



Reinforcing effects of opioid/cannabinoid mixtures in rhesus monkeys responding under a food/drug choice procedure

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Abstract

Objective Cannabinoid receptor agonists such as delta-9-tetrahydrocannabinol (Δ^9 -THC) enhance the antinociceptive potency of mu opioid receptor agonists such as morphine, indicating that opioid/cannabinoid mixtures might be effective for treating pain. However, such enhancement will be beneficial only if cannabinoids do not also enhance adverse effects of opioids, including those related to abuse. In rhesus monkeys, cannabinoids fail to enhance and often decrease self-administration of the mu opioid receptor agonist heroin, suggesting that opioid/cannabinoid mixtures do not have greater reinforcing effects (abuse potential) compared with opioids alone. Previous studies on the self-administration of opioid/cannabinoid mixtures used single-response procedures, which do not easily differentiate changes in reinforcing effects from other effects (e.g., rate decreasing).

Methods In this study, rhesus monkeys ($n = 4$) responded under a choice procedure wherein responding on one lever delivered sucrose pellets and responding on the other lever delivered intravenous infusions of the mu opioid receptor agonist remifentanyl (0.032–1.0 $\mu\text{g}/\text{kg}/\text{infusion}$) alone or in combination with either Δ^9 -THC (10–100 $\mu\text{g}/\text{kg}/\text{infusion}$) or the synthetically derived cannabinoid receptor agonist CP55940 (3.2–10 $\mu\text{g}/\text{kg}/\text{infusion}$).

Results Remifentanyl dose-dependently increased choice of drug over food, whether available alone or in combination with a cannabinoid, and the potency of remifentanyl was not significantly altered by coadministration with a cannabinoid. Mixtures containing the largest doses of cannabinoids decreased response rates in most subjects, confirming that behaviorally active doses were studied.

Conclusion Overall, these results extend previous studies to include choice behavior and show that cannabinoids do not substantially enhance the reinforcing effects of mu opioid receptor agonists.

Keywords Opioids · Cannabinoids · Drug mixtures · Choice procedures · Drug self-administration · Rhesus monkeys

Introduction

Pain continues to be a significant clinical problem and mu opioid receptor agonists such as morphine and oxycodone are the gold standard for treating moderate to severe pain. However, numerous adverse effects (e.g., tolerance, dependence, abuse, and overdose) limit the legitimate medical use of opioids. Lowering the dose of opioid required to treat pain adequately could limit the risks associated with larger doses

(Dowell et al. 2016). One strategy for reducing the dose of the opioid required for adequate pain treatment while maintaining effectiveness is to combine an opioid with another (non-opioid) drug such that the combination produces the same therapeutic effect as a larger dose of the opioid alone.

Cannabinoid receptor agonists such as delta-9-tetrahydrocannabinol (Δ^9 -THC), the primary psychoactive constituent of cannabis, as well as synthetically derived agonists such as CP55940 increase the antinociceptive potency of mu opioid receptor agonists such as morphine in non-human primates as measured with the warm water tail withdrawal procedure (Li et al. 2008; Maguire et al. 2013b; Maguire and France 2014). Moreover, cannabinoids have been shown to enhance analgesic effects of opioids in humans (e.g., Cooper et al. 2018) including in pain patients (see Nielson et al. 2017 for a review). However, the therapeutic utility of opioid/cannabinoid mixtures depends upon whether

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cannabinoids also enhance the potency of opioids to produce other unwanted effects, particularly those contributing to abuse. In rhesus monkeys, response-contingent and/or non-contingent administration of Δ^9 -THC or CP55940 fails to enhance and often decreases self-administration of the mu opioid receptor agonist heroin (Li et al. 2012; Maguire et al. 2013b; Maguire and France 2016b), suggesting that opioid/cannabinoid mixtures do not have greater reinforcing effects compared with opioids alone. However, previous studies used a single-response, fixed-ratio schedule that does not easily differentiate changes in reinforcing effects from other (e.g., generalized rate suppression) effects (e.g., Katz 1989); thus, the interaction between opioids and cannabinoids with regard to reinforcing effects remains unclear.

Several procedures (e.g., progressive ratio, demand, and second-order schedules) were developed, in part, to address the interpretational challenges associated with the use of single-response, fixed-ratio schedules to characterize the reinforcing effectiveness of drugs. Although each approach has strengths and weaknesses, the current study used a choice procedure to compare the reinforcing effects of an opioid alone to those of an opioid/cannabinoid mixture, with food as the non-drug alternative. Under choice procedures, subjects can choose between two or more alternatives (Catania 1966) with the primary measure of reinforcing effects based on allocation of behavior among the alternatives rather than overall response output (i.e., response rate), which can be influenced by many factors not directly related to reinforcing effectiveness. Moreover, many of the adverse outcomes associated with drug abuse reflect disproportionate allocation of behavior to drug seeking and taking (e.g., Lamb and Ginsburg 2018), and choice procedures have been useful for assessing factors that impact drug taking (e.g., Banks and Negus 2017; Lamb et al. 2016; Perkins and Freeman 2018).

