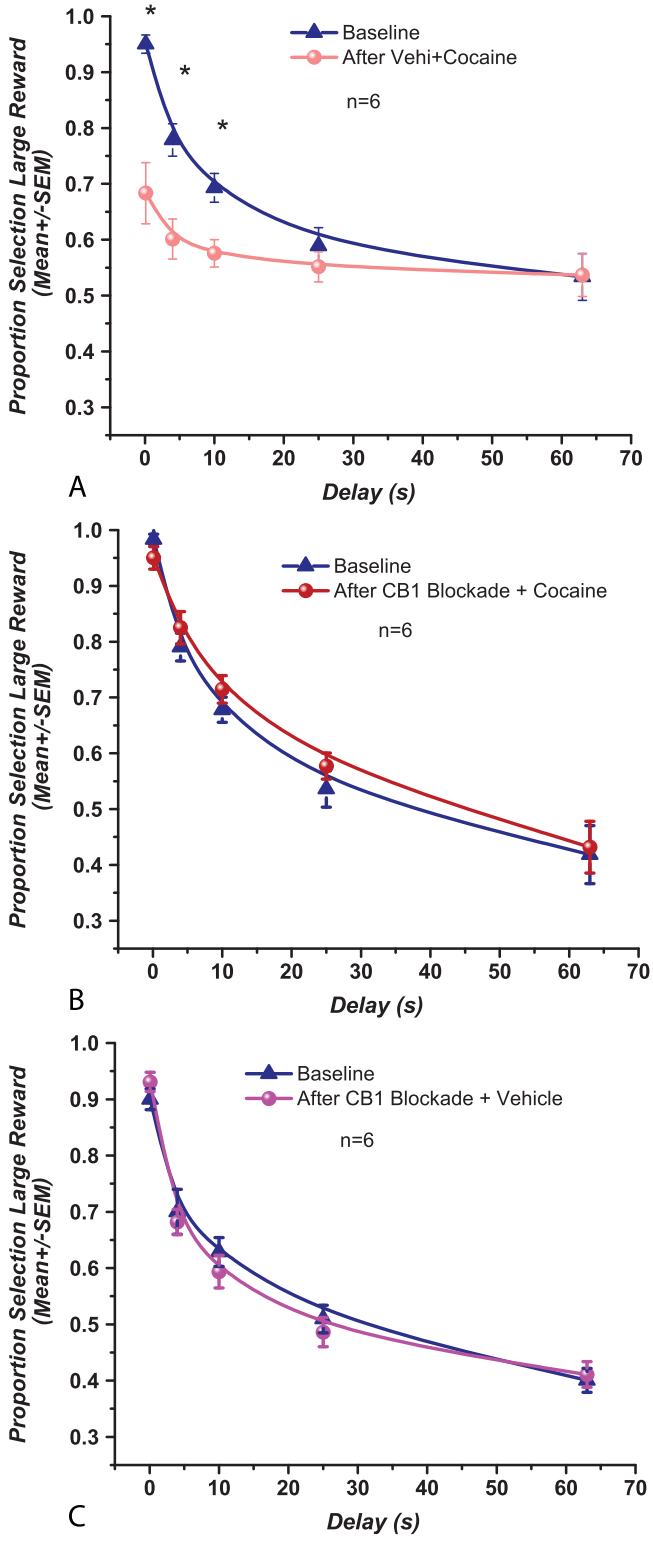
Fast-Scan Cyclic Voltammetry. During the DDt, DA was detected from fast-scan cyclic voltammograms collected at the carbon fiber electrode every 100 milliseconds (initial waveform: −.4 V to 1.3 V, 400 V/sec) (63). Principal component regression was used as previously described to extract the DA component from the raw voltammetric data (69). Dopamine concentration was estimated based on the average postimplantation sensitivity of electrodes (63,70).

Data Analysis. Behavioral performance across the different phases of the task, as well as phasic DA release, were evaluated using repeated-measures analysis of variance to the arcsine transformation to limit the effect of an artificially imposed ceiling (71). When significant interactions or main effects were obtained ($p < .05$), differences between groups were tested using Dunnett's multiple comparison test. Omissions, response latencies, and locomotor counts were analyzed using paired *t* tests. All analyses were carried out using Statistica (Statsoft, Tulsa, Oklahoma).

