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Effects of Morphine/CP55940 Mixtures on an Impulsive Choice Task in Rhesus Monkeys

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Abstract

Mu opioid receptor agonists are commonly used to treat pain despite their adverse effects. In preclinical studies, cannabinoid receptor agonists increase the potency of opioids for producing antinociceptive but not reinforcing effects. It is unknown whether other adverse effects of these drugs, such as impairment of complex behavior, are enhanced by their co-administration. This study characterized the effects of morphine (*mu* opioid receptor agonist; 0.32 – 5.6 mg/kg, s.c.) and CP55940 (CB₁/CB₂ cannabinoid receptor agonist; 0.0032 – 0.32 mg/kg, s.c.), alone and in mixtures, in monkeys (n=3) choosing between 1 pellet delivered immediately and 2 pellets delivered after a delay. Two consecutive choices of the immediate or delayed reward decreased or increased, respectively, the delay. The median adjusted delay, indicating indifference between the immediate and delayed reinforcers, was increased by morphine (3.2 mg/kg) and CP55940 (0.01 – 0.032 mg/kg). Performance after administration of morphine (0.32 and 1 mg/kg)/CP55940 (0.0032 – 0.032 mg/kg) mixtures was not different from performance after CP55940 alone. Neither morphine, CP55940, nor mixtures decreased the median adjusted delay (i.e., increased impulsivity). These findings failed to confirm previous studies showing that morphine increases impulsivity, perhaps because of procedural differences among studies. Treating pain often requires repeated drug administration; thus, it remains to be determined whether the present findings predict the effects of chronically administered morphine/CP55940 mixtures on impulsive choice.

Keywords

opioid; cannabinoid; morphine; CP55940; drug mixture; delay discounting; impulsivity; monkey

INTRODUCTION

Mu opioid receptor agonists are commonly prescribed to treat moderate to severe pain, despite their well-documented adverse effects (e.g., constipation, abuse). One strategy for reducing the likelihood of adverse effects of opioids is to combine them with drugs that produce the desired therapeutic effect through non-opioid mechanisms. In a mixture, smaller doses of each drug might achieve the desired therapeutic effect while also avoiding the

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adverse effects that occur with larger doses of either drug administered alone (see Smith, 2008). In preclinical studies, small doses of a *mu* opioid receptor agonist combined with a cannabinoid receptor agonist have antinociceptive effects that are comparable to those produced by much larger doses of the opioid alone; the interaction between *mu* opioid receptor agonists and cannabinoid receptor agonists is supra-additive (synergistic) for antinociceptive effects in mice (Cichewicz and McCarthy, 2003; Tham et al., 2005; Kazantzis et al., 2016).

Both opioids and cannabinoids are widely abused (Volkow et al., 2014; Panlilio et al. 2015) and previous studies evaluated whether abuse-related effects of these drugs were similarly enhanced in mixtures (Li et al., 2008; Maguire and France, 2014). The cannabinoid receptor agonists ⁹-tetrahydrocannabinol (THC) and CP55940 did not enhance positive reinforcing effects of heroin (*mu* opioid), and CP55940 attenuated the discriminative stimulus effects of morphine in rhesus monkeys discriminating morphine from saline (Li et al., 2012; Maguire et al., 2013; Maguire and France, 2016a, 2016b). Smaller doses of each drug in mixtures achieved the desired antinociceptive effect without enhancing an abuse-related effect, supporting the notion that opioid/cannabinoid mixtures might be useful for treating pain without the risk of abuse that currently limits the therapeutic use of *mu* opioids. It is unknown whether other adverse effects of opioids and cannabinoids are enhanced by their co-administration.

The tendency to prefer smaller immediate over larger delayed reinforcers reflects a form of impulsivity, termed delay discounting, which is thought to be associated with drug abuse (Perry and Carroll, 2008; Odum, 2011; Bickel et al., 2014). Humans who are dependent on an opioid discount delayed monetary reinforcers more rapidly than non-users (Madden et al., 1997; Kirby and Petry, 2004). In rats the *mu* opioid receptor agonist morphine increases choice for a small amount of food delivered immediately and concomitantly decreases choice for a large amount of food delivered after a delay (Pits and McKinney, 2005; Pattij et al., 2009). Enhanced delay discounting of non-drug reinforcers might represent a general predisposition to prefer immediately available reinforcers rather than larger delayed reinforcers, thereby increasing an individual's vulnerability for drug abuse. This view is supported by studies in rats showing that steeper discounting of food reinforcers predicted cocaine self-administration and reinstatement of previously extinguished responding in the presence of a cocaine-paired stimulus (Perry et al., 2005; Anker et al., 2009). Another possibility is that enhanced discounting might be a result of drug use. Acute administration of morphine can decrease preference for delayed versus immediate food reinforcers (i.e., increased discounting) in pigeons (Eppolito et al., 2013), rats (Kieres et al., 2004; Harvey-Lewis and Franklin, 2015), and rhesus monkeys (Maguire et al., 2016), and repeated administration of an opioid can increase delay discounting as well other forms of impulsivity, such as response inhibition (Verdejo-Garcia et al., 2007; Harvey-Lewis et al., 2012).

Compared with opioids, much less is known about the effects of cannabinoids on delay discounting, and the findings reported to date have varied among studies. For example, THC can decrease or increase impulsive choice (Wiskerke et al., 2011; Tanno et al., 2014) in rats choosing between smaller immediate and larger delayed food reinforcers. In humans, THC

significantly enhanced motor impulsivity (i.e., a failure to inhibit responding; Metrik et al. 2012). Nonetheless, opioids and cannabinoids can affect attention, inhibition, and decision-making, as reflected by impairments in various behavioral tasks (McDonald et al., 2003; Ramaekers et al., 2006; Friswell et al., 2008; Crean et al., 2011; Dhingra et al., 2015; Irimia et al., 2015); however, the effects of opioid/cannabinoid mixtures on complex behavior have not been thoroughly investigated. Given the hypothesized relationship between delay discounting and drug abuse, it is important to assess for potential adverse effects of opioid/cannabinoid mixtures on delay discounting performance and whether the effects of mixtures might be greater than either drug administered alone.