In the current study, responding on one lever delivered food and responding on the other lever delivered an intravenous (i.v.) infusion of remifentanyl alone or remifentanyl combined with a dose of Δ^9 -THC or CP55940. Like other mu opioid receptor agonists, such as heroin, remifentanyl is self-administered readily by non-human subjects. The faster onset and shorter duration of action of remifentanyl, compared with other opioids (e.g., Ko et al., 2002), are preferable under experimental conditions in which subjects make repeated choices because accumulation of the opioid is limited or avoided (Maguire et al. 2013a; Maguire et al. 2013c; Maguire et al. 2016). Δ^9 -THC and CP55940 were studied because both drugs increase the potency of some mu opioid receptor agonists to produce antinociceptive effects; however, the magnitude of enhancement appears to differ between them possibly related to their different intrinsic efficacies (Maguire and France 2014). If cannabinoids attenuate the reinforcing effects of remifentanyl per se, then its potency should be diminished when in a mixture with a cannabinoid, compared

with remifentanyl alone. On the other hand, if cannabinoids do not attenuate the reinforcing effects of remifentanyl, then its potency should not be altered up to unit doses of the cannabinoid that suppress overall response output.

Materials and methods

Animals Four adult rhesus monkeys (3 males [WI, GI, MO] and 1 female [RI]), weighing between 8.7 and 10.8 kg during this study, were housed individually in stainless steel cages with interior space measuring 81 cm tall by 81 cm wide by 72 cm deep; the home cage also served as the experimental chamber (see below). The colony room was maintained under a 14/10-h light/dark cycle (lights on at 0600 h). Monkeys were fed chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI, USA), fresh fruit, peanuts, and other edible treats daily at approximately 0730 h with water available continuously. Studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the United States National Institutes of Health (National Research Council 2011) and were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Surgery and equipment Monkeys were sedated with 10 mg/kg (i.m.) of ketamine (Henry Schein Animal Health, Dublin, OH, USA), intubated, and then maintained on 2 l/min oxygen and isoflurane anesthesia (Butler Animal Health Supply, Grand Prairie, TX, USA). A 5-french polyurethane catheter (Access Technologies, Skokie, IL, USA) was inserted into a jugular or femoral vein and tunneled subcutaneously to an exit point in the mid-scapular region of the back. Penicillin B&G (35,000 IU/kg) and meloxicam (0.2 mg/kg) were given postoperatively.

The catheter was passed through a flexible stainless steel tether (Lomir Biomedical, Quebec, Canada) and connected to an 18-g single-channel fluid swivel (Lomir Biomedical) that was secured to the rear wall of the cage. Outside of the cage, the swivel was attached to a 30-ml polypropylene syringe mounted in a syringe pump (Razel Scientific Instruments, Fairfax, VT, USA) that infused at a rate of 2.3 ml/min. Monkeys wore a jacket (Lomir Biomedical) that protected the catheter and secured the tether. A stainless steel instrument panel (20 cm high by 28 cm wide) was mounted on one wall of the cage that contained two 4.5-cm wide response levers, positioned 23 cm above the cage floor, and spaced 15 cm apart center to center; two metal partitions attached to the instrument panel between the levers prevented responding on both levers simultaneously with the same limb. Three stimulus lights were horizontally aligned 5 cm above the levers. Directly above the instrument panel was a 6 cm high by 5 cm wide aperture through which 300-mg raspberry flavored

sucrose pellets (5TUT, Test Diet, Richmond, IN, USA) were delivered via activation of a pellet dispenser (Med Associates, Inc., Fairfax, VT, USA).