Results

Blockade of CB1 Receptors Reverses Cocaine-Induced Changes in Preference for the Large Reward

The initial injection of cocaine produced similar increases in locomotor activity in both groups, the group that would be injected with the CB1R antagonist and the one injected with vehicle ($t_{10} = -1.17$, $p = .26$). Rats of both groups were sensitized to the locomotor effects of cocaine ($t_{5\ \text{vehicle-group}} = 2.86$, $p = .035$; $t_{5\ \text{CB1 blockade-group}} = 3.32$, $p = .020$) (Figure 1A,B; Figure S2 in Supplement 1). Saline-injected rats showed no statistical difference between the first and last saline injection ($t_{5\ \text{saline-group}} = .14$, $p = .88$) (Figure 1C; Figure S2 in Supplement 1). Cocaine pretreatment produced a significant change in self-control (Figure 2A,B), as a significant delay ($F_{4,348\ \text{vehicle-group}} = 141.57$, $p = .000$; $F_{4,348\ \text{CB1 blockade-group}} = 128.23$, $p < .001$) or pretreatment effect ($F_{2,87\ \text{vehicle-group}} = 7.41$, $p = .000$; $F_{2,87\ \text{CB1 blockade-group}} = 10.75$, $p < .001$), and a significant interaction ($F_{8,348\ \text{vehicle-group}} = 2.35$, $p = .017$; $F_{8,348\ \text{CB1 blockade-group}} = 4.17$, $p < .001$) were observed for both groups. Post hoc analysis confirms that behavior observed across the first four delays (0, 4, 10, 25 sec) was significantly different, showing an orderly decrease in the preference for the large reward as the delay to obtain it increased. However, behavior observed for the last two delays (25 and 63 sec) was statistically similar. Pretreatment with cocaine decreased the preference for the large reward, in particular during the first 3 delays (0, 4, 10 sec) (Figure 2A; Figure S6A in Supplement 1); this change in preference was stable (Figure S3C, D in Supplement 1)



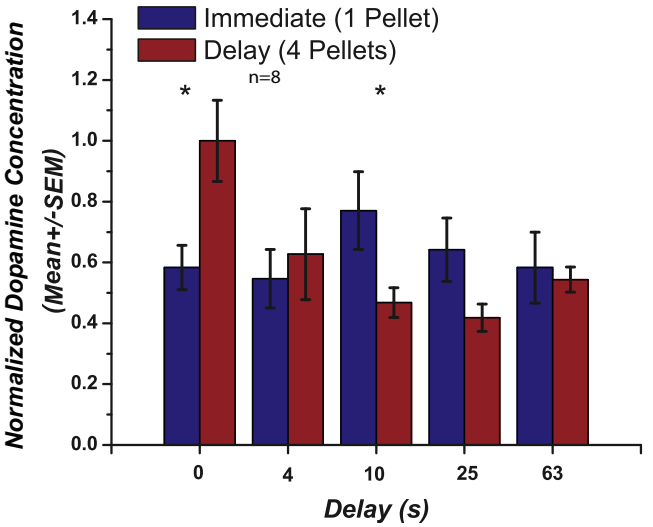


Figure 5. Phasic dopamine release for both reward magnitudes at different delays. Release for the four reward pellets (dark blue) is higher for the two initial delays (0 and 4 sec) when compared with the one pellet immediate option (dark red). However, it was only statistically higher ($p < .05$ denoted by *) at 0-second delay. There is a peak of phasic dopamine release for the immediate one-pellet option when the delay for the four pellets reaches 10 seconds; this difference in release between the options is statistically significant ($p < .05$).

Figure 4. Rimonabant (1.5 mg/kg/intraperitoneal) prevents the effects of cocaine pre-exposure on choice of the delayed reward. **(A)** When contrasted against baseline (blue triangles), vehicle (Vehi) administration 30 minutes before cocaine injections (light red circles) consistently reduces the selection of the delayed large reward. The reductions in the selection of the large delayed reward observed after vehicle and cocaine are statistically significant ($p < .05$ denoted by *). These differences are particularly pronounced for the first three delays (0, 4, 10 sec). These observations contrast to the results obtained when rimonabant (red circles) is administered 30 minutes before the cocaine injections **(B)**. Performance during baseline and following treatment with rimonabant and cocaine are not statistically different ($p > .05$). Similar results are obtained when rimonabant is administered 30 minutes before saline (magenta circles) **(C)**. CB1, cannabinoid type 1 receptor.