Enhanced impulsivity by opioids and cannabinoids in combination would limit the therapeutic potential of these mixtures. The present study characterized the effects of the *mu* opioid receptor agonist morphine, the CB₁/CB₂ cannabinoid receptor agonist CP55940, and morphine/CP55940 mixtures in rhesus monkeys responding under an impulsive choice task. Additionally this study assessed the feasibility of using an adjusting delay procedure (Mazur, 1988) to study behavioral and pharmacological interventions in monkeys.

METHODS

Subjects

Male rhesus monkeys (*Macaca mulatta*) were 7.1, 8.9, and 9.1 years old at the start of the experiment and weighed 8.5 – 10.0 kg. The monkeys were housed individually under a 14/10-h light/dark cycle. Monkeys had unrestricted access to water while in their home cages and were fed primate chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI), fresh fruit, and peanuts daily after experimental sessions. The monkeys were maintained at a healthy body weight by food received in sessions and in the home cage. Monkeys were previously trained to press levers for food pellets and had received drug injections (Maguire et al., 2016). The monkeys had not received any drug for at least 2 months prior to the current study. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and was in accordance with guidelines set forth by the Guide for the Care and Use of Laboratory Animals.

Apparatus

Sessions were conducted in ventilated, sound-attenuating operant-conditioning chambers while monkeys were seated in commercially available primate chairs (Model R001, Primate Products, Miami, FL). The chambers contained a custom-made response panel with two retractable response levers (Model ENV-612M, Med Associates, St Albans, VT), a pellet receptacle, and three stimulus lights horizontally aligned 15.5 cm apart (center-to-center). Monkeys were seated approximately eye-level with the stimulus lights, which could be transilluminated red, green (left and right lights), or white (center light). The response levers were positioned 14 cm below the left and right stimulus lights. A food hopper (Model ENV-203, Med Associates) dispensed 300-mg raspberry-flavored sucrose pellets (5TUT, TestDiet, Richmond, IN) to a receptacle centrally located between the two levers. Med-PC

IV software and a PC-compatible interface (Med Associates) controlled stimulus events and recorded data.

Procedure

General Procedure—Sessions comprised up to 15 blocks of 4 trials (i.e., a maximum of 60 trials). The first two trials of each block were forced trials in which either the left lever and light or the right lever and light were presented. The lever position (i.e., left or right) was selected at random for the first forced trial, and the alternative lever was presented in the second forced trial. Completing 30 responses on the lever (fixed ratio [FR] 30) retracted the lever, extinguished the stimulus light, and delivered a reinforcer. For immediately delivered reinforcers, the food hopper dispensed a pellet (or pellets) while the center light simultaneously was illuminated white for 0.2 sec. For delayed reinforcers, the center white light remained illuminated for the length of the delay until the pellets were dispensed. Each food delivery was followed by a 30-s timeout, during which the lights were extinguished. The timeout between lever retraction and presentation of the next trial was held constant in each block; after an immediate reinforcer was delivered, the length of the timeout was 30 s plus the length of the delay for the alternative reinforcer in that block. When both reinforcers were delivered immediately, the timeout was 30 s.

The next two trials of each block were choice trials in which the contingencies were identical to the previous two forced trials, except that both the left and right levers and lights were presented concurrently. Monkeys could respond on either lever; 30 consecutive responses on a lever delivered the corresponding reinforcer. If a monkey chose the delayed reinforcer in both choice trials, then the delay increased by 25% in the next block. Conversely, if a monkey chose the immediate reinforcer in both choice trials, then the delay decreased by 25% in the next block. If a monkey chose one of each reinforcer in the two choice trials of a block, the delay remained the same in the subsequent block. Sessions ended after 15 blocks or 100 min, whichever occurred first.

Environmental Manipulations—Prior to testing drugs, several environmental manipulations were conducted to assess the sensitivity of this procedure to the experimental contingencies (i.e., reinforcer amounts and delay). The stimulus color and lever position (i.e., green/left and red/right; or red/left and green/right) always remained constant for a block of 4 trials, with the order being pseudo-randomized each session so that same color/lever configuration did not occur in more than three consecutive blocks or in more than 8 blocks total. Therefore, to assess whether there was a bias towards a stimulus light based on its color, rather than the consequences it signals, the reinforcers associated with the red and green stimulus lights were reversed twice. Initially, a green stimulus light above a lever signaled 1 pellet delivered immediately and a red stimulus light 3 pellets delivered immediately. Once the monkeys chose 3 pellets over 1 pellet (80% choice for at least 2 consecutive sessions), the stimulus conditions were reversed such that the green stimulus and red stimulus signaled 3 pellets and 1 pellet, respectively. The stimulus conditions were reversed once more, restoring the initial conditions with green signaling 1 pellet and red signaling 3 pellets. For the remainder of this study, the green stimulus signaled a smaller number of pellets relative to the amount signaled by the red stimulus.

To explore whether the difference between reinforcer amounts impacted performance (adjusted delay), the number of pellets for the larger and smaller reinforcers were manipulated in the following three conditions: 3 pellets versus 1 pellet, 2 pellets versus 1 pellet, and 3 pellets versus 2 pellets. These pellet amounts were chosen because the relative differences of 3, 2, and 1.5 fold could be compared to the absolute differences of 2, 1, and 1, respectively. The conditions were tested when both reinforcers were delivered immediately, as well as when the larger amount was delayed (according to the adjusting delay procedure described above). The first time a delay was introduced in a given condition, the delay was set to 10 s for the first block. Thereafter, the delay for the first block was set to the median adjusted delay from the preceding session. Performance was considered stable when the following criteria were satisfied: 1) the median adjusted delay for 3 consecutive sessions was within $\pm 20\%$ of the average of those three sessions; 2) the median adjusted delay was neither increasing nor decreasing across 3 consecutive sessions; and (3) the adjusted delay in the last 3 blocks of the third consecutive session was neither increasing nor decreasing.