Choice procedure Prior to the start of the session, catheter lines were flushed with 3 ml of heparinized saline (100 U/ml; Hospira Inc., Lake Forest, IL, USA), and a syringe containing the solution available for self-administration that day was connected to the catheter. The pump was then activated to fill the catheter with the new solution. At the beginning of the session (1200 h), one of the side lights was illuminated green. Thirty consecutive responses on the lever directly below the green light turned that light off, turned the white center light on for 2 s, and delivered the reinforcer associated with that lever for that day, either 3 sucrose pellets or an i.v. infusion. Food pellets were delivered at a rate of one every 0.5 s. Infusion durations ranged from 22 to 28 s, with each infusion delivering 1 ml of solution per 10 kg of body weight. Completion of the response requirement also initiated a 10-min timeout, during which all lights were off (except for illumination of the center light for 2 s) and responses were recorded but had no programmed consequence. After completion of the first post-reinforcer timeout, the other side light was illuminated green, and 30 consecutive responses on the lever located below that light delivered the associated reinforcer followed by a timeout. Each session began with four of these sample trials (2 per side), the order of which varied quasi-randomly across trials and sessions. Choice trials began once all sample trials were completed. During choice trials both side lights were illuminated green, and 30 consecutive responses on either lever delivered the reinforcer associated with that lever. The consequences of responding on each lever during choice trials were identical to those presented during the sample trials. For all trials, responses on one lever reset the response counter for the other lever. There was no limited hold for individual trials, and sessions ended after completion of 20 choice trials or 8 h, whichever occurred first.

Experimental design Dose-effect curves were determined for remifentanyl alone and in combination with varying unit doses of Δ^9 -THC or CP55940 using a within-subjects design wherein data for each monkey served as his or her own control. All monkeys had experience lever pressing for food and/or i.v. drug infusions under various schedules of reinforcement (e.g., Maguire et al. 2016), so no preliminary training was required. Infusions were always available for responding on one lever and food was always available for responding on another lever; however, the lever designations switched during the experiment as indicated below. Dose-effect curves were determined by varying the solution available across conditions. Each solution and lever designation (e.g., food, left; infusion, right) was in effect within and across sessions until responding was stable, as defined by three consecutive

sessions in which the percentage of choices for the infusion across sessions did not vary by more than 20% or for up to 8 sessions, whichever occurred first. Once responding was stable, the same solution continued to be available, but the lever designations were switched, confirming that behavior allocation reflected sensitivity to the consequences of responding on each lever rather than other factors (e.g., side bias). After responding was stable once again, the solution changed while the lever designations remained the same.

For each determination of a dose-effect curve, the dose of remifentanyl (0.032–1.0 $\mu\text{g}/\text{kg}/\text{infusion}$) available alone or in combination with a fixed unit dose of Δ^9 -THC (10.0–100.0 $\mu\text{g}/\text{kg}/\text{infusion}$) or CP55940 (3.2–10.0 $\mu\text{g}/\text{kg}/\text{infusion}$) varied in half-log unit increments until the range of remifentanyl doses tested included at least one dose that maintained not more than 30% of drug choice and at least one dose that maintained at least 70% of drug choice (see “Data and statistical analyses” section for further details about calculating the percentage of drug choice). Each unit dose of a cannabinoid was tested once per monkey in combination with a range of remifentanyl doses. Remifentanyl dose-effect curves determined in combination with different doses of a cannabinoid or different cannabinoids were separated by re-determination of a dose-effect curve for remifentanyl alone, which required that at least 12 sessions intervened between drug mixture tests. Saline was substituted for drug at the beginning and at the end of the study. During the course of the study, monkeys were sedated every 2 weeks with 10 mg/kg of ketamine to obtain an updated body weight, confirm catheter patency, perform routine health checks, and inspect the equipment.

Drugs Remifentanyl hydrochloride and delta-9-tetrahydrocannabinol (Δ^9 -THC) were provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (Bethesda, MD). 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP55940) was provided by NIDA or purchased (Sigma, St. Louis, MO, USA). Δ^9 -THC and CP55940 were stored at -20°C in absolute ethanol, and dilutions were made by mixing the ethanol solution with an equivalent volume of Alkamuls EL-620 (Rhodia, Cranbury, NJ, USA) and by adding sterile 0.9% saline to obtain the required concentration of drug. Saline made up at least 94% of the total volume for all solutions. Remifentanyl was dissolved in saline when administered alone and in the cannabinoid vehicle when administered in combination with a cannabinoid.

Data and statistical analyses Percentage of drug choice for each session was calculated by dividing the total number of ratios completed on the drug lever during choice trials by the total number of choice trials completed and multiplying by

100. Percentage of drug choice for each condition was obtained by calculating the mean percentage across six sessions, comprising the last three sessions under the first lever designation and the last three sessions following a lever designation switch. Response rate for each session was calculated by dividing the total number of responses on both levers, excluding timeout responses, by the total time that at least one green light was illuminated. Response rate for each condition was obtained by calculating the mean response rate across the six sessions indicated above.