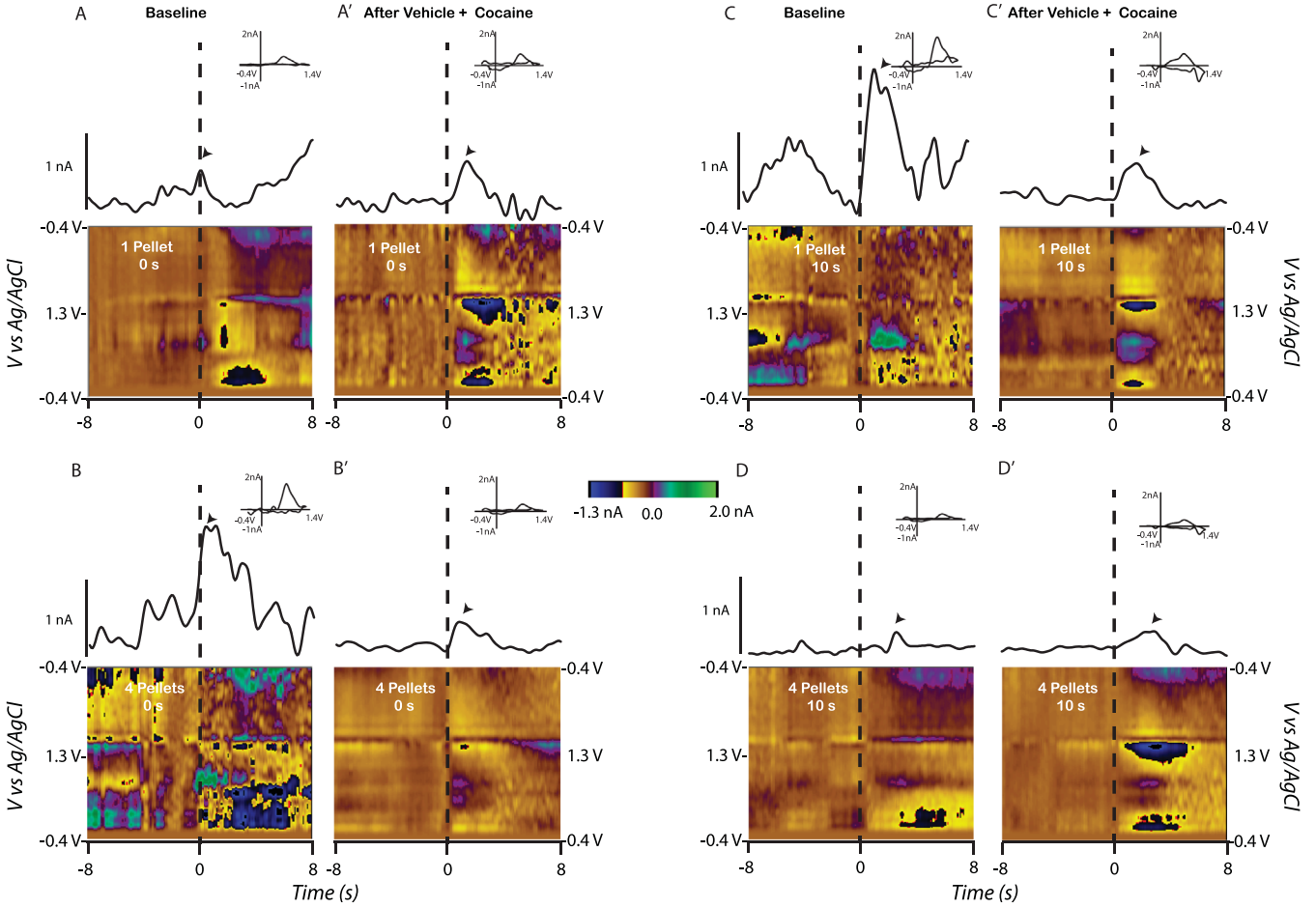


Figure 6. Representative example of dopamine (DA) release to reward delivery at different delays for vehicle pretreatment. Dopamine release for the one-pellet **(A)** and four-pellet **(B)** options at 0 seconds and following pre-exposure to vehicle and cocaine **(A′, B′)**. Current at the peak oxidation potential of DA is plotted as function of time, the insets showing the cyclic voltammogram identifying the detected peak current, denoted by the arrow head, as DA. Below are two-dimensional pseudocolor plots of cyclic voltammograms over time. Dashed lines denote reward delivery. During baseline, release for the four-pellet option is higher at 0 seconds **(B)** when contrasted against the one-pellet option **(A)**. However, following cocaine and vehicle, this pattern reverses **(A′, B′)**. At 10 seconds, during baseline, the release for the immediate one-pellet option **(C)** is higher than that of the four-pellet delay option **(D)**. This pattern remains following vehicle and cocaine pre-exposure **(C′, D′)**. Ag/AgCl, silver/silver chloride; V, volt.

and was not reversed by administration of vehicle. Interestingly, rats whose CB1Rs were blocked, reversed their behavior to a level similar to that observed during baseline (Figure 2B; Figure S6B in Supplement 1). Visual inspection of the different sessions revealed that the effect of CB1R blockade on performance was not immediate (Figure S3E, F in Supplement 1). Temporal factors cannot explain the changes in performance observed following cocaine pretreatment, since performance under saline was not different from baseline ($F_{1,58\ \text{saline-group}} = .33$, $p = .562$) (Figure 2C; Figure S6C in Supplement 1). When contrasted against baseline, none of the treatments had an effect on other performance measures, latency to press ($t_{5\ \text{vehicle-group}} = .84$, $p = .217$; $t_{5\ \text{CB1 blockade-group}} = .60$, $p = .286$; $t_{5\ \text{saline-group}} = .51$, $p = .314$) or omissions, which were extremely rare ($t_{5\ \text{vehicle-group}} = .99$, $p = .183$; $t_{5\ \text{CB1 blockade-group}} = .20$, $p = .423$; $t_{5\ \text{saline-group}} = .25$, $p = .404$).