To test whether responding alternated on both levers independent of delay, the delay for the first block of the session was set to 100 s for 3 consecutive sessions. This test was conducted in the 3 versus 2 pellet condition for two subjects (Monkey AN and Monkey GE) and in the 3 versus 1 pellet condition for the other subject (Monkey BR). If monkeys simply alternated responding across levers at random, then the delay would not be expected to increase or decrease significantly, with the median adjusted delay remaining close to 100 s. Conversely, if the median adjusted delay was sensitive to delay, then the pattern of responding would decrease the adjusted delay, eventually approximating the median adjusted delay from the 3 versus 2 pellet condition for Monkey AN and Monkey GE and the 3 versus 1 pellet condition for Monkey BR. The order of the environmental manipulations and the number of sessions in each condition varied for each monkey, except that 2 pellets versus 1 pellet (with delays) was always implemented last.

Dose-Effect Determinations: Morphine, CP55940, and Morphine/CP55940

Mixtures—The condition in which drugs were tested for all monkeys was 2 pellets versus 1 pellet. Monkeys received saline injections (0.3 ml) 15-min before every session, except when morphine was tested. At least three consecutive saline sessions preceded each drug test, and performance was considered stable when the median adjusted delay was within $\pm 20\%$ of the average of those three sessions and neither increasing nor decreasing across consecutive sessions. Additionally, if three sequential increases or decreases to the delay occurred in the last three blocks, then performance did not meet stability criteria for drug testing. For any session that immediately followed a drug test, the delay for the first block was set to the median adjusted delay from the most recent saline session.

Dose-response functions were determined twice for morphine alone then twice for CP55940 alone. Doses were tested in an irregular order until the dose-response function ranged from at least one ineffective dose (within $\pm 20\%$ of saline control values both for median adjusted delay and for response rate) and up to a dose that decreased responding to $\sim 20\%$ of control values. Doses were increased or decreased by 1/2-log units, except from 3.2 to 5.6 mg/kg morphine. For Monkey GE, 0.32 mg/kg morphine was tested after the dose-response function for CP55940 was completed.

The major goal of this study was to assess the potential interaction between morphine and CP55940 in the delay discounting task. The doses of morphine chosen for mixtures were 0.32 mg/kg and 1.0 mg/kg because, given alone, they were the largest doses that did not decrease control response rates by more than 20%.

Drugs

Morphine sulfate was provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD) and was dissolved in sterile water. 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP55940; Sigma-Aldrich, St. Louis, MO) was dissolved in pure ethanol (2 ml ethanol per bottle containing 10 mg CP55940) then diluted with a solution of 1:1:18 ethanol:emulphor:saline. Drugs were administered subcutaneously in a volume of 0.2 – 0.8 ml. Pretreatment times were 15 min for morphine and 60 min for CP55940; behavioral effects of morphine and CP55940 are apparent with 15 and 60 min, respectively, and last at least 90 min in various operant-conditioning tasks, including delay discounting (e.g., Maguire et al., 2012, 2013, 2016). All doses were expressed as the weight of the forms noted above.

Data Analyses

When both reinforcers were delivered immediately (no delay), the primary measure was the proportion choice for the larger reinforcer (number of choices for the larger reinforcer divided by total number of choice trials completed [i.e., maximum 30 choice trials]). For sessions in which delay adjusted for the larger reinforcer, there were two primary measures: the median adjusted delay (based on up to 15 delays per session) and overall response rate (total number of responses divided by total duration the levers were extended).

To compare the median adjusted delays in each of the pellet conditions (3 versus 1; 2 versus 1; and 3 versus 2), the first three sessions that met the stability criterion were examined. Because Monkey GE did not meet stability criteria in the 3 versus 1 pellet condition (the median adjusted delay was still increasing even by Session 10), the last 3 sessions were examined. A one-way repeated measures ANOVA with Bonferroni's post hoc test was conducted for each monkey and then again for each condition to compare among subjects.

To analyze the effects of morphine and CP55940 administered alone, the mean and 95% confidence interval from each dose were compared to the mean and 95% CI from control (saline) sessions; if the 95% CIs did not overlap, the effects were considered significant. Because the potency of CP55940 varied among subjects, the dose producing the largest increase in the median adjusted delay was determined for each monkey (i.e., E_{MAX}) with smaller and larger doses expressed in log-unit doses relative to the E_{MAX} (see Manvich et al., 2012). A two-way repeated-measures ANOVA was used to analyze the effects of morphine/CP55940 mixtures on the ascending limb of the dose-response curve for median adjusted delay. To analyze the effects of morphine/CP55940 mixtures on response rate, the data were converted to a percentage of the control (vehicle) response rate. ED_{50} values were obtained for individual monkeys by fitting a linear regression to the data between 20% and 80% (including not more than one dose producing less than 20% effect and not more than one dose producing more than 80% effect). Potency ratios for each subject were calculated

by dividing the ED_{50} of CP55940 by the ED_{50} of CP55940 with morphine. Data were then averaged among the 3 subjects; when the 95% CI did not contain 1 the shift was considered significant. Analyses were conducted using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

RESULTS

With no delay, monkeys chose the larger amount of pellets over the smaller amount (Figure 1) and completed all trials (not shown). The number of sessions needed to meet criteria (0.8 proportion choice for the larger amount) when 3 pellets were signaled by the red stimulus and 1 pellet was signaled by the green stimulus, was 1 session for Monkeys BR and GE and 2 sessions for Monkey AN. When the stimuli signaling each reinforcer were reversed, Monkeys AN and GE met criteria in 2 sessions, and Monkey BR in 4 sessions. When the stimuli were reversed again, Monkeys BR and GE required 4 and 5 sessions, respectively, to meet criteria, whereas Monkey AN met criteria in 1 session. Finally, when the number of pellets for the smaller reinforcer was increased from 1 pellet to 2 pellets and when the number of pellets for the larger reinforcer was decreased from 3 pellets to 2 pellets, monkeys chose the larger amount and met criteria within 2 sessions.

