Title

Effects of the selective dopamine D_3 receptor antagonist PG01037 on morphine-induced hyperactivity and antinociception in mice

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Abstract

Dopamine D_3 receptor (D_3R) antagonists have been reported to attenuate the abuse-related effects of opioids in preclinical studies and have therefore garnered interest as novel pharmacotherapeutics for the treatment of opioid use disorder (OUD). However, some disparities among the effects of different D_3R antagonists have been observed, most notably in their modulation of the behavioral effects of psychostimulants. For example, pretreatment with the highly-selective D_3R antagonist PG01037 enhances the locomotor-activating effects of cocaine, yet other D_3R antagonists attenuate this response. It is unclear whether such differences among D_3R antagonists may also extend to opioids, partially because PG01037 has not been investigated in this context. The purpose of this study was to assess the impact of PG01037 administration on the locomotor-activating, antinociceptive, and cataleptic effects of morphine in mice. C57Bl/6J mice were pretreated with PG01037 (0 – 10 mg/kg) and tested for 1) locomotor activity induced by acute morphine administration (5.6 – 56 mg/kg), 2) locomotor sensitization following repeated morphine administration (56 mg/kg), 3) antinociception following acute morphine administration (18 mg/kg), and 4) catalepsy following administration of PG01037 alone or in combination with morphine (56 mg/kg). PG01037 attenuated the acute locomotor-activating and antinociceptive effects of morphine, but did not prevent the induction of morphine-induced locomotor sensitization and did not induce catalepsy alone or in combination with morphine. These results suggest that attenuation of opioid-induced hyperactivity is a behavioral effect shared among selective D_3R antagonists, lending further support to their investigation as treatments for OUD. However, PG01037 is unlike more D_3R-selective antagonists in its attenuation of opioid-induced analgesia.

Keywords

morphine; dopamine D_3 receptor; PG01037; locomotor activity; opioid analgesia; catalepsy

1 Introduction

The abuse of prescription and illicit opioids has culminated in a growing national healthcare crisis, with efforts being devoted to the development of novel pharmacotherapeutics that can safely and more effectively reduce opioid misuse and dependence as compared to currently-available medications (Blanco and Volkow, 2019; Kreek et al., 2019; Volkow et al., 2019). The abuse-related effects of opioids are predominantly mediated by their capacity to increase dopamine (DA) neurotransmission within the mesolimbic reward system (for review, see Di Chiara and North, 1992; McBride et al., 1999; Pierce and Kumaresan, 2006; Reiner et al., 2019; Wise, 1989), a projection arising from DAergic neurons located in the ventral tegmental area (VTA) and terminating in the nucleus accumbens (NAc) (Bjorklund and Dunnett, 2007; Moore and Bloom, 1978). Opioids administered either systemically (Chefer et al., 2003; Gysling and Wang, 1983) or directly into the VTA (Devine et al., 1993; Gysling and Wang, 1983; Leone et al., 1991; Spanagel et al., 1992) produce increases in NAc DA levels by disinhibiting VTA DA neurons, via activation of G_i-coupled mu opioid receptors located on the cell bodies and terminals of GABAergic neurons that normally provide inhibitory tone (Johnson and North, 1992; Matsui and Williams, 2011). Accordingly, the locomotor-activating, reinforcing, and reinstatement-inducing effects of opioids are dampened following perturbation of NAc DA neurotransmission (Hand and Franklin, 1985; Kelley et al., 1980; Phillips et al., 1983;

Shippenberg et al., 1993; Smith et al., 1985; Spyraki et al., 1983; Stinus et al., 1980; Wang et al., 2003).

DA binds to five G protein-coupled receptor subtypes which are divided into two families. The D_1-like receptor family includes the G_s-coupled D_1 and D_5 receptor subtypes (D_1R and D_5R) while the D_2-like receptor family includes the G_i-coupled D_2, D_3, and D_4 receptor subtypes (D_2R, D_3R, D_4R) (Beaulieu and Gainetdinov, 2011). Antagonism of either D_1-like receptors or D_2-like receptors reduces opioid-induced locomotor activation, opioid self-administration, and opioid seeking (for review, see Di Chiara and North, 1992; Pierce and Kumaresan, 2006; Reiner et al., 2019; Wise, 1989). However, adverse side effect profiles associated with these compounds have hampered their potential clinical utility (Cho et al., 2010; Haney et al., 2001; Kishi et al., 2013; Millan et al., 1995). Attention has therefore shifted towards selective interrogation of subtypes within these DA receptor families in an effort to maintain or improve pharmacotherapeutic efficacy for treatment of opioid use disorder (OUD) while reducing undesirable side effects.

The D_3R has emerged as an appealing target in this regard for reasons that have been reviewed extensively elsewhere (Galaj et al., 2020; Heidbreder and Newman, 2010; Sokoloff and Le Foll, 2017). Of most relevance to the present report is preclinical evidence that selective D_3R antagonism attenuates opioid self-administration under various schedules of reinforcement as well as opioid-seeking behaviors, without producing adverse side effects associated with nonselective D_2-like receptor antagonists (Boateng et al., 2015; de Guglielmo et al., 2019; Galaj et al., 2015; Hu et al., 2013; Jordan et al., 2019a; Lv et al., 2019; You et al., 2019). Moreover, two recently-developed D_3R antagonists have been reported to potentiate the desirable analgesic properties of opioids (Jordan et al., 2019a; You et al., 2019). Despite these promising findings,

the underlying neuropharmacological mechanisms by which D_3R blockade alters the neurochemical and/or behavioral effects of opioids remain unresolved.