Blockade of CB1 Receptors Curtails Cocaine-Induced Changes in Preference for the Large Reward

Blockade of CB1R prevented the effects on cocaine-induced locomotion (Figure 3A; Figure S4 in Supplement 1) ($t_{5\ \text{CB1 blockade + cocaine}} = 1.63$, $p = .16$). In contrast, a significant increase in cocaine-induced locomotion was observed in vehicle-treated animals (Figure 3B; Figure S4 in Supplement 1) ($t_{5\ \text{vehicle + cocaine}} = 2.62$, $p = .046$). Blockade of CB1R along with saline did not produce a discernible effect in locomotion (Figure 3C; Figure S4 in Supplement 1) ($t_{5\ \text{CB1 blockade + saline}} = .23$, $p = .41$). Locomotor differences between pharmacologic treatments were a reliable predictor of subsequent self-control. Indeed, vehicle-pretreated rats showed a decrease in preference for the large reward (Figure 4A; Figure S7A in Supplement 1). As observed before, there was a significant delay effect between baseline and following treatment ($F_{4,232} = 51.97$, $p < .001$), the pretreatment ($F_{1,58} = 14.74$, $p < .001$), and for the interaction between delay and pretreatment ($F_{4,232} = 8.84$, $p < .001$). Behavior observed across the first four delays (0, 4, 10, 25 sec) was significantly different. In this group, cocaine reduced the preference for the large reward during the first 3 delays (0, 4, 10 sec). Importantly, CB1R blockade prevented all changes in self-control associated with cocaine pre-exposure (Figure 4B; Figure S7B in Supplement 1), as no significant differences were observed during baseline or after treatment ($F_{1,58} = .124$, $p = .725$) or for the interaction between delay and treatment ($F_{4,232} = 1.36$, $p = .247$). Similarly, blockade of CB1R alone before saline injections did not affect self-control (Figure 4C; Figure S7C in Supplement 1)

($F_{1,58} = .04$, $p = .950$) and there was no effect for the interaction between delay and treatment ($F_{4,232} = .64$, $p = .627$). None of the treatments altered the latency to press ($t_{5\ \text{CB1 blockade + cocaine}} = .72$, $p = .251$; $t_{5\ \text{vehicle + cocaine}} = 1.28$, $p = .128$; $t_{5\ \text{CB1 blockade + saline}} = .57$, $p = .296$) or the omissions ($t_{5\ \text{CB1 blockade + cocaine}} = .97$, $p = .186$; $t_{5\ \text{vehicle + cocaine}} = .70$, $p = .255$; $t_{5\ \text{CB1 blockade + saline}} = .47$, $p = .326$).

Phasic DA Associated with Reward Delivery Recapitulates Behavioral Effects

Dopamine release measurements showed that prior to cocaine exposure, release was greater for the four-pellet option when the delay was minimal. However, when the delay reached 10 seconds, this pattern reversed, i.e., there was greater release for the immediate one-pellet option compared with the delayed four-pellet alternative ($F_{4,28} = 7.15$, $p < .001$) (Figure 5). This pattern of DA release was significantly disrupted by cocaine in vehicle-treated rats (Figure 6A–D′). Dopamine release for the four-pellet option was consistently lower at small and moderate delays compared with baseline and this difference was particularly salient when the delay between the two options was identical ($F_{4,12} = 3.36$, $p = .045$) (Figure 7A–D). Importantly, blockade of CB1R before cocaine injections maintained DA release patterns observed before cocaine exposure ($F_{4,12} = .412$, $p = .796$) (Figure 8A–D′; Figure 9A–D).