When the relative difference between the larger and smaller number of pellets was decreased, the adjusted delays were shorter (Figure 2). The difference in the adjusted delay between conditions was statistically significant for Monkey AN ($F_{2,8}=996.2$, $p<0.01$; $t=35.0$, $p<0.05$ for 3 v. 1 pellets and 2 v. 1 pellets; $t=41.5$, $p<0.05$ for 3 v. 1 pellets and 3 v. 2 pellets; $t=6.5$, $p<0.05$ for 2 v. 1 pellets and 3 v. 2 pellets) and for Monkey BR ($F_{2,8}=58.6$, $p<0.01$; $t=5.4$, $p<0.05$ for 3 v. 1 pellets and 2 v. 1 pellets; $t=10.8$, $p<0.05$ for 3 v. 1 pellets and 3 v. 2 pellets; $t=5.5$, $p<0.05$ for 2 v. 1 pellets and 3 v. 2 pellets). For Monkey GE, the difference was significant only for 3 versus 1 pellets compared to 3 versus 2 pellets ($F_{2,8}=11.6$, $p<0.02$; $t=4.81$, $p<0.05$).

In the 3 versus 1 pellet condition, the adjusted delay for Monkey BR was significantly shorter than the adjusted delay for Monkey GE ($F_{2,8}=9.1$, $p=0.03$; $t=4.3$, $p<0.05$) but not Monkey AN; Monkey AN was not significantly different from Monkey GE. In the 2 versus 1 pellet condition and the 3 versus 2 pellet condition, Monkey GE had significantly longer adjusted delays than Monkey AN ($F_{2,8}=34.7$, $p<0.01$; $t=7.3$, $p<0.05$ for 2 v. 1 pellets; $F_{2,8}=56.1$, $p<0.01$; $t=9.3$, $p<0.05$ for 3 v. 2 pellets) and Monkey BR ($t=7.2$, $p<0.05$ for 2 v. 1 pellets; $t=9.1$, $p<0.05$ for 3 v. 2 pellets), but Monkey AN and Monkey BR did not differ significantly.

When the initial delay of a session was 100 s, the monkeys adjusted the delay downward to values that approximated the median adjusted delay for each monkey (right panels, Figure 2). The median adjusted delay in 3 representative sessions of the 3 versus 2 pellet condition was 9.9, 10.0, and 10.0 for Monkey AN and 34.0, 26.3, and 32.8 s for Monkey GE; these monkeys adjusted the delay to a minimum 12.5 sec (Monkey AN) and 24.4 s (Monkey GE) when the initial delay was 100 sec for 3 sessions. The median adjusted delay was 33.0, 33.9, and 31.9 s for Monkey BR in the 3 versus 1 pellet condition, and the delay was adjusted downward to a minimum of 22.2 sec when the initial delay was 100 s.

Morphine increased the median adjusted delay in all monkeys (Figure 3). The increase was significant in Monkey AN at 1.0, 3.2, and 5.6 mg/kg, in Monkey BR at 5.6 mg/kg, and in Monkey GE at 1.0 mg/kg. Morphine significantly decreased response rates at 3.2 and 5.6 mg/kg in all monkeys, and also at 1.0 mg/kg in Monkey AN. At least one dose of CP55940 increased the median adjusted delay in each monkey (upper panels, Figure 4). Compared with the control values (3 saline sessions before each CP55940 test; see means and 95% CIs in Table 1), the increase was significant in Monkey AN at 0.01 and 0.032 mg/kg, in Monkey BR at 0.032 mg/kg, and in Monkey GE at 0.01 mg/kg. CP55940 dose-dependently decreased responding in all monkeys (middle panels, Figure 4). The rate-decreasing effect of CP55940 was significant at 0.1 mg/kg for all monkeys and at 0.32 mg/kg for Monkey BR (compared to mean control response rates and 95% CIs; see Table 1). The number of choice trials completed (lower panels, Figure 4) tended to co-vary inversely with the median adjusted delay but did not necessarily co-vary with response rate.

The potency of CP55940 differed among individual monkeys (i.e., an E_{MAX} of 0.01 mg/kg for Monkeys AN and GE and 0.032 mg/kg for Monkey BR); therefore, median adjusted delay and response rate were plotted as a function of the log-unit dose relative to the E_{MAX} (Figure 4). CP55940 increased the group median adjusted delay 1.9-fold from 59.9 sec (mean for vehicle) to 109.5 sec (mean for E_{MAX}). A two-way repeated measures ANOVA revealed a significant main effect of CP55940 ($F_{2,12}=6.68$, $p=0.01$) but not morphine ($F_{2,6}=0.06$, NS), and there was not a significant interaction between CP55940 and morphine ($F_{4,12}=1.65$, NS). The dose of CP55940 that significantly increased the median adjusted delay was without effect on response rate. For all monkeys, the CP55940 dose-response curve for response rate shifted leftward when CP55940 was given in combination with either 0.32 or 1.0 mg/kg morphine, with a significantly increased potency of CP55940 to decrease responding (Table 2).