Stimulation of locomotor activity in rodents is a useful behavioral measure with which to selectively interrogate NAc DA neurotransmission following systemic administration of drugs of abuse, including opioids and psychostimulants (Broekkamp et al., 1979; Delfs et al., 1990; Kalivas et al., 1983; Kelly and Iversen, 1976; Kelly et al., 1975). We recently reported that pretreatment with the highly-selective D_3R antagonist PG01037 enhances cocaine-induced locomotor activity and locomotor sensitization, and that these potentiating effects of PG01037 are likely due to augmented DA-induced excitation of D_1-expressing medium spiny neurons (MSNs) within the NAc (Manvich et al., 2019). Based on these findings, one might predict that PG01037 treatment, or perhaps D_3R antagonism in general, would similarly enhance the locomotor-activating effects of other DA-increasing drugs of abuse such as opioids. However, D_3R antagonists other than PG01037 have recently been shown to attenuate this and other behavioral effects of opioids (i.e. locomotor-increasing effects, reinforcing effects) while enhancing or having no effect on others (e.g. analgesia, cardiovascular responses) (Jordan et al., 2019a; You et al., 2019; You et al., 2017). The mechanisms by which D_3R antagonism attenuates some effects of opioids and not others are not known, nor is it clear as to whether these modulatory patterns are consistent across all D_3R antagonists. One particular obstacle to addressing these questions is that the impact of PG01037 treatment upon the effects of opioids has not been evaluated.

In this study, we sought to determine the impact of pretreatment with the highly-selective D_3R antagonist PG01037 (133-fold selectivity for the D_3R over D_2R (Grundt et al., 2005)) on acute morphine-induced hyperactivity and antinociception, as well as the induction of locomotor

sensitization to repeated morphine administration, in mice. Moreover, because nonselective blockade of D_2-like receptors produces catalepsy alone and potentiates morphine-induced catalepsy (Kiritsy-Roy et al., 1989; Rodriguez-Arias et al., 2000; Wilcox et al., 1983), we also investigated whether administration of PG01037 alone, or in combination with morphine, would induce cataleptic effects.

2 Materials and Methods

2.1 Subjects

Subjects used in this study were 64 adult male and female C57BL/6J mice (32/sex), 8-12 weeks old at the start of study. Mice were either acquired from Jackson Laboratory (Bar Harbor, ME; n = 40) or from a breeding colony at the National Institute on Drug Abuse (n = 24). Mice were housed in same-sex groups of 3-5 per cage in a climate-controlled vivarium with a 12-hr light cycle and had *ad libitum* access to food and water in the home cage. Procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Research Council and were approved by Institutional Animal Care and Use Committees at Emory University, Rowan University, or the National Institute on Drug Abuse of the National Institutes of Health. All behavioral testing was performed during the light cycle.

2.2 Locomotor Activity Apparatus

Locomotor activity was assessed in transparent polycarbonate cages (22 x 43 x 22 cm) that allowed passage of 8 infrared beams through the long wall and 4 infrared beams through the short wall of the enclosure at 4.9 cm intervals (San Diego Instruments; San Diego, California).

Horizontal ambulations, defined as the sequential disruption of two adjacent infrared beams, were recorded in 5-min bins. The test chambers were prepared with a thin layer of clean bedding prior to each test session. Before the onset of experiments, mice were injected i.p. with saline and placed in the test chambers for 30 min for 3 consecutive days in order to habituate the mice to injections and the test apparatus.

2.3 Acute Morphine-Induced Locomotion

The effects of PG01037 on acute morphine-induced locomotion were evaluated in 16 mice (8 males, 8 females) using a within-subjects design. Animals were initially placed in the center of the locomotor chamber and ambulations were recorded for 90 min. Next, animals were briefly removed from the chamber, injected with PG01037 (vehicle, 0.1, 1, or 10 mg/kg i.p.), and returned to the locomotor chamber for 30 min. Finally, mice were again removed from the chamber, injected with morphine (vehicle, 5.6, 18, or 56 mg/kg i.p.), and placed back in the chamber for 120 min. The dose range for PG01037 was carefully selected for use based on our previous work showing that doses up to 10 mg/kg do not appreciably disrupt basal locomotion but significantly modulate the locomotor-activating effects of cocaine in C57BL/6J mice (Manvich et al., 2019). The 5.6 – 56 mg/kg dose range of morphine was selected based on our own pilot studies showing that it captures both the ascending and descending limbs of the morphine dose-response curve. Dose administration of PG03017 was pseudorandomized and counterbalanced across animals within each dose of morphine. All doses of PG01037 were assessed for a given morphine dose before switching to a different morphine dose. The order of morphine dose testing was 18 mg/kg, 56 mg/kg, 5.6 mg/kg, vehicle. All test sessions were

separated by at least 1 week to prevent the development of locomotor sensitization to morphine. All mice received all treatments.

2.4 Morphine-Induced Locomotor Sensitization

Sensitization induction took place over 5 consecutive days and was performed in 2 separate groups of mice (n = 8/group, 4 male and 4 female). Mice were initially placed in the center of the locomotor chamber, and locomotor activity was recorded for 90 min. They were then briefly removed from the chamber, injected with PG01037 (vehicle or 10 mg/kg, i.p.), and returned to the locomotor chamber for 30 min. Mice were again removed and injected with morphine (56 mg/kg i.p.), then placed back in the chamber for 120 min. Mice received the same dose of PG01037 across each of the 5 induction days. Seven days following the last induction session, locomotor activity was again assessed as described above with the exception that all mice received vehicle as the pretreatment 30 min prior to challenge with 56 mg/kg morphine.