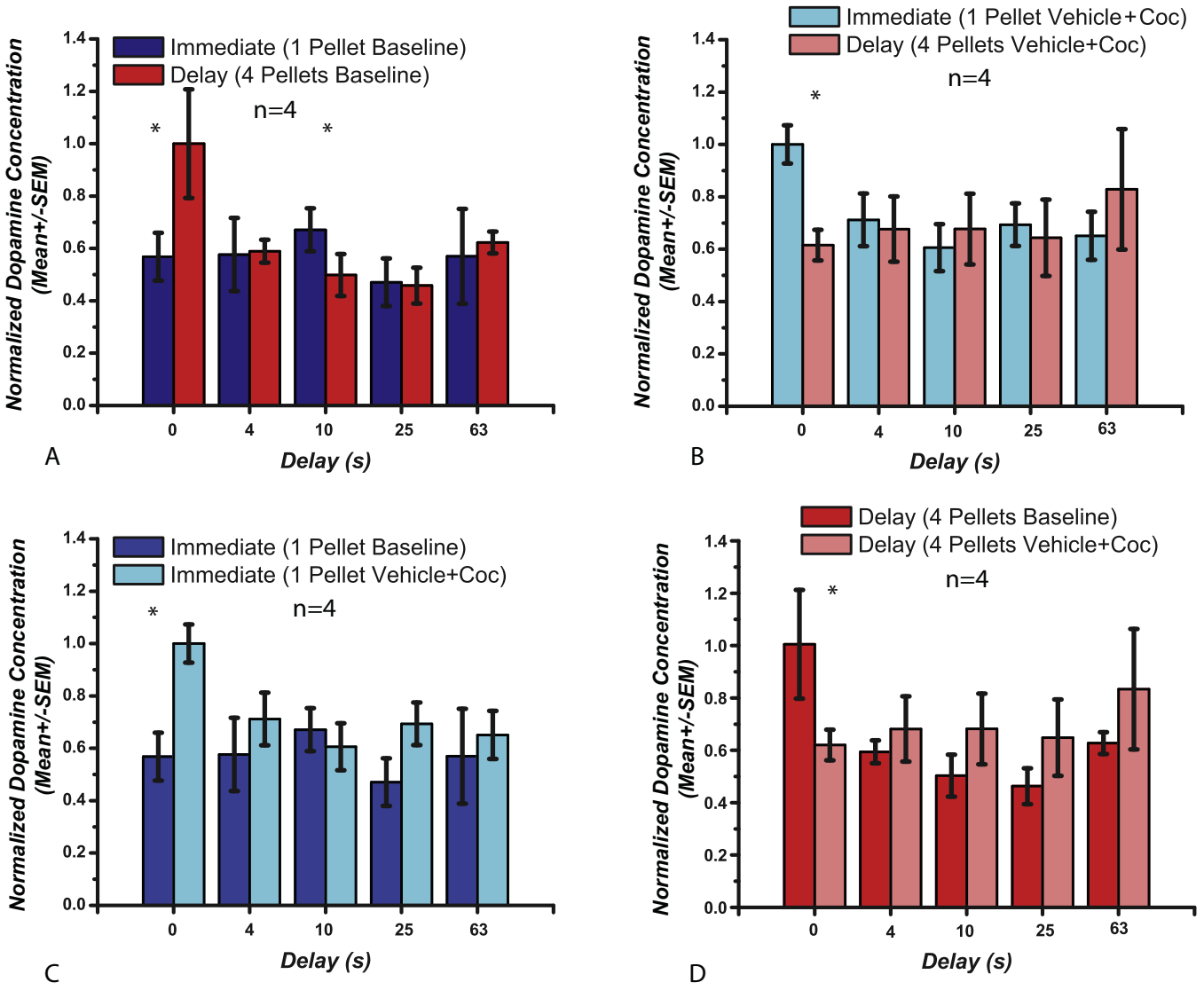


Figure 7. Bar graphs of pooled dopamine (DA) release to reward delivery at different delays for vehicle-treated rats. **(A)** Shows the DA profile obtained during baseline. Release observed for the immediate one-pellet option (blue bar) is paired with release obtained for the four-pellet option (red bar) across different delays. Release for the four-pellet option is statistically higher (*$p < .05$) at 0 seconds delay compared with its immediate counterpart. When the delay for the four-pellet option reaches 10 seconds, there is a significant peak ($p < .05$) of phasic DA release associated with the immediate one-pellet alternative. **(B)** Injections of vehicle and cocaine (Coc) produced a change in the DA release profile; following treatment, release for the immediate option (cyan bar) is significantly higher than that observed for the four-pellet option (light red bar) at zero-delay, whereas release for the four-pellet option remains stable across delays. Directly contrasting the effect of pre-exposure to vehicle and cocaine on DA release against baseline for the immediate **(C)** and delay option **(D)** shows that highest differences are observed when the delay is 0 seconds. At this delay, there is a significant increase in release for the one-pellet option following pre-exposure to vehicle and cocaine, whereas there is a significant decrease for the four-pellet option ($p < .05$).