Effects of the cannabinoids were analyzed by comparing the potency of remifentanyl to increase drug choice when available alone with the potency of remifentanyl in combination with each unit dose of cannabinoid. Percentage of drug choice was plotted as a function of the log-transformed (base 10) unit dose of remifentanyl. Dose-effect data for individual curve determinations were fit with a straight line using linear regression and only data comprising the linear portion of the curve, ranging from the largest dose that produced not more than 30% of drug choice to the smallest dose that produced at least 70% drug choice. The slope and the y-intercept were then used to estimate the dose of remifentanyl producing 50% drug choice (ED_{50}). The ED_{50} values for all dose-effect curves for remifentanyl alone (7 or 8 determinations across monkeys) were averaged for individual monkeys to determine the control ED_{50} . Potency ratios for remifentanyl in combination with each unit dose of a cannabinoid were calculated for individual monkeys by dividing the control ED_{50} by the ED_{50} for each drug mixture; potency ratios were calculated using anti-log transformed (i.e., arithmetic) ED_{50} values. Potency ratios greater than 1.0 indicate a leftward shift in the remifentanyl dose-effect curve (increased potency) whereas ratios less than 1.0 indicate a rightward shift in the dose-effect curve (decreased potency). For each unit dose of a cannabinoid, 95% confidence limits around the mean potency ratio for the group were calculated, and changes in the potency of remifentanyl were considered significant if the confidence limits did not include 1.0. All curve fitting and data analyses were conducted using Microsoft Excel® 2016 (Redmond, WA, USA).

Results

When given a choice between 3 sucrose pellets and saline, monkeys responded predominantly for food (not more than 8% choice of infusions) at rates ranging from 0.8 to 3.0 responses per second, completing all choice trials (Fig. 1, circles above “S”). When available alone, remifentanyl dose-dependently increased choice of drug, with 0.32 or 1.0 $\mu\text{g}/\text{kg}/\text{infusion}$ maintaining near exclusive choice of drug over food (Fig. 1, top row, circles); in 3 of 4 monkeys, response rates also increased as a function of remifentanyl dose, ranging from 1.8 to 3.2 responses per second at the largest doses tested (Fig. 1,

middle row, circles). Control ED_{50} values for choice of remifentanyl available alone ranged from 0.13 to 0.25 $\mu\text{g}/\text{kg}/\text{infusion}$ across monkeys, with a group mean of 0.17 $\mu\text{g}/\text{kg}/\text{infusion}$ (Table 1; Fig. 1 rightmost column).

When available in a mixture with Δ^9 -THC or CP55940 (Figs. 1 and 2, respectively), remifentanyl increased drug choice in a dose-dependent manner. In some cases, responding increased for particular doses of remifentanyl, compared with remifentanyl alone, whereas in other cases responding for remifentanyl decreased. For most drug mixtures, the potency of remifentanyl to increase drug choice was not significantly altered by the addition of a cannabinoid (Table 1). However, a mixture with 32 $\mu\text{g}/\text{kg}/\text{infusion}$ of Δ^9 -THC significantly decreased the potency of remifentanyl, as indicated by a nearly 2-fold shift rightward in the remifentanyl dose-effect curve (Fig. 1, top row, upright triangles). In some cases, mixtures of remifentanyl with the largest doses of Δ^9 -THC (100 $\mu\text{g}/\text{kg}/\text{infusion}$) or CP55940 (10 $\mu\text{g}/\text{kg}/\text{infusion}$) decreased response rates compared with remifentanyl alone (middle row, Figs. 1 and 2, respectively); however, monkeys continued to complete most choice trials (bottom row, Figs. 1 and 2).

Discussion

Cannabinoid receptor agonists such as Δ^9 -THC and CP55940 increase the antinociceptive potency of some mu opioid receptor agonists in non-human primates without enhancing their reinforcing or discriminative stimulus effects (Li et al. 2008; Li et al. 2012; Maguire et al. 2013b; Maguire and France 2016a,b), suggesting opioid/cannabinoid mixtures could be used to treat pain without increasing, and possibly decreasing, abuse. In previous studies, cannabinoids reduced self-administration of heroin; those results could be interpreted to suggest that cannabinoids decrease the reinforcing effects of opioids and, thus, potential for abuse. However, those studies used a single-response, fixed-ratio schedule of self-administration that does not easily differentiate changes in reinforcing effects from other (e.g., generalized rate decreasing) effects, so the interaction between opioids and cannabinoids with regard to reinforcing effects remains unclear. The current study used a choice procedure to compare reinforcing effects of an opioid alone with those of an opioid/cannabinoid mixture. One advantage of choice procedures is that the primary measure of reinforcing effects is allocation of behavior among alternatives, rather than overall response output, which can be sensitive to many factors other than reinforcing effectiveness.