2.5 Hot Plate Test for Thermal Nociception

Antinociception was assessed in mice using a hot plate system (Model 39, IITC Life Science Inc., Woodland Hills, CA, USA) set to 52 ± 0.2°C. Mice were placed on the platform surrounded by transparent Plexiglas walls and removed after the first sign of thermal distress (paw licking, jumping, hind paw stomping). The latency to the first indicator of pain was recorded. A maximal cutoff of 60 s was instituted to prevent tissue damage. The antinociceptive effects of PG01037 alone were assessed in one group of 8 mice (4 males, 4 females). Subjects were first placed on the hot plate prior to any drug treatment to measure baseline response latencies (time point 0). Next, mice were administered PG01037 (vehicle, 0.1, 1, or 10 mg/kg,

i.p.) and tested on the hot plate at 30, 60, 90, and 120 min post-injection. PG01037 doses were counterbalanced across subjects. The effects of PG01037 pretreatment on morphine-induced antinociception were examined in a separate group of 16 mice (8 males, 8 females). Following baseline testing (time point 0), mice were administered PG01037 (vehicle, 0.1, 1, or 10 mg/kg, i.p.) followed 30 min later by morphine (18 mg/kg i.p.). Hot plate testing was assessed at 30, 60, 90, and 120 min post-morphine injection. Each mouse received 1-3 doses of PG01037 in a counterbalanced manner whereby all possible PG01037 x morphine dose combinations consisted of n = 8. For each experiment, hot plate test sessions were separated by 2-3 days.

2.6 Catalepsy

The capacity of PG01037 alone or in combination with morphine to produce catalepsy was assessed in 8 mice (4 males, 4 females). Mice received a pretreatment of either PG01037 (vehicle or 10 mg/kg, i.p.) followed 30 min later by morphine (vehicle or 56 mg/kg, i.p.). Catalepsy was evaluated using the “bar test” (Sanberg et al., 1988), during which a thin bar was secured horizontally 1.75 in above a flat surface. Each test was conducted by lifting the mouse by the tail and allowing it to grab the bar with its front paws, then releasing the tail so that the mouse was positioned sitting upright on its hind legs. Upon assuming this position, the latency to remove at least one paw from the bar was recorded. The test was stopped if the subject failed to withdraw one paw within 60 s. Mice that could not be placed in the testing position after 3 attempts received a latency score of 0 s. In each test, catalepsy was measured 0, 15, 30, 60, and 120 min following administration of morphine. The order of dose-combinations was randomized across mice. Mice were tested once per week until each mouse received all treatment combinations. After PG01037/morphine testing was completed, all mice received a final

catalepsy test in which they were administered risperidone (3 mg/kg, i.p.) followed by saline i.p. 30 min later. Catalepsy in these tests was measured up to 60 min following saline injection. The 3 mg/kg risperidone test was included as a positive control as it induces prominent catalepsy in mice (Fink-Jensen et al., 2011).

2.7 Drugs

Morphine sulfate (National Institute on Drug Abuse Drug Supply Program, Bethesda, MD) was dissolved in sterile saline. PG01037 was synthesized by Ms. J. Cao in the Medicinal Chemistry Section, National Institute on Drug Abuse Intramural Research Program as described previously (Grundt et al., 2005) and dissolved in sterile water. Risperidone (Sigma-Aldrich; St. Louis, MO) was dissolved in vehicle containing ethanol:CremophorEL (Sigma-Aldrich):saline (5:10:85 v/v). All drugs were administered i.p. at a volume of 10 ml/kg.

2.8 Statistical Analyses

For acute morphine-induced locomotion studies, total ambulations during the 2 h following morphine administration were analyzed via two-way ANOVA with repeated measures on both factors (PG01037 dose × morphine dose), followed by post hoc Dunnett's multiple comparisons tests to compare each dose of PG01037 to its vehicle within each dose of morphine. Locomotor activity in the 30-min period after PG01037 administration (prior to morphine administration) was analyzed using one-way repeated measures ANOVA. Effects of vehicle or 10.0 mg/kg PG01037 alone on locomotor activity were assessed by paired t-test. For the induction phase of sensitization (i.e. days 1-5), total ambulations during the 2 h following morphine administration were analyzed via mixed two-way ANOVA with repeated measures on

one factor (day) and independent measures on the other factor (PG01037 dose). Dunnett's multiple comparisons tests were used to compare morphine-induced locomotor activity on each of induction days 2-5 vs. day 1. For the challenge day of sensitization studies (day 12), total ambulations during the 2 h following morphine administration were analyzed via independent two-tailed t-test. The antinociceptive effects of PG01037 alone or in combination with morphine were analyzed using a two-way ANOVA with repeated measures on one factor (time) and independent measures on the other factor (PG01037 dose), followed by Dunnett's or Tukey's multiple comparisons tests, as specified in the text. Latency scores in catalepsy experiments were analyzed using two-way ANOVA with repeated measures on both factors (treatment × time). The effect of 3 mg/kg risperidone + saline was excluded from statistical analyses because risperidone was included only as a positive control to validate the catalepsy detection procedure. All data were plotted and analyzed using GraphPad Prism v8.4 (GraphPad Software, La Jolla, CA, USA). Significance was set at $p < 0.05$ for all tests.