DISCUSSION

Mu opioid receptor agonists are commonly used to treat moderate to severe pain; however, opioids are also the most widely abused of all prescription medications with fatal overdoses having reached epidemic levels (Volkow, 2014; Center for Disease Control and Prevention [CDC], 2016). Consequently, there is a need to identify pharmacological treatments for pain with fewer adverse effects than those currently limiting the use of *mu* opioid receptor agonists. Previous studies showed that cannabinoid receptor agonists increase the potency of opioids for producing antinociceptive but not reinforcing effects (e.g., Maguire et al., 2013). Abuse of *mu* opioid receptor agonists is associated with enhanced impulsivity (delay discounting) in humans, and experimenter-administered opioids can enhance delay discounting in nonhumans (e.g., Kirby and Petry, 2004; Pitts and McKearney, 2005). Therefore, this study evaluated the effects of the *mu* opioid receptor agonist morphine and the CB₁/CB₂ receptor agonist CP55940, alone and in mixtures, on delay discounting performance in rhesus monkeys, to determine whether the effects of mixtures are greater than either drug administered alone. The results showed that neither morphine nor CP55940 alone enhanced delay discounting, and at least some doses decreased delay discounting (increased the median adjusted delay) in every monkey. Co-administration of morphine (0.32 and 1.0 mg/kg) and CP55940 in mixtures also decreased discounting, with the effects

on median adjusted delay not being significantly different from the effects of either drug administered alone (i.e., no interaction). Morphine significantly increased the potency of CP55940 to decrease response rates, even though 0.32 and 1.0 mg/kg morphine did not decrease response rates by more than 20% when administered alone.

The design of the delay discounting procedure used in the present study was based on the adjusting delay procedure described by Mazur (1988, 1997) and Cardinal et al. (2002). Stable performance in this task occurs when a subject allocates responding approximately equally between the immediate and delayed reinforcers. The adjusted delay represents the point at which a subject is indifferent between a smaller immediate reinforcer and a larger delayed reinforcer. For each monkey in this study, there was a delay at which they were indifferent between a smaller immediate and larger delayed reinforcer, regardless of baseline delay. However, there were differences among monkeys; for example, under control conditions Monkey GE had significantly longer baseline delays compared with Monkeys AN and BR. Monkey GE also showed greater variability in the adjusted delay under control and drug conditions. Because the delay increased or decreased by 25% depending on performance, the absolute values of the adjustments for Monkey GE were larger than the adjustments for other monkeys. Cardinal et al. (2002) reported that larger adjustments can produce greater variability, and Mazur (1988) noted that larger adjustments might lead to relatively larger stable delays. Monkey GE also had much lower response rates compared with the other two monkeys, possibly related to the longer delays. In pigeons and rats, with longer delays the latency to respond increases and the response rate decreases (Green et al. 1981; Green and Estle 2003). Notwithstanding the differences among monkeys under control conditions, the same pattern of performance occurred (increased delay) when morphine and CP55940 were administered; thus, drug effects did not appear to depend on baseline performance.

Previous studies reported enhanced discounting following the administration of an opioid receptor agonist (Harvey-Lewis and Franklin, 2015; Maguire et al., 2016), suggesting that a potential adverse effect of opioids is increased impulsivity. In this study, neither morphine nor CP55940 increased impulsivity, which would be reflected by a decrease in the median adjusted delay. Conversely, both morphine and CP55940, administered alone, increased the median adjusted delay (i.e., decreased impulsivity). This finding cannot easily be attributed to or interpreted as a lever-position bias or perseverative responding because the lever position associated with the delayed reinforcer alternated within session. Previous studies used a different procedure in which the delay to the larger reinforcer increased by a fixed duration within session and the lever position associated with each reinforcer remained constant (Evenden and Ryan, 1996). Previous studies also used the same discriminative stimuli for the immediate and delayed reinforcer, whereas the present study used distinct stimuli (i.e., green stimulus for the immediate reinforcer and red stimulus for the delayed reinforcer). While it is possible that increases in the median adjusted delay result from a stimulus (color) bias, that appears unlikely because the monkeys were trained to discriminate between larger and smaller reinforcer amounts when the stimulus colors were reversed. Thus, procedural differences between the present study and previous studies might account for different effects of morphine on impulsivity. The adjusting delay procedure might be sensitive to different aspects of impulsivity compared with procedures using fixed,

ascending, or descending delays (e.g., Maguire et al., 2016; Tanno et al., 2014), much in the same way that delay discounting is sensitive to different aspects of impulsivity compared with motor (impulsivity) tasks. There is no *a priori* reason to expect that different procedures will necessarily measure the same features of something as complex and multifaceted as impulsivity. The current findings underscore the importance of testing the generality of drug effects on different aspects of impulsivity.

Statistical analyses indicated a significant effect of CP55940 but not morphine on delay discounting, and there was no significant interaction between morphine and CP55940. Morphine/CP55940 mixtures did not increase impulsivity (i.e., decrease median adjusted delay) in this task. Rather, morphine/CP55940 mixtures increased the median adjusted delay, which might reflect less impulsivity. This interpretation is consistent with previous studies showing that cannabinoids selectively enhance some (antinociceptive) but not other (positive reinforcing) effects of opioids (Maguire and France, 2016a) and attenuate the discriminative stimulus effects of opioids (e.g., Maguire and France, 2016b). There are at least three other possible explanations for these drug-induced increases in the median adjusted delay. First, cannabinoids have well-known hyperphagic effects (e.g., Berry and Mechoulam, 2002), which might have increased preference for the larger (sometimes delayed) amount of food; however, it is worth noting that the effects of drugs under free-feeding conditions often are not predictive of effects on operant behavior maintained by food reinforcement (e.g., LeSage et al., 2004). Second, cannabinoids can alter the time perception, which is an important feature in delay discounting (Wittmann and Paulus, 2008); however, this interpretation is not consistent with findings in humans that THC caused relatively short (2 – 4 s) but not long (8 – 32 s) intervals to be overestimated. Third, both morphine and CP55940 have sedative effects (Dhingra et al., 2015), which may have increased other behavior associated with non-programmed reinforcement (e.g., sleep) to occur during the delay, in turn, making the delay to the programmed food reinforcement more tolerable. This interpretation is consistent with the finding in the present study that rate-decreasing effects were greatest with morphine/CP55940 mixtures.