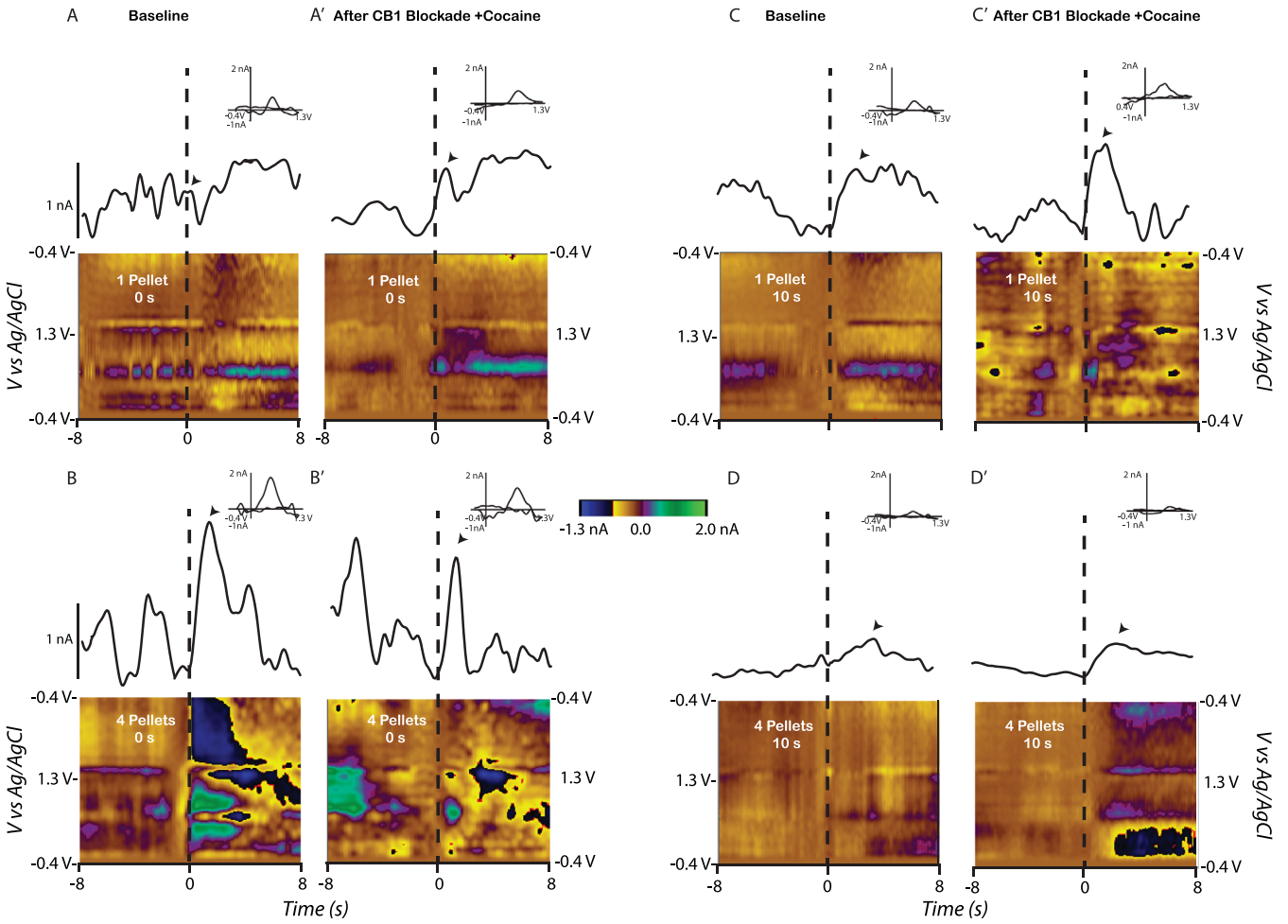


Figure 8. Example of dopamine (DA) release to reward delivery at different delays in rimonabant-treated rats. Release for the one-pellet **(A)** and four-pellet **(B)** options at 0 seconds and following rimonabant pretreatment and cocaine **(A′, B′)**. Current at the peak oxidation potential of DA is plotted as function of time, the insets showing the cyclic voltammogram identifying the detected peak current, denoted by the arrow head, as DA. Below are two-dimensional pseudocolor plots of cyclic voltammograms over time. Dashed lines denote reward delivery. During baseline recording, DA for the four-pellet option is higher at 0 seconds **(B)** when compared with the one-pellet option **(A)**. Following rimonabant and cocaine, this pattern persists **(A′, B′)**. At 10 seconds, release for the immediate one-pellet option at baseline **(C)** is higher than that of the four-pellet delay option **(D)**. Following rimonabant and cocaine pre-exposure, this pattern remains **(C′, D′)**. Ag/AgCl, silver/silver chloride; CB1, cannabinoid type 1 receptor; V, volt.

Phasic DA Associated with Cue Presentation Does Not Encode Delay or Reward Magnitude

Cue presentation during forced trials produced phasic DA release; however, its amplitude did not differ between different reward magnitudes ($F_{1,7} = 1.450$, $p = .267$) or delays ($F_{4,28} = 1.26$, $p = .308$) (Figure S5A in Supplement 1). Likewise, cocaine exposure did not produce changes in release in vehicle-treated rats ($F_{1,3\ \text{immediate}} = .831$, $p = .429$; $F_{1,3\ \text{delay}} = .697$, $p = .465$) (Figure S5B, C in Supplement 1) or in rats in which CB1Rs were blocked ($F_{1,3\ \text{immediate}} = 1.369$, $p = .326$; $F_{1,3\ \text{delay}} = 2.083$, $p = .244$) (Figure S5D, E in Supplement 1) compared with baseline.