3 Results

3.1 Effects of PG01037 on Acute Morphine-Induced Locomotion

Administration of morphine after vehicle pretreatment resulted in increased locomotor activity with a typical inverted U-shaped dose-response function (Fig. 1). Two-way repeated measures ANOVA of PG01037 in combination with 5.6 – 56 mg/kg morphine indicated significant main effects of morphine dose ($F_{(2,30)} = 5.82$, $p = 0.007$), PG01037 dose ($F_{(3,45)} = 29.27$, $p < 0.0001$), and a significant morphine × PG01037 interaction ($F_{(6,90)} = 5.90$, $p < 0.0001$). Post hoc comparisons revealed that pretreatment with 0.1 mg/kg PG01037 slightly elevated

hyperlocomotion produced by 18 mg/kg morphine, whereas higher doses of PG01037 dose-dependently and more robustly attenuated the effects of 18 and 56 mg/kg morphine. The inhibitory actions of PG01037 on morphine-induced locomotion typically emerged within 5-15 min following morphine administration and persisted for the duration of the 120-min observation period (Fig. 2A-C). PG01037 administration did not significantly alter locomotor activity in the 30 min following its administration, prior to morphine injection (one-way repeated measures ANOVA, ($F_{(3,45)} = 0.33$, $p = 0.801$) (Fig. S1). To further confirm a lack of effect by PG01037 alone, we administered vehicle or 10 mg/kg PG01037 followed 30 min later by saline and monitored locomotion for 120 min. 10 mg/kg PG01037 pretreatment did not alter total ambulations during this longer observation period (paired t-test, $t_{(15)} = 1.58$, $p = 0.13$) (Fig. S2A-B).

3.2 Effects of PG01037 on Morphine-Induced Locomotor Sensitization

To test the impact of selective D_3R antagonism on the development of morphine-induced locomotor sensitization, mice were pretreated daily for 5 consecutive days with vehicle or PG01037 (10 mg/kg i.p.) 30 min prior to morphine (56 mg/kg, i.p.). 10 mg/kg PG01037 was selected for use in this experiment because it produced the greatest attenuation of morphine's acute locomotor activity and did not disrupt basal locomotion in the preceding experiment. Two-way mixed factors ANOVA revealed significant main effects of induction day ($F_{(4, 56)} = 17.66$, $p < 0.0001$) and PG01037 dose ($F_{(1, 14)} = 4.67$, $p = 0.049$), but not a significant day × PG01037 interaction ($F_{(4, 56)} = 0.51$, $p = 0.73$). Post hoc analyses indicated that, collapsed across vehicle and 10 mg/kg PG01037 pretreatments, mice exhibited a sensitized locomotor response to morphine by day 3 of the induction phase (Fig. 3; time course, Fig. 4A-E). The significant main

effect of PG01037 treatment without a significant day x PG01037 interaction indicates that PG01037 attenuated morphine-induced hyperlocomotion equally across all 5 days, effectively reducing it on average by ~ 36.5% (Fig. 3; time course, Fig. 4A-E). One week after the final induction session, all mice received vehicle followed by a morphine challenge (56 mg/kg, i.p.). Independent t-test showed no difference in locomotor activity between mice that had previously been pretreated with 10 mg/kg PG01037 during induction as compared to mice pretreated with vehicle during induction ($t_{(14)} = 0.67$, $p = 0.52$) (Fig. 3; time course, Fig. 4F).

3.3 Effects of PG01037 on Morphine-Induced Antinociception

The effects of PG01037 administration alone on nociception in the hot plate test are shown in Fig. 5A. Two-way mixed-factors ANOVA indicated a significant main effect of time ($F_{(4, 112)} = 4.02$, $p = 0.004$) but not of PG01037 dose ($F_{(3, 28)} = 0.82$, $p = 0.49$) or a time × PG01037 interaction ($F_{(12, 112)} = 0.18$, $p = 0.18$). Post hoc Tukey's tests revealed that, collapsed across PG01037 dosing conditions, reaction latencies decreased slightly but significantly at the 30-min and 60-min time points as compared to 0 min ($p < 0.05$).

In mice pretreated with vehicle, 18 mg/kg morphine increased reaction latency with maximal efficacy 30 min after morphine administration that gradually returned to near-baseline levels by the 120-min time point (Fig. 5B). Two-way mixed factors ANOVA indicated a significant main effect of time ($F_{(4, 112)} = 23.41$, $p < 0.0001$), no main effect of PG01037 dose ($F_{(3, 28)} = 2.29$, $p = 0.10$), and a significant time × PG01037 interaction ($F_{(12, 112)} = 2.77$, $p = 0.003$). Post hoc Dunnett's tests revealed that compared to vehicle pretreatment, administration of 1 or 10 mg/kg PG01037 significantly attenuated the antinociceptive effects of morphine at the 30-min time point, when morphine's effects were maximal. This attenuating effect of PG01037

fell just short of statistical significance at the 60-min time point ($p = 0.06$ for both 1 and 10 mg/kg PG01037 compared to vehicle).

3.4 Effects of PG01037 Alone or in Combination with Morphine on Catalepsy

To determine whether selective D_3R antagonism induces catalepsy either alone or in combination with morphine, mice were administered PG01037 (vehicle or 10 mg/kg, i.p.) 30 min prior to morphine (vehicle or 56 mg/kg, i.p.). We purposely selected the highest doses administered of each compound in our locomotor and nociception experiments in order to maximize the potential detection of catalepsy. Neither administration of PG01037 alone, morphine alone, nor their combination resulted in catalepsy (Fig. 6). Analysis of these treatment conditions using two-way repeated measures ANOVA showed no significant main effect of treatment ($F_{(2,14)} = 1.00$, $p = 0.39$), time ($F_{(4,28)} = 1.00$, $p = 0.42$), or a treatment × time interaction ($F_{(8,56)} = 1.00$, $p = 0.45$). For all mice tested, the latency to withdraw a forepaw in any of the aforementioned conditions did not exceed 1 s. By contrast, 3 mg/kg risperidone produced a robust increase in catalepsy across the 60-min test period, ranging on average from ~ 48 s up to the procedural maximum time allowed of 60 s (Fig. 6).

4 Discussion

The primary objective of the present study was to examine the impact of treatment with the highly-selective dopamine D_3R antagonist PG01037 on several behavioral effects of opioids in mice. The major findings are that PG01037 dose-dependently attenuates the locomotor-activating effects of acute morphine but does not disrupt the induction of morphine-induced

locomotor sensitization. Furthermore, although PG01037 reduced the analgesic effects of acutely administered morphine in mice, it did not produce cataleptic effects alone or in combination with morphine.