This study used acute drug administration to examine possible interactions between morphine and CP55940 on complex choice behavior. Particularly relevant to the clinical use of opioids are conditions of chronic drug administration, under which enhanced discounting can occur (Setlow et al., 2009; Zancy and de Wit, 2009); the potential for morphine/CP55940 mixtures to adversely affect complex operant behavior (e.g., impulsive choice) has yet to be examined under chronic administration conditions. Opioid abuse and opioid dependence in humans are associated with increased delay discounting and probability discounting (Madden et al., 1997; Kirby et al., 1999; Odum et al., 2000; Kirby and Petry, 2004), and humans who self-report regular or daily marijuana use have steeper discounting functions than non-users (Johnson et al., 2010; Aston et al., 2016; Mejía-Cruz et al., 2016). Thus, compared to acute dosing conditions, the effects of opioid/cannabinoid mixtures might be qualitatively and/or quantitatively different during repeated administration or after discontinuation of repeated treatment.

In conclusion, in this study the effects of CP55940 and morphine on response rate, but not adjusted delay, in an impulsive choice task, were enhanced by their co-administration in

rhesus monkeys. These results extend previous research showing that cannabinoids enhance antinociceptive effects but not reinforcing or discriminative stimulus effects of opioids, and add to a growing body of evidence supporting the therapeutic potential of opioid/cannabinoid mixtures. It is unclear whether the present findings are predictive of the effects of opioid/cannabinoid mixtures on other forms of impulsivity (e.g., motor impulsivity) or during chronic drug treatment, in part because different procedures appear to be sensitive to different measures of impulsivity. Because pain patients often require repeated drug administration, the effects of morphine/CP5540 mixtures on various complex behaviors should be evaluated during and after daily drug administration. The relationship among pain, impulsivity, and opioids appears to be complex; emerging evidence shows that chronic pain increases motor impulsivity and acute administration of the *mu* opioid receptor agonist remifentanyl further increases motor impulsivity in an additive manner, even at pain-relieving doses (Kurita et al., 2015). If opioid/cannabinoid mixtures enhance impulsivity, then the therapeutic potential of these drugs likely would be limited.

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References

- Anker JJ, Perry JL, Gliddon LA, Carroll ME. Impulsivity predicts the escalation of cocaine self-administration in rats. *Pharmacol Biochem Behav.* 2009; 93:343–8. [PubMed: 19490925]
- Aston ER, Metrik J, Amlung M, Kahler CW, MacKillop J. Interrelationships between marijuana demand and discounting of delayed rewards: Convergence in behavioral economic methods. *Drug Alcohol Depend.* 2016; 169:141–7. [PubMed: 27810657]
- Berry EM, Mechoulam R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol Ther.* 2002; 95:185–90. [PubMed: 12182965]
- Bickel WK, Johnson MW, Koffarnus MN, MacKillop J, Murphy JG. The behavioral economics of substance use disorders: Reinforcement pathologies and their repair. *Ann Rev Clin Psychol.* 2014; 10:641–77. [PubMed: 24679180]
- Cardinal RN, Daw N, Robbins TW, Everitt BJ. Local analysis of behaviour in the adjusting-delay task for assessing choice of delayed reinforcement. *Neural Networks.* 2002; 15:617–34. [PubMed: 12371516]
- Centers for Disease Control and Prevention(CDC). Injury Prevention & Control: Opioid Overdose. 2016 Mar 14. Retrieved from <http://www.cdc.gov/drugoverdose/index.html>
- Cichewicz DL, McCarthy EA. Antinociceptive synergy between 9-tetrahydrocannabinol and opioids after oral administration. *J Pharmacol Exp Ther.* 2003; 304:1010–5. [PubMed: 12604676]
- Crean RD, Tapert SF, Minassian A, Macdonald K, Crane NA, Mason BJ. Effects of chronic, heavy cannabis use on executive functions. *J Addict Med.* 2011; 5:9–15. [PubMed: 21643485]
- Dhingra L, Ahmed E, Shin J, Scharaga E, Magun M. Cognitive effects and sedation. *Pain Med.* 2015; 16(Suppl 1):S37–43. [PubMed: 26461075]
- Eppolito AK, France CP, Gerak LR. Effects of acute and chronic morphine on delay discounting in pigeons. *J Exp Anal Behav.* 2013; 99:277–89. [PubMed: 23553726]