Monkeys chose food over saline and over small doses of remifentanyl and increasingly chose remifentanyl as the unit dose of remifentanyl increased. Dose-effect curves for remifentanyl alone were very stable over the course of the study, with potency not varying by more than a half a log unit

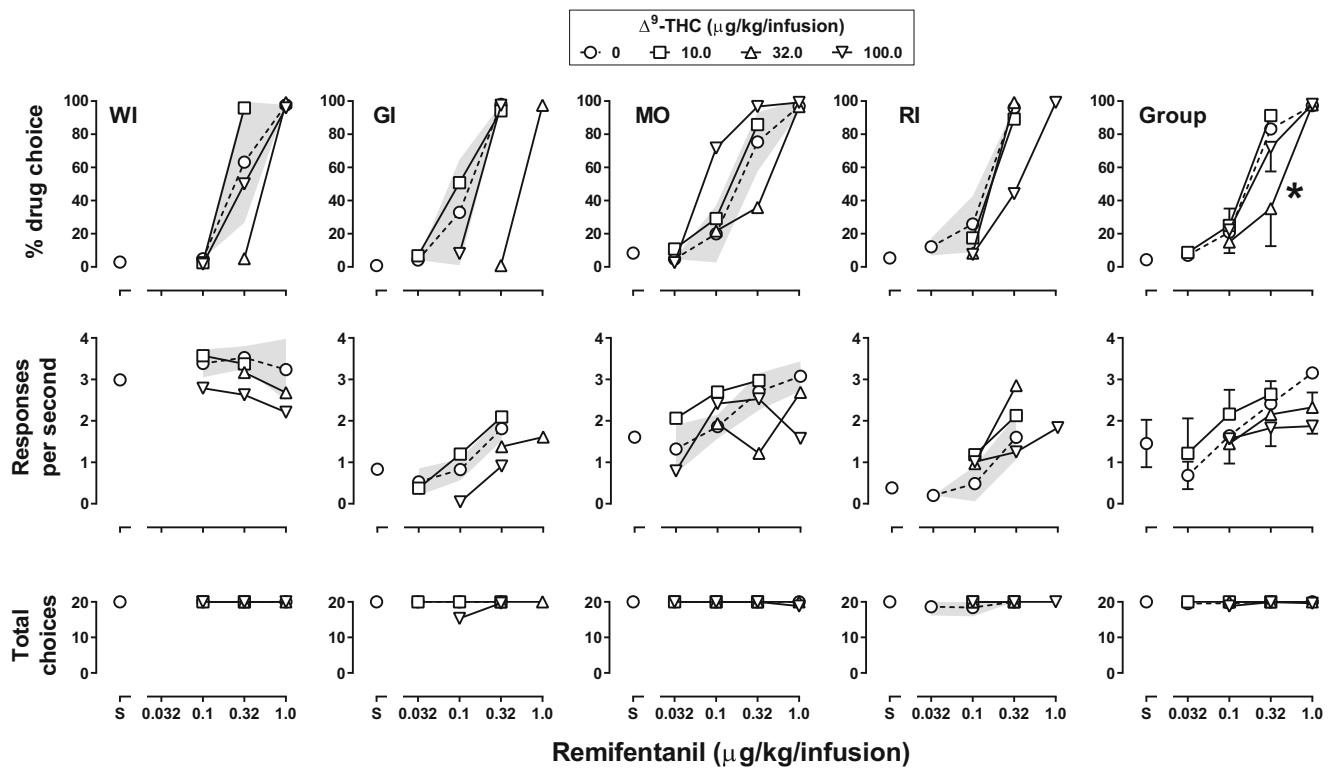


Fig. 1 Dose-effect curves for remifentaniil alone (circles) or in combination with varying unit doses of Δ^9 -THC (squares, upright triangles, and inverted triangles). Percentage choice of drug during choice trials (top row), overall response rate in responses per second (middle row), and total number choice trials completed (bottom row) are plotted as a function of unit dose of remifentaniil ($\mu\text{g}/\text{kg}/\text{infusion}$) for individual monkeys as well as for the grouped data (rightmost panels). The shaded region for the individual plots indicates ± 1 standard deviation of the mean of

multiple (7 or 8) determinations of the dose-effect curve for remifentaniil alone; error bars for the group plots indicate ± 1 standard error of the group. The asterisk next to the group dose-effect curve for remifentaniil in combination with 32 $\mu\text{g}/\text{kg}/\text{infusion}$ of Δ^9 -THC for percentage of drug choice (upright triangles, top-right panel) indicates that this unit dose of Δ^9 -THC significantly decreased the potency of remifentaniil (see Table 1 for quantification of this effect)

for individual monkeys, which is critical for studies using within-subject experimental designs. The potency of remifentaniil to increase drug choice over three 300-mg

sucrose pellets in the current study, with 0.32–1.0 $\mu\text{g}/\text{kg}/\text{infusion}$ producing exclusive drug choice, was comparable to previous studies using choice procedures wherein the alternative