4.1 PG01037 attenuates acute morphine-induced hyperlocomotion

The observation that D_3R antagonism significantly reduces the locomotor-activating effects of opioids such as morphine is in agreement with several previous studies that have used compounds either modestly or highly selective for the D_3R. For example, pretreatment with nafadotride, which exhibits ~10-fold selectivity for D_3R vs. D_2R (Audinot et al., 1998), produces a significant but less effective attenuation of morphine-induced hyperlocomotion as compared to that produced by the nonselective D_2-like receptor antagonist eticlopride (Cook and Beardsley, 2003). A similar pattern of results has also been reported in a comparison between the nonselective D_2-like receptor antagonist haloperidol and the modestly-selective D_3R antagonist, U99194A (Manzanedo et al., 1999). More recent studies utilizing D_3R antagonists that exhibit high D_3R vs. D_2R selectivity such as VK4-116, BAK4-54, CAB2-015, and YQA14 also demonstrate significant but modest reductions in the locomotor-activating effects of opioids (Kumar et al., 2016; Lv et al., 2019; You et al., 2017). Based on these prior findings and our present results with PG01037, we conclude that D_3R antagonism reliably attenuates the locomotor-activating effects of opioids regardless of the specific compound used, suggesting a D_3R receptor antagonist class effect. Because the locomotor-activating effects of opioids are most often attributed to increased neurotransmission within the mesolimbic DA system (Broekkamp et al., 1979; Kalivas et al., 1983; Kelley et al., 1980), we hypothesize that attenuated opioid-induced hyperlocomotion following D_3R antagonism primarily reflects disrupted NAc

output in some as-yet unidentified manner. It could be argued that the attenuation of morphine-induced hyperactivity following PG01037 pretreatment in the present study may have been more effective had higher doses of the antagonist been tested. Although we cannot presently rule this out, we avoided testing higher doses for two main reasons. First, we have previously tested higher doses of PG01037 (up to 30 mg/kg) and did not find them to be more efficacious at altering cocaine-induced hyperlocomotion than 10 mg/kg (unpublished observations). Second, testing higher doses of PG01037 runs the risk of disrupting basal locomotion and/or limiting D_3R vs. D_2R selectivity *in vivo*, each of which would undoubtedly confound the interpretation of the data. It is interesting to note that while various D_3R antagonists all appear to attenuate opioid-induced hyperlocomotion, their impact on psychostimulant-induced hyperlocomotion is more variable. While we and others have reported that PG01037 and the selective D_3R antagonist NGB294 enhance the locomotor-activating effects of cocaine or amphetamine, respectively (Manvich et al., 2019; Pritchard et al., 2007), other D_3R antagonists have been found to either reduce or not affect psychostimulant-induced hyperlocomotion (Galaj et al., 2014; Jordan et al., 2019b; Song et al., 2012). The reasons as to why D_3R antagonists share in common the capacity to dampen opioid-induced hyperlocomotion but exhibit more diverse effects on stimulant-induced hyperlocomotion remain unclear and will require further research to resolve.

4.2 PG01037 does not alter morphine-induced locomotor sensitization

In contrast to its effects on acute morphine-induced locomotor activation, PG01037 did not disrupt the induction of sensitization, as mice appeared sensitized when challenged with morphine alone one week after the last drug combination was administered. It is generally accepted that changes in mesolimbic DA neurotransmission mediate opioid-induced locomotor

sensitization, and that the primary site at which these neuroadaptations take place is the VTA (Kalivas and Duffy, 1987; Vanderschuren and Kalivas, 2000; Vezina et al., 1987). Although D_3Rs are expressed as autoreceptors within the VTA (Beaulieu and Gainetdinov, 2011; Diaz et al., 2000; Lejeune and Millan, 1995; Mercuri et al., 1997; Missale et al., 1998), it is possible that they do not exert influence over the development of opioid-induced sensitization. On the other hand, the NAc does not play a prominent role in the induction of opioid-induced locomotor sensitization (Vanderschuren and Kalivas, 2000; Vezina et al., 1987), but contributes to the locomotor-activating effects of acute systemic morphine administration (Schildein et al., 1998; Teitelbaum et al., 1979; Vaccarino and Corrigall, 1987; Vezina et al., 1987). Intra-NAc administration of the highly-selective D_3R antagonist SB-277011A has been reported to attenuate the *expression* of morphine-induced locomotor sensitization (Liang et al., 2011), suggesting that the NAc, rather than the VTA, may be the primary site at which D_3R antagonists like PG01037 act to inhibit opioid-induced locomotor increases. Therefore, our finding that systemic D_3R antagonism attenuates the acute locomotor-activating effects of morphine but does not alter induction of sensitization to this phenomenon could be due to differential modulatory impacts on neural activity within the NAc and VTA, respectively. Consequently, D_3R antagonism may "mask" the overt appearance of sensitization during the induction phase due to its capacity to attenuate morphine-induced hyperactivity, but the development of the underlying sensitization mechanisms can be "unmasked" when morphine is subsequently tested in the absence of D_3R antagonism. It is noteworthy that this hypothesis may contradict a report that pretreatment with the highly-selective D_3R antagonist VK4-116 attenuated the induction of oxycodone-induced locomotor sensitization (Kumar et al., 2016) as assessed by a challenge test with oxycodone alone. However, some key procedural differences may underlie these discrepant

findings including the use of different opioids (morphine vs. oxycodone), use of different D_3R antagonists (PG01037 vs. VK4-116), and imposition of 7 days vs. 2 days between the final induction session and the expression test.