- Evenden JL, Ryan CN. The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology (Berl)*. 1996; 128:161–70. [PubMed: 8956377]
- Friswell J, Phillips C, Holding J, Morgan CJA, Brandner B, Curran HV. Acute effects of opioids on memory functions of healthy men and women. *Psychopharmacology (Berl)*. 2008; 198:243–50. [PubMed: 18379759]
- Green L, Estle SJ. Preference reversals with food and water reinforcers in rats. *J Exp Anal Behav*. 2003; 79:233–42. [PubMed: 12822689]
- Green L, Fisher EB, Perlow S, Sherman L. Preference reversal and self-control: choice as a function of reward amount and delay. *Behav Anal Lett*. 1981; 1:43–51.
- Harvey-Lewis C, Perdriet J, Franklin KB. The effect of morphine dependence on impulsive choice in rats. *Psychopharmacology (Berl)*. 2012; 223:477–87. [PubMed: 22588252]
- Irimia C, Polis IL, Stouffer D, Parsons LH. Persistent effects of chronic 9-THC exposure on motor impulsivity in rats. *Psychopharmacology (Berl)*. 2015; 232:3033–43. [PubMed: 25925779]
- Johnson MW, Bickel WK, Baker F, Moore BA, Badger GJ, Budney AJ. Delay discounting in current and former marijuana-dependent individuals. *Exp Clin Psychopharmacol*. 2010; 18:99–107. [PubMed: 20158299]
- Kazantzis NP, Casey SL, Seow PW, Mitchell VA, Vaughan CW. Opioid and cannabinoid synergy in a mouse neuropathic pain model. *Br J Pharmacol*. 2016; 173:2521–31. [PubMed: 27278681]
- Kieres AK, Hausknecht KA, Farrar AM, Acheson A, de Wit H, Richards JB. Effects of morphine and naltrexone on impulsive decision making in rats. *Psychopharmacology (Berl)*. 2004; 173:167–74. [PubMed: 14752586]
- Kirby KN, Petry NM. Heroin and cocaine abusers have higher discount rates for delayed rewards than alcoholics or non-drug-using controls. *Addiction*. 2004; 99:461–471. [PubMed: 15049746]
- Kirby KN, Petry NM, Bickel WK. Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. *J Exp Psychol Gen*. 1999; 128:78–87. [PubMed: 10100392]
- Kurita GP, Malver LP, Andresen T, Polianskis R, Drewes AM, Christrup L, Hojsted J, Sjogren P. Does mutual compensation of the cognitive effects induced by pain and opioids exist? An experimental study. *Psychopharmacology (Berl)*. 2015; 232:1373–81. [PubMed: 25345731]
- LeSage MG, Stafford D, Glowa JR. Effects of anorectic drugs on food intake under progressive-ratio and free-access conditions in rats. *J Exp Anal Behav*. 2004; 82:275–92. [PubMed: 15693523]
- Li JX, McMahon LR, Gerak LR, Becker GL, France CP. Interactions between 9-tetrahydrocannabinol and μ opioid receptor agonists in rhesus monkeys: discrimination and antinociception. *Psychopharmacology (Berl)*. 2008; 199:199–208. [PubMed: 18470505]
- Li JX, Koek W, France CP. Interactions between 9-tetrahydrocannabinol and heroin: self-administration in rhesus monkeys. *Behav Pharmacol*. 2012; 23:754–761. [PubMed: 23044830]
- Madden GJ, Petry NM, Badger GJ, Bickel WK. Impulsive and self-control choices in opioid-dependent patients and non-drug-using control participants: drug and monetary rewards. *Exp Clin Psychopharmacol*. 1997; 5:256–262. [PubMed: 9260073]
- Maguire DR, France CP. Impact of efficacy at the μ -opioid receptor on antinociceptive effects of combinations of μ -opioid receptor agonists and cannabinoid receptor agonists. *J Pharmacol Exp Ther*. 2014; 351:383–9. [PubMed: 25194020]
- Maguire DR, France CP. Effects of daily delta-9-tetrahydrocannabinol treatment on heroin self-administration in rhesus monkeys. *Behav Pharmacol*. 2016a; 27:249–57. [PubMed: 26397756]
- Maguire, France. Interactions between cannabinoid receptor agonists and μ opioid receptor agonists in rhesus monkeys discriminating fentanyl. *Eur J Pharmacol*. 2016b; 784:199–206. [PubMed: 27184925]
- Maguire DR, Li JX, France CP. Impulsivity and drugs of abuse: a juice-reinforced operant procedure for determining within-session delay discounting functions in rhesus monkeys. *J Pharmacol Toxicol Methods*. 2012; 66:264–9. [PubMed: 22944347]
- Maguire DR, Yang W, France CP. Interactions between μ -opioid receptor agonists and cannabinoid receptor agonists in rhesus monkeys: antinociception, drug discrimination, and drug self-administration. *J Pharmacol Exp Ther*. 2013; 345:354–362. [PubMed: 23536317]

- Maguire DR, Gerak LR, France CP. Effect of daily morphine administration and its discontinuation on delay discounting of food in rhesus monkeys. *Behav Pharmacol.* 2016; 27:155–64. [PubMed: 26397762]
- Manvich D, Kimmel HL, Howell LL. Effects of Serotonin 2C Receptor Agonists on the Behavioral and Neurochemical Effects of Cocaine in Squirrel Monkeys. *J Pharmacol Exp Ther.* 2012; 341:424–34. [PubMed: 22328576]
- Mazur JE. Estimation of indifference points with an adjusting-delay procedure. *J Exp Anal Behav.* 1988; 49:37–47. [PubMed: 3346621]
- Mazur JE. Choice, delay, probability, and conditioned reinforcement. *Animal Learning & Behavior.* 1997; 25:131–147.
- McDonald J, Schleifer L, Richards JB, de Wit H. Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology.* 2003; 28:1356–1365. [PubMed: 12784123]
- Mejía-Cruz D, Green L, Myerson J, Morales-Chaine S, Nieto J. Delay and probability discounting by drug-dependent cocaine and marijuana users. *Psychopharmacology (Berl).* 2016; 233:2705–14. [PubMed: 27180181]
- Metrik J, Kahler CW, Reynolds B, McGeary JE, Monti PM, Haney M, de Wit H, Rohsenow DJ. Balanced placebo design with marijuana: pharmacological and expectancy effects on impulsivity and risk taking. *Psychopharmacology (Berl).* 2012; 223:489–499. [PubMed: 22588253]
- Odum AL. Delay discounting: I'm a k, you're a k. *J Exp Anal Behav.* 2011; 96:427–39. [PubMed: 22084499]
- Odum AL, Madden GJ, Badger GJ, Bickel WK. Needle sharing in opioid-dependent outpatients: psychological processes underlying risk. *Drug Alcohol Depend.* 2000; 60:259–66. [PubMed: 11053760]
- Panlilio LV, Goldberg SR, Justinova Z. Cannabinoid abuse and addiction: clinical and preclinical findings. *Clin Pharmacol Ther.* 2015; 97:616–27. [PubMed: 25788435]
- Pattij T, Schetters D, Janssen MC, Wiskerke J, Schoffelemeier AN. Acute effects of morphine on distinct forms of impulsive behavior in rats. *Psychopharmacology (Berl).* 2009; 205:489–502. [PubMed: 19436995]
- Perry JL, Carroll ME. The role of impulsive behavior in drug abuse. *Psychopharmacology (Berl).* 2008; 200:1–26. [PubMed: 18600315]
- Perry JL, Larson EB, German JP, Madden GJ, Carroll ME. Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. *Psychopharmacology (Berl).* 2005; 178:193–201. [PubMed: 15338104]
- Pitts RC, McKinney AP. Effects of methylphenidate and morphine on delay-discounting functions obtained within sessions. *J Exp Anal Behav.* 2005; 83:297–314. [PubMed: 16047609]
- Ramaekers JG, Kauert G, van Ruitenbeek P, Theunissen EL, Schneider E, Moeller MR. High-potency marijuana impairs executive function and inhibitory motor control. *Neuropsychopharmacology.* 2006; 31:2296–303. [PubMed: 16572123]
- Setlow B, Mendez IA, Mitchell MR, Simon NW. Effects of chronic administration of drugs of abuse on impulsive choice (delay discounting) in animal models. *Behav Pharmacol.* 2009; 20:380–9. [PubMed: 19667970]
- Smith HS. Combination opioid analgesics. *Pain Physician.* 2008; 11:201–14. [PubMed: 18354712]
- Tanno T, Maguire DR, Henson C, France CP. Effects of amphetamine and methylphenidate on delay discounting in rats: interactions with order of delay presentation. *Psychopharmacology (Berl).* 2014; 231:85–95. [PubMed: 23963529]
- Tham SM, Angus JA, Tudor EM, Wright CE. Synergistic and additive interactions of the cannabinoid agonist CP55,940 with *mu* opioid receptor and alpha2-adrenoceptor agonists in acute pain models in mice. *Br J Pharmacol.* 2005; 144:875–884. [PubMed: 15778704]
- Verdejo-García AJ, Perales JC, Pérez-García M. Cognitive impulsivity in cocaine and heroin polysubstance abusers. *Addict Behav.* 2007; 32:950–966. [PubMed: 16876962]
- Volkow ND, Frieden TR, Syde PS, Cha SS. Medication-assisted therapies – tackling the opioid-overdose epidemic. *N Engl J Med.* 2014; 370:2063–66. [PubMed: 24758595]