Discussion

The current study documents the role of eCBs in the development and maintenance of changes in impulsive choice that arise from cocaine exposure and adds to a growing body of evidence related to the modulatory role that eCBs play in self-control—in particular when it is altered by psychostimulants (72–74). We demonstrate that cocaine produces a decrease in self-control that is reversed and blocked by interfering with CB1R signaling. Rats exposed to cocaine show a preference for immediate rewards over delayed ones, even when the imposed delay is minimal. Since eCBs also modulate the reinforcing properties of natural rewards and the motivation to obtain food (54,75–77), it could be argued that the results in the present study are the product of a generalized reduction in motivation. However, this seems unlikely, as we did not observe differences between any of the groups in indirect measures of motivation, neither the latencies to respond nor omissions. Others have shown, using higher doses than the one used here, that CB1R antagonists fail to change the subject's sensitivity toward the delayed large reward (72,73). Furthermore, CB1R blockade had no long-term effects on motivation, since we did not observe changes in performance in the blockade experiment when rats were treated with the antagonist along with saline. The indiscriminate preference for the immediate reward in the present study is in contrast to previous studies (10,11) in which the preference for the smaller immediate reward was evident only after the 10-second delay. A factor that may

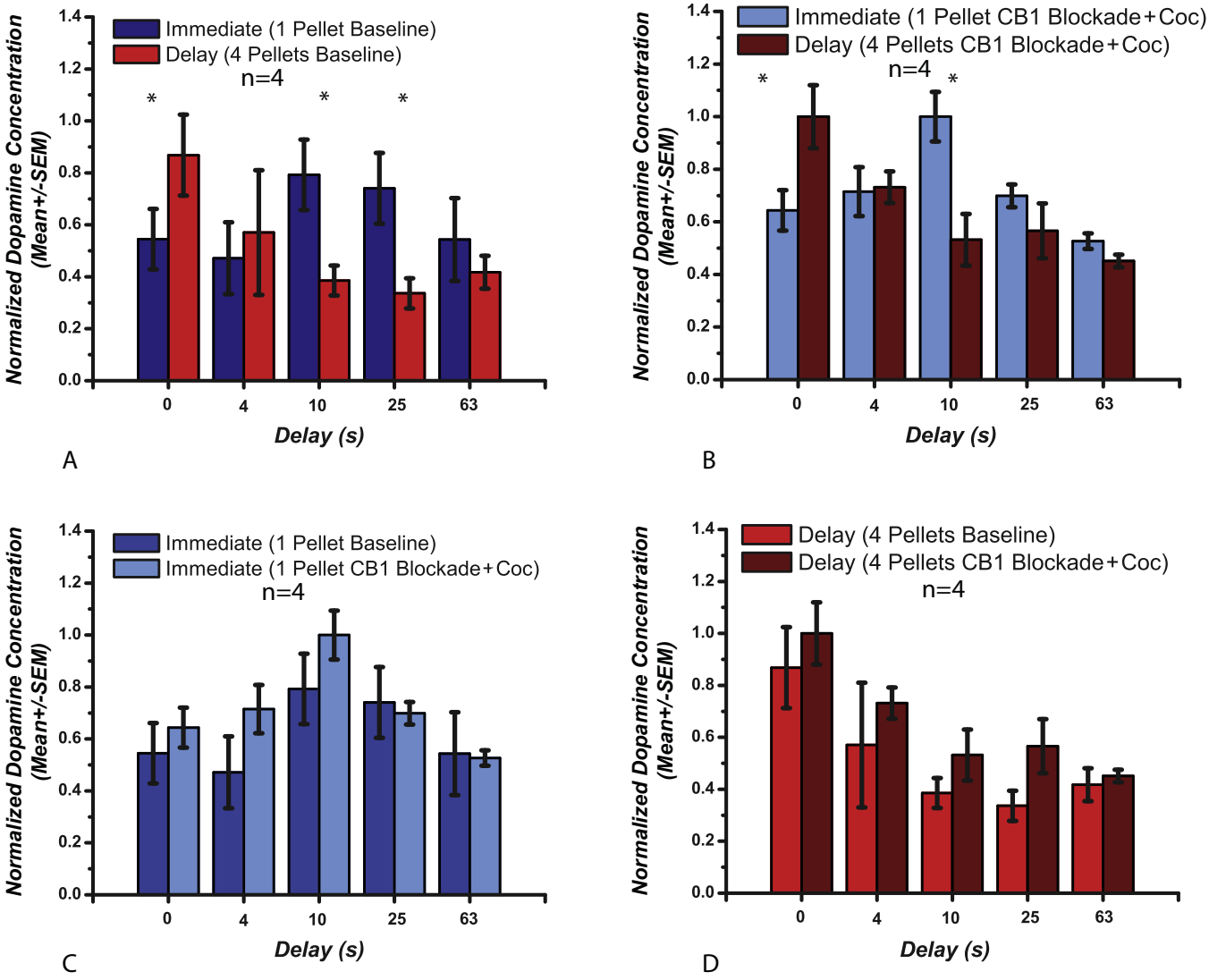


Figure 9. Bar graphs of pooled dopamine release to reward delivery at different delays for rimonabant-treated animals. **(A)** Shows the dopamine profile obtained during baseline. Release observed for the immediate one-pellet option (blue bar) is paired with release obtained for the four-pellet option (red bar) across different delays. Release for the four-pellet option is statistically higher (*$p < .05$) at 0 seconds delay compared with its immediate counterpart. When the delay for the four-pellet option reaches 10 seconds, there is a significant peak ($p < .05$) in release associated for the immediate one-pellet alternative. **(B)** Injections of rimonabant before cocaine (Coc) treatment maintained the release pattern observed during baseline; at 0-delay, release is higher for the four-pellet option (wine bars) than for the immediate option (light blue bars). Similar to baseline, when the delay for the four-pellet option reaches 10 seconds, there is a significant peak ($p < .05$) of phasic release associated for the immediate one-pellet alternative. Directly contrasting the effect of pre-exposure to vehicle and cocaine on release to baseline for the immediate **(C)** and across delay options **(D)** shows that rimonabant preserves the baseline release pattern since no significant difference ($p > .05$) in release at any of the delays for any of the options are observed. CB1, cannabinoid type 1 receptor.

explain this difference is that in our protocol the location of the levers changed randomly across trial blocks. This modification possibly reduced the presentation of stereotyped lever selection, and it revealed that in addition to impulsivity, cocaine produces a generalized aversion to temporal delays in the pursuit of reward. It is also possible that reduced constitutive activity of CB1Rs due to inverse agonism might have contributed to the effects reported herein.

Increases in impulsivity following cocaine treatment have been reported by others (3,9–11,78,79) and have been linked to heightened sensitivity to DA (9,18), which is theorized to bias responding toward the subjectively more valuable reward (18) or immediate reward (80,81). Our electrochemical results are the first to report patterned DA release during a DDt, where evidence of temporal bias can be observed. Phasic DA release associated with reward delivery is higher when the delay is below 10 seconds and decreases to a level that is below that observed for the immediate option. However, following cocaine treatment, phasic release for the immediate option is above that observed for the delay option regardless of the delay. This observation correlates with behavioral performance, in which cocaine exposure increases the preference for immediate rewards and confirms electrophysiological recordings suggesting that phasic DA firing encodes subjective reward value (18). Importantly, blockade of CB1Rs reverted and prevented this change in self-control. Therefore, blockade of eCB signaling may play a previously unrecognized role in facilitating and maintaining long-term changes in reward valuation arising from cocaine exposure. It is possible that the changes observed at