4.3 PG01037 attenuates morphine-induced analgesia

Although PG01037 administration alone did not disrupt thermal nociception in the present study, it dose-dependently attenuated the antinociceptive effects of acute morphine, evidenced by an apparent downward shift of morphine's time course across the 120-min assessment period. This result is in opposition to recent studies using the newer and highly-selective D_3R antagonists VK4-116 and R-VK4-40, as pretreatment with either of these compounds enhances, rather than attenuates, the antinociceptive effects of oxycodone in rats (Jordan et al., 2019a; You et al., 2019). Why PG01037 produces an opposite effect on opioid-mediated antinociception in the present study remains unclear, although VK4-40 and VK4-116 notably exhibit some other effects that are discordant with older D_3R antagonists; for example, they do not potentiate the cardiovascular effects of cocaine (Jordan et al., 2019b). It is also noteworthy that our present study examined the antinociceptive effects of morphine rather than oxycodone, and did so in mice rather than rats. These procedural differences aside, more research will be needed to clarify the mechanisms by which highly-selective D_3R antagonists modulate the analgesic properties of clinically-utilized opioid analgesics (Galaj et al., 2020).

4.4 PG01037 does not produce catalepsy alone or in combination with morphine

Combined administration of nonselective D_2-like receptor antagonists with opioids induces catalepsy in mice that is substantially greater than that produced by either drug alone

(Kiritsy-Roy et al., 1989; Rodriguez-Arias et al., 2000). It is generally believed that D_2-like receptor antagonists exert these effects via actions at the D_2R subtype, because selective D_2R blockade alone produces catalepsy in mice (Hattori et al., 2006), rats (Millan et al., 2000), and nonhuman primates (Achat-Mendes et al., 2010), whereas catalepsy has not been detected following treatment with the D_3R antagonists S33084 or SB-277011A (Millan et al., 2000; Reavill et al., 2000). In agreement with these latter findings, PG01037 in the present study showed no evidence of inducing cataleptic effects alone at a dose which significantly modulated both morphine-induced hyperactivity and antinociception. More notably, whether selective D_3R antagonism in combination with opioids induces cataleptic effects had not previously been investigated. Our study is the first to examine this question, and the results indicate that concurrent administration of a selective D_3R antagonist with morphine does not produce cataleptic effects. Given that D_3R antagonists are being considered as potential pharmacotherapeutics for OUD, the lack of measurable catalepsy following D_3R antagonism alone or in combination with morphine adds to accumulating evidence that D_3R antagonists exhibit a desirable safety profile with no appreciable extrapyramidal side effects as compared to nonselective D_2-like receptor antagonists, even when opioids are concurrently administered (Jordan et al 2019). Moreover, because PG01037 did not alter basal locomotion or induce catalepsy alone or in combination with morphine, the attenuations in morphine-induced hyperlocomotion and antinociception we observed are unlikely to be attributable to nonspecific disruptions in motor function.

4.4 PG01037 and modulation of locomotor activity: potential mechanisms

One key question remaining is how pretreatment with PG01037 attenuates opioid-induced hyperactivity (present results) but potentiates cocaine-induced hyperactivity (Manvich et al., 2019). The enhancement of cocaine-induced locomotion by D_3R antagonism occurs coincidentally with an increase in DA-induced excitability and activity of DA D_1 receptor-expressing MSNs within the NAc (Manvich et al., 2019). However, it is unknown whether this or other modulations of MSN activity within the NAc underlie the inhibitory influence of D_3R antagonism on opioid-induced hyperlocomotion. There are some notable disparities between hyperactivity produced by opioids vs. psychostimulants that may partially or wholly explain the bidirectional effect of PG01037. For example, cocaine increases locomotion in a DA-dependent manner via blockade of the dopamine reuptake transporter (DAT) and a consequent rise in extracellular DA levels within the NAc (Giros et al., 1996; Ritz et al., 1987; Yamamoto et al., 2013). By contrast, opioid receptor agonists like morphine are capable of increasing locomotion via both DA-dependent and DA-independent processes. Intra-VTA opioid receptor agonist administration induces locomotor activity via disinhibition of DAergic neurons, and as such requires an intact mesolimbic DA projection (Devine et al., 1993; Gysling and Wang, 1983; Johnson and North, 1992; Matsui and Williams, 2011; Spanagel et al., 1992), whereas intra-NAc opioid receptor agonists induce locomotion via direct actions on NAc MSNs, an effect that does not require intact DA neurotransmission (Churchill and Kalivas, 1992; Kalivas et al., 1983; Pert and Sivit, 1977; Stinus et al., 1985). It is possible that PG01037 potentiates drug-induced hyperactivity that is DA-dependent but attenuates drug-induced hyperactivity that is DA-independent. This hypothesis is further supported by findings that intra-NAc administration of a selective D_3R antagonist potentiates the locomotor-activating effects of cocaine (Manvich et al., 2019) but attenuates the locomotor-activating effects of morphine (Liang et al., 2011).