Table 1 ED_{50} values ($\mu\text{g}/\text{kg}/\text{infusion}$) for remifentaniil alone or in combination with different unit doses of Δ^9 -THC or CP55940 ($\mu\text{g}/\text{kg}/\text{infusion}$) as a drug mixture for individual monkeys and averaged for the group [95% confidence limits (CL)] as well as potency ratios [95% CL]

Drug or drug mixture	Monkey				Group mean ED_{50} ^a	Group mean potency ratio ^b
	WI	GI	MO	RI		
Remifentaniil alone	0.25	0.13	0.19	0.14	0.17 [0.12, 0.23]	
+ Δ^9 -THC						
10	0.18	0.10	0.15	0.17	0.15 [0.11, 0.19]	1.19 [0.94, 1.45]
32	0.55	0.57	0.30	0.17	0.36 [0.20, 0.62]	0.53 [0.29, 0.78] ^c
100	0.32	0.17	0.08	0.31	0.20 [0.11, 0.36]	1.09 [0.24, 1.95]
+ CP55940						
3.2	0.18	0.20	0.19	0.53	0.24 [0.15, 0.40]	0.83 [0.35, 1.30]
10	0.18	0.30	0.12	0.08	0.15 [0.09, 0.26]	1.29 [0.71, 1.87]

^a Mean and 95% confidence limits of ED_{50} values were calculated initially using the \log_{10} transform of the ED_{50} and then all values were converted back to arithmetic values by taking the anti-log

^b Potency ratios were calculated by dividing the ED_{50} for remifentaniil alone by the ED_{50} for remifentaniil in each mixture

^c Confidence limits that do not include 1.0 indicate that the potency of remifentaniil in a mixture is significantly different from remifentaniil alone

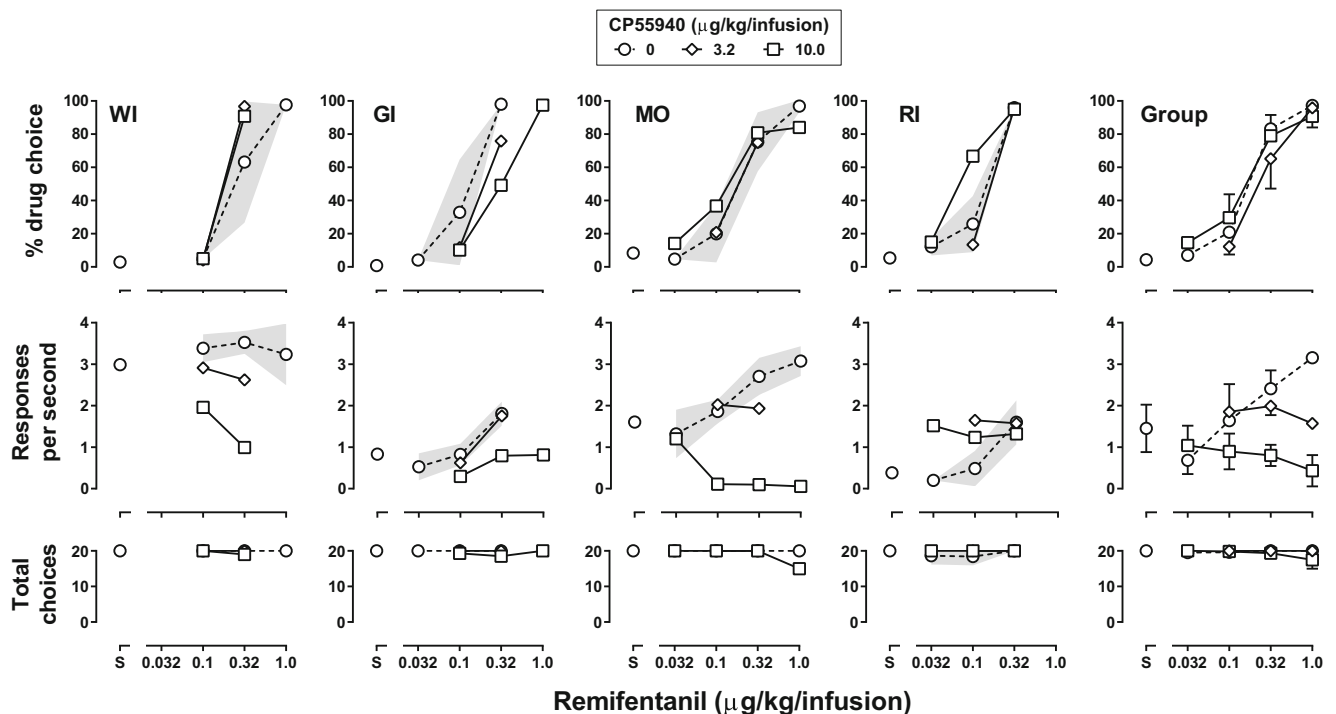


Fig. 2 Dose-effect curves for remifentanil alone (circles) or in combination with varying unit doses of CP55940 (diamonds and squares). Other details are the same as in Fig. 1. Control data are replotted from Fig. 1 for comparison