- Wiskerke J, Stoop N, Schetters D, Schoffelmeer ANM, Pattij T. Cannabinoid CB1 Receptor Activation Mediates the Opposing Effects of Amphetamine on Impulsive Action and Impulsive Choice. *PLoS One*. 2011; 6:e25856. [PubMed: 22016780]
- Wittmann M, Paulus MP. Decision making, impulsivity and time perception. *Trends in Cognitive Sciences*. 2008; 12:7–12. [PubMed: 18042423]
- Zancy JP, de Wit H. The prescription opioid, oxycodone, does not alter behavioral measures of impulsivity in healthy volunteers. *Pharmacol Biochem Behav*. 2009; 94:108–13. [PubMed: 19660489]

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Figure 1.

Proportion choice for the larger number of pellets (vertical axes; from a maximum of 30 choice trials) for sessions (horizontal axes) in which the larger and the smaller number of pellets were delivered immediately (i.e., no delay). Each symbol denotes an individual subject. The color of the stimulus light signaling the availability of the larger amount is indicated for each condition.

Figure 2.

The adjusted delay (vertical axes) in sec for each block (horizontal axes; up to 15 per 100-min session) in three representative sessions from the 3 versus 1 pellet, 2 versus 1 pellet, and 3 versus 2 pellet conditions; the three sessions shown for each condition were consecutive and satisfied the criteria for stability (see text for details). The horizontal solid lines through the open symbols represent the median delay for each session. Filled symbols represent the adjusted delay when the initial delay for the session was 100 s. Dashed horizontal lines through the filled symbols are the means for the adjusted delay (the median adjusted delay

average for three sessions) in the 3 versus 2 pellet condition (Monkeys AN and GE) or the 3 versus 1 pellet condition (Monkey BR). Each symbol denotes an individual subject (same symbols as in Figure 1).

Figure 3.

Morphine dose-response curves for median adjusted delay (s; upper panels), response rate (responses/s; middle panels), and choice trials completed (lower panels) for individual subjects. Each data point represents the mean of two determinations and error bars denote $\pm 95\%$ CI. The horizontal shaded bars represent the 95% CI from control sessions (three consecutive saline sessions preceding each drug test). See text for statistical analyses.

Figure 4.

Dose-response curves for CP55940 administered alone and in combination with morphine (0.32 and 1.0 mg/kg) for median adjusted delay, response rate, and choice trials completed for individual subjects. In the rightmost panels data for the median adjusted delay, response rate (percentage of the control), and choice trials (percentage of the control) are averaged for the 3 subjects and plotted as a function of the log-unit dose relative to the E_{MAX} dose for CP55940 alone. Each data point, including symbols above "V" (CP55940 vehicle), represents the mean of two determinations and error bars denote ± 1 SEM. See text and Table 2 for details regarding statistical analyses. The median adjusted delay was not calculated if a monkey completed fewer than 8 trials (i.e., at least 2 blocks had to be completed, allowing for the opportunity to adjust the delay).

Table 1

Median adjusted delay, response rate, and the number of choice trials completed for individual monkeys under control conditions (i.e., three consecutive saline sessions immediately preceding each CP55940 test). Values shown are the mean and 95% confidence interval (CI).

Monkey	Median Adjusted Delay, sec (95% CI)	Responses per sec (95% CI)	Choice Trials Complete (95% CI)
AN	28.8 (7.2)	5 (0.2)	30 (0)
BR	38.1 (7.7)	4.7 (0.2)	29.9 (0.2)
GE	109.2 (26.2)	2.6 (0.4)	20.4 (2.7)

ED₅₀ values for CP55940 alone with morphine (0.32 and 1.0 mg/kg) for response rate. The potency ratio (CP55940/CP55940+morphine) is shown for individual monkeys, and for the group mean with 95% confidence intervals (CI).

Table 2

Monkey	CP55940		+ 0.32 mg/kg morphine		+ 1.0 mg/kg morphine	
	ED ₅₀	Potency Ratio	ED ₅₀	Potency Ratio	ED ₅₀	Potency Ratio
AN	0.032	0.015	2.05	0.011	2.96	
BR	0.092	0.029	3.18	0.018	5.07	
GE	0.032	0.017	1.85	0.006	5.37	
Group Mean (95% CI)			2.36 (1.54, 3.18)		4.47 (2.99, 5.95)	