Alternatively, the temporal patterns of cocaine-induced vs. morphine-induced increases in NAc DA are different (Di Chiara and Imperato, 1988; Pontieri et al., 1995; Rouge-Pont et al., 2002; Sorge and Stewart, 2006; Zocchi et al., 2003), likely owing to their dependence upon DAT inhibition vs. VTA DA neuron disinhibition respectively, and it is plausible that these temporally-distinct influences on DA signaling may be differentially modulated by D_3R antagonism. Still another potential explanation for the opposing influence of D_3R antagonism on stimulant- vs. opioid-induced hyperactivity may be that the reliance of cocaine and related psychostimulants on the DAT to induce increases in locomotion renders them potentially susceptible to neuropharmacological modulation by direct interactions between DAT and the D_3R (Castro-Hernandez et al., 2015; McGinnis et al., 2016; Zapata et al., 2007). DAT/D_3R interactions would not be predicted to play a prominent role in the DA-independent drivers of opioid-induced locomotion since they do not require functional DAergic terminals to exert their behavioral effects (Churchill and Kalivas, 1992; Kalivas et al., 1983; Stinus et al., 1985). Such hypotheses remain speculative and additional studies would be required to elucidate the specific mechanisms and neuroanatomical substrates underlying the ability of PG01037 to oppositely modulate the locomotor-activating effects of psychostimulants and opioids. Regardless, the capacity to potentiate any psychostimulant-induced behavioral and/or neurochemical effect will limit the therapeutic potential of a treatment for OUD, since opioid use frequently co-occurs with use of psychostimulants such as cocaine (Liu et al., 2020; Zhu and Wu, 2020). Hence, selective D_3R antagonists that reduce the abuse-related effects of opioids but do not enhance the effects of psychostimulants, such as VK4-40 and VK4-116, have been prioritized as lead candidates for the treatment of OUD (Newman et al., 2021).

4.5 Conclusions

In summary, we show that pretreatment with the highly-selective D_3R antagonist PG01037 attenuates acute morphine-induced hyperactivity and morphine-induced nociception in mice in the absence of any cataleptic effects. The reduction in opioid-induced hyperlocomotion and the lack of catalepsy aligns with the reported behavioral effects of other D_3R antagonists and are therefore likely to be features common to all compounds in this drug class, lending further support to their potential use and safety in the treatment of OUD. Recently-developed selective D_3R antagonists such as VK4-40 and VK4-116 exhibit favorable behavioral profiles and should be prioritized for translational studies, although further investigation is necessary to identify the neuropharmacological mechanisms by which D_3R antagonists alter the behavioral effects of opioids and, in some instances, psychostimulants.

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Declaration of Interest

The authors have no declarations of interest to disclose.

References

Achat-Mendes, C., Grundt, P., Cao, J., Platt, D. M., Newman, A. H., Spealman, R. D., 2010. Dopamine D3 and D2 receptor mechanisms in the abuse-related behavioral effects of cocaine: studies with preferential antagonists in squirrel monkeys. J Pharmacol Exp Ther 334, 556-565.

Audinot, V., Newman-Tancredi, A., Gobert, A., Rivet, J. M., Brocco, M., Lejeune, F., Gluck, L., Desposte, I., Bervoets, K., Dekeyne, A., Millan, M. J., 1998. A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D3 receptor antagonists (+)-S 14297, nafadotride, GR 103,691 and U 99194. J Pharmacol Exp Ther 287, 187-197.

Beaulieu, J. M., Gainetdinov, R. R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63, 182-217.

Bjorklund, A., Dunnett, S. B., 2007. Dopamine neuron systems in the brain: an update. Trends Neurosci 30, 194-202.

Blanco, C., Volkow, N. D., 2019. Management of opioid use disorder in the USA: present status and future directions. Lancet 393, 1760-1772.

Boateng, C. A., Bakare, O. M., Zhan, J., Banala, A. K., Burzynski, C., Pommier, E., Keck, T. M., Donthamsetti, P., Javitch, J. A., Rais, R., Slusher, B. S., Xi, Z. X., Newman, A. H., 2015. High Affinity Dopamine D3 Receptor (D3R)-Selective Antagonists Attenuate Heroin Self-Administration in Wild-Type but not D3R Knockout Mice. J Med Chem 58, 6195-6213.

Broekkamp, C. L., Phillips, A. G., Cools, A. R., 1979. Stimulant effects of enkephalin microinjection into the dopaminergic A10 area. Nature 278, 560-562.

Castro-Hernandez, J., Afonso-Oramas, D., Cruz-Muros, I., Salas-Hernandez, J., Barroso-Chinea, P., Moratalla, R., Millan, M. J., Gonzalez-Hernandez, T., 2015. Prolonged treatment with pramipexole promotes physical interaction of striatal dopamine D3 autoreceptors with dopamine transporters to reduce dopamine uptake. Neurobiol Dis 74, 325-335.

Chefer, V. I., Kieffer, B. L., Shippenberg, T. S., 2003. Basal and morphine-evoked dopaminergic neurotransmission in the nucleus accumbens of MOR- and DOR-knockout mice. Eur J Neurosci 18, 1915-1922.

Cho, D. I., Zheng, M., Kim, K. M., 2010. Current perspectives on the selective regulation of dopamine D(2) and D(3) receptors. Arch Pharm Res 33, 1521-1538.

Churchill, L., Kalivas, P. W., 1992. Dopamine depletion produces augmented behavioral responses to a mu-, but not a delta-opioid receptor agonist in the nucleus accumbens: lack of a role for receptor upregulation. Synapse 11, 47-57.

Cook, C. D., Beardsley, P. M., 2003. The modulatory actions of dopamine D2/3 agonists and antagonists on the locomotor-activating effects of morphine and caffeine in mice. Pharmacol Biochem Behav 75, 363-371.

de Guglielmo, G., Kallupi, M., Sedighim, S., Newman, A. H., George, O., 2019. Dopamine D3 Receptor Antagonism Reverses the Escalation of Oxycodone Self-administration and