reinforcer was a grain-based food pellet (Maguire et al. 2013a) or an infusion of cocaine (Wade-Galuska et al. 2007; Koffarnus and Woods 2008; Wade-Galuska et al. 2011; Freeman and Woolverton 2011). These doses are also within the range of doses that maintained self-administration under a variety of single-response procedures (Woods and Winger 2002; Ko et al. 2002; Winger et al. 2006; Woolverton et al. 2008; Podlesnik et al. 2011; Koffarnus et al. 2012; Lagorio and Winger 2014). Remifentanil was used in the current study to minimize accumulation of opioid during the session that can occur with longer acting opioids such as heroin, which can reduce response rates (e.g., Stevenson et al. 2005; Negus 2005a, 2006). Rate-decreasing effects of the opioid in combination with those of a second drug in a mixture could limit the range of doses studied. In the current study, when remifentanil was available alone, response rates remained high despite high levels of drug choice, likely due to the combination of a relatively long post-infusion timeout (10 min) and the short duration of action of remifentanil (< 10 min; for example, see Ko et al. 2002).

Across multiple unit doses, Δ^9 -THC and CP55940 did not significantly enhance the potency of remifentanil to increase drug choice, and the absence of enhancement was evident in grouped data as well as at the individual-subject level (Figs. 1 and 2), indicating that group data were representative of individual subjects. These data are consistent with previous results showing that cannabinoid receptor agonists fail to enhance self-administration of heroin (e.g., Li et al. 2012; Maguire et

al. 2013b; Maguire and France 2016b) and extend these studies to include another opioid receptor agonist (remifentanil) and procedure (choice). One unit dose of Δ^9 -THC (32 $\mu\text{g}/\text{kg}/\text{infusion}$) decreased the potency of remifentanil, although the magnitude of effect was modest, producing less than a 2-fold shift on average across monkeys, and was not clearly dose related as the larger unit dose of Δ^9 -THC was ineffective with regard to choice.

In previous studies, co-administration of a cannabinoid receptor agonist dose-dependently decreased heroin self-administration, as indicated by a flattening of the self-administration dose-effect curve (e.g., Li et al. 2012). The absence of marked, dose-related rightward shifts in the remifentanil dose-effect under the choice procedure suggests that effects of cannabinoids reported in previous studies were likely due to general rate-suppressant effects of the cannabinoid (possibly combined with those of heroin) rather than a specific attenuation of reinforcing effects. Likewise, in a recent study with healthy human participants, smoked cannabis enhanced the antinociceptive effects of oxycodone with experimental cold pain at doses that did not substantially increase or decrease abuse-related effects of oxycodone (Cooper et al. 2018). Recent reports suggest that the use of opioids for pain (e.g., Bradford et al. 2018; Boehnke et al. 2016; Wen and Hockenberry 2018) as well as fatalities from opioid overdose (Bachhuber et al. 2014) have decreased concomitantly with the increased legalization of cannabis for medicinal and/or recreational

use. However, the extent to which those associations are causally related and whether increased access to cannabis alters rates opioid abuse and dependence remain open questions (e.g., see discussions by Hall et al. 2018; Hayes and Brown 2014).

Monkeys responded for mixtures of remifentanyl with Δ^9 -THC or CP55940 despite significant reductions in response rate, exemplifying the dissociation of response allocation and response rate in this choice procedure. Δ^9 -THC or CP55940 have aversive effects under some conditions (e.g., Elsmore and Fletcher 1972; McGregor et al. 1996), and decreased rates of drug self-administration have been interpreted as reflecting aversive effects of drugs (e.g., Riley 2011). However, in the current study, it is unlikely that aversive effects contributed to lowered rates of responding. Otherwise, monkeys would be expected to reallocate behavior toward the food alternative and avoid taking infusions of the drug mixture (e.g., Negus 2005b). Rather, monkeys self-administered nearly all of the available drug on a daily basis, albeit at longer inter-injection intervals. On the other hand, cannabinoids also have well-documented hyperphagic effects (e.g., Abel 1975; Kirkham and Williams 2001; Bellocchino et al. 2010), which could increase the reinforcing effectiveness of food and decrease the relative reinforcing effectiveness of an alternative drug reinforcer (e.g., Nader and Woolverton 1991, 1992). In such a case, choice of remifentanyl would decrease when combined with a cannabinoid; however, that was not the case in the current study.