the behavioral level, as well as the neurochemical correlates, result from preventing the binding of eCBs to CB1Rs present on glutamatergic terminals located in several nuclei that send projections to the VTA (82) or by interfering with eCB binding to presynaptic CB1Rs on gamma-aminobutyric acid terminals in the VTA (48,83,84). These changes may, in turn, prevent cocaine-induced increases in DA transients (52,53) in brain areas related to decision making and therefore exert the observed changes in self-control. However, as drugs were delivered systemically, other targets for these effects could underlie the pharmacologic responses.

In striking contrast to the changes in phasic DA release observed following reward delivery, release associated with cue onset at different delays did not encode subjective reward value and was not altered by cocaine exposure. A possible explanation for the lack of cue encoding by phasic DA release in our experimental conditions is that what is encoded in this signal is the effort threshold that has to be overcome to obtain a reward (85). Since the cost for the delay and immediate options was the same, the effort threshold and the DA signal that accompanies it should theoretically remain unaltered. Our results agree with Wanat *et al.* (70), who reported that DA release at cues is unaltered across different delays. However, other neurochemical studies (86–89) have found that reward value is encoded by phasic DA release at the presentation of cues. It is likely that methodological differences between the tasks account for some of the discrepancies. Our results were obtained using a task in which the only variable that changed across trials was the time at which the large reward was delivered following a response at the corresponding lever, whereas in prior studies, the access to different response requirements (86,88), a fixed delay (88), or the probability to obtain a reward (87) have been compared. In addition, most of the above-mentioned studies recorded from the NAc core [but see (70)]. Our electrodes were aimed at the shell, so it is possible, as previously proposed, that NAc core and shell mediate different aspects of reward-cue encoding (90).

Here, phasic DA release observed during the reward did not shift toward cues. During learning, phasic DA neuron firing that occurs at reward delivery decreases as rewards become fully predicted and firing activity shifts toward the first predictor of the reward in Pavlovian (89) and operant paradigms (90,91). However, phasic firing at the first predictors critically depends on the duration of the stimulus-reward interval. As the stimulus-reward interval increases beyond 2 seconds, the phasic response to the conditioned stimulus decreases and the phasic response to the reward increases (17,92). This observation is confirmed by our data, since in our procedure, the cue was presented 2 seconds before lever extension and rats had an opportunity to press it and obtain a reward.

In conclusion, our results show that the eCB system plays an important role in decision-making processes, in particular when these have been modified as a result of exposure to cocaine. Endocannabinoids are well-known synaptic modulators of different limbic and motor inputs required to organize goal-directed behaviors. Here, we show that this system is also crucially involved in the development and maintenance of enduring DA adaptations that result from cocaine exposure. The present data advance our understanding of the role of eCbs in aberrant decision making in cocaine addiction.

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