Decreases Withdrawal-Induced Hyperalgesia and Irritability-Like Behavior in Oxycodone-Dependent Heterogeneous Stock Rats. Front Behav Neurosci 13, 292.
Delfs, J. M., Schreiber, L., Kelley, A. E., 1990. Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J Neurosci 10, 303-310.
Devine, D. P., Leone, P., Pocock, D., Wise, R. A., 1993. Differential involvement of ventral tegmental mu, delta and kappa opioid receptors in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. J Pharmacol Exp Ther 266, 1236-1246.
Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85, 5274-5278.
Di Chiara, G., North, R. A., 1992. Neurobiology of opiate abuse. Trends Pharmacol Sci 13, 185-193.
Diaz, J., Pilon, C., Le Foll, B., Gros, C., Triller, A., Schwartz, J. C., Sokoloff, P., 2000. Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. J Neurosci 20, 8677-8684.
Fink-Jensen, A., Schmidt, L. S., Dencker, D., Schulein, C., Wess, J., Wortwein, G., Woldbye, D. P., 2011. Antipsychotic-induced catalepsy is attenuated in mice lacking the M4 muscarinic acetylcholine receptor. Eur J Pharmacol 656, 39-44.
Galaj, E., Ananthan, S., Saliba, M., Ranaldi, R., 2014. The effects of the novel DA D3 receptor antagonist SR 21502 on cocaine reward, cocaine seeking and cocaine-induced locomotor activity in rats. Psychopharmacology (Berl) 231, 501-510.
Galaj, E., Manuszak, M., Babic, S., Ananthan, S., Ranaldi, R., 2015. The selective dopamine D3 receptor antagonist, SR 21502, reduces cue-induced reinstatement of heroin seeking and heroin conditioned place preference in rats. Drug Alcohol Depend 156, 228-233.
Galaj, E., Newman, A. H., Xi, Z. X., 2020. Dopamine D3 receptor-based medication development for the treatment of opioid use disorder: Rationale, progress, and challenges. Neurosci Biobehav Rev 114, 38-52.
Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., Caron, M. G., 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606-612.
Grundt, P., Carlson, E. E., Cao, J., Bennett, C. J., McElveen, E., Taylor, M., Luedtke, R. R., Newman, A. H., 2005. Novel heterocyclic trans olefin analogues of N-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D3 receptor. J Med Chem 48, 839-848.
Gysling, K., Wang, R. Y., 1983. Morphine-induced activation of A10 dopamine neurons in the rat. Brain Res 277, 119-127.
Hand, T. H., Franklin, K. B., 1985. 6-OHDA lesions of the ventral tegmental area block morphine-induced but not amphetamine-induced facilitation of self-stimulation. Brain Res 328, 233-241.
Haney, M., Ward, A. S., Foltin, R. W., Fischman, M. W., 2001. Effects of ecopipam, a selective dopamine D1 antagonist, on smoked cocaine self-administration by humans. Psychopharmacology (Berl) 155, 330-337.
Hattori, K., Uchino, S., Isosaka, T., Maekawa, M., Iyo, M., Sato, T., Kohsaka, S., Yagi, T., Yuasa, S., 2006. Fyn is required for haloperidol-induced catalepsy in mice. J Biol Chem 281, 7129-7135.

Heidbreder, C. A., Newman, A. H., 2010. Current perspectives on selective dopamine D(3) receptor antagonists as pharmacotherapeutics for addictions and related disorders. Ann N Y Acad Sci 1187, 4-34.

Hu, R., Song, R., Yang, R., Su, R., Li, J., 2013. The dopamine D(3) receptor antagonist YQA14 that inhibits the expression and drug-prime reactivation of morphine-induced conditioned place preference in rats. Eur J Pharmacol 720, 212-217.

Johnson, S. W., North, R. A., 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12, 483-488.

Jordan, C. J., Humburg, B., Rice, M., Bi, G. H., You, Z. B., Shaik, A. B., Cao, J., Bonifazi, A., Gadiano, A., Rais, R., Slusher, B., Newman, A. H., Xi, Z. X., 2019a. The highly selective dopamine D3R antagonist, R-VK4-40 attenuates oxycodone reward and augments analgesia in rodents. Neuropharmacology 158, 107597.

Jordan, C. J., Humburg, B. A., Thorndike, E. B., Shaik, A. B., Xi, Z. X., Baumann, M. H., Newman, A. H., Schindler, C. W., 2019b. Newly Developed Dopamine D3 Receptor Antagonists, R-VK4-40 and R-VK4-116, Do Not Potentiate Cardiovascular Effects of Cocaine or Oxycodone in Rats. J Pharmacol Exp Ther 371, 602-614.

Kalivas, P. W., Duffy, P., 1987. Sensitization to repeated morphine injection in the rat: possible involvement of A10 dopamine neurons. J Pharmacol Exp Ther 241, 204-212.

Kalivas, P. W., Widerlov, E., Stanley, D., Breese, G., Prange, A. J., Jr., 1983. Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. J Pharmacol Exp Ther 227, 229-237.

Kelley, A. E., Stinus, L., Iversen, S. D., 1980. Interactions between D-ala-met-enkephalin, A10 dopaminergic neurones, and spontaneous behaviour in the rat. Behav Brain Res 1, 3-24.

Kelly, P. H., Iversen, S. D., 1976. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur J Pharmacol 40, 45-56.

Kelly, P. H., Seviour, P. W., Iversen, S. D., 1975. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94, 507-522.

Kiritsy-Roy, J. A., Standish, S. M., Terry, L. C., 1989. Dopamine D-1 and D-2 receptor antagonists potentiate analgesic and motor effects of morphine. Pharmacol Biochem Behav 32, 717-721.

Kishi, T., Matsuda, Y., Iwata, N., Correll, C. U., 2013. Antipsychotics for cocaine or psychostimulant dependence: systematic review and meta-analysis of randomized, placebo-controlled trials. J Clin Psychiatry 74, e1169-1180.

Kreek, M. J., Reed, B., Butelman, E. R., 2019. Current status of opioid addiction treatment and related preclinical research. Sci Adv 5, eaax9140.

Kumar, V., Bonifazi, A., Ellenberger, M. P., Keck, T. M., Pommier, E., Rais, R., Slusher, B. S., Gardner, E