It is possible that the cannabinoids were not studied at doses large enough to alter significantly choice for remifentanyl; however, this is unlikely for several reasons. First, mixtures of remifentanyl and larger unit doses of each cannabinoid decreased response rates in some cases, indicating that monkeys received behaviorally active doses. Second, the largest dose of Δ^9 -THC (100 $\mu\text{g}/\text{kg}/\text{infusion}$) tested in the current study was 3-fold larger than the dose (32 $\mu\text{g}/\text{kg}/\text{infusion}$) that markedly decreased responding for heroin in monkeys responding under a single-response procedure (Li et al. 2012). Third, the largest unit doses of Δ^9 -THC and CP55940 tested in the current study have discriminative stimulus effects and decrease rates of operant behavior in rhesus monkeys when given as a single bolus injection by the same route (i.v.) of administration (e.g., McMahon 2006; McMahon 2011; Hrubá and McMahon 2014). In fact, in the current study, when cannabinoids were studied in combination with larger doses of remifentanyl, monkeys completed most trials, resulting in cumulative intake of up to 2200 $\mu\text{g}/\text{kg}$ of Δ^9 -THC or 220 $\mu\text{g}/\text{kg}$ of CP55940 per session. These total doses are at least 20-fold larger than discriminable doses of these drugs in rhesus monkeys trained to discriminate 100 $\mu\text{g}/\text{kg}$ of Δ^9 -THC (e.g., McMahon 2006). Moreover, the total cumulative dose of Δ^9 -THC achieved in the currently study was at least twice as large as a dose that enhanced the

antinociceptive effects of some opioids when given subcutaneously as a bolus injection (Li et al. 2008; Maguire and France 2014).

The cannabinoid receptor agonists Δ^9 -THC and CP55940 have a rapid onset of action in rhesus monkeys (e.g., Hrubá and McMahon 2014) and man (e.g., Ohlsson et al. 1980; Grotenhermen 2003) following i.v. administration; however, behavioral effects can last longer than an hour following a single infusion (e.g., Hrubá and McMahon 2014; Ohlsson et al. 1980). For studies in which subjects make repeated choices with drug as an alternative, long-lasting drug effects could interfere with choices on subsequent trials. For tests with remifentanyl alone, carryover effects are less of a concern due to its very short duration of action (less than 10 min) and the 10-min inter-trial interval (discussed above; see Ko et al. 2002). However, it is possible, in fact likely, that effects of cannabinoid infusions early in the session persisted during subsequent choices trials and that the cannabinoid accumulated during the session with repeated choices of the drug mixture. Persistent effects of the cannabinoid could influence the ability to discriminate between the sources of each reinforcer later in the session. For example, it might decrease the ability to determine whether delivery of the previous cannabinoid infusion was the result of responding on the left or right lever, or whether it was delivered with food or remifentanyl. However, this seems unlikely for several reasons. First, drug was never administered concurrent with food delivery. Given the quick onset of action of remifentanyl and both cannabinoids following i.v. administration, the onset of drug action would not have overlapped significantly with food delivery in subsequent trials. Second, each dose of remifentanyl alone or in a mixture was studied under steady state conditions wherein a single drug or drug mixture was available for at least six sessions (with a minimum of three sessions under each lever designation) and until choice was stable. There were multiple opportunities across sessions to sample either option at the beginning of the session while in a “drug-free” state, and switching lever designations tested whether responding was sensitive to the reinforcement contingencies in effect at that time. Third, failure to discriminate between the two options would likely result in a flattening of the remifentanyl dose-effect curve and/or a lever bias (i.e., responding only or predominantly on the left or right lever). However, the slope of the remifentanyl dose-effect curve did not differ across conditions, and it spanned the entire range of the ordinate. Moreover, responding typically adjusted quickly following a change in dose or lever designation, suggesting adequate discriminability between the food and drug alternatives.

In summary, this study found that the cannabinoid receptor agonists Δ^9 -THC and CP55940 failed to alter reliably the potency of remifentanyl in rhesus monkeys responding under a food/drug choice procedure. These data confirm and extend previous results showing that, in non-human primates,

cannabinoid receptor agonists do not appear to enhance the reinforcing effects of mu opioid receptor agonists. Moreover, this study is relevant to interpretation of prior results with the current study suggesting that decreased opioid self-administration reported previously (e.g., Li et al. 2012) was the result of a generalized rate-suppressant effect of the cannabinoid or the mixture of a cannabinoid and heroin, rather than reduced reinforcing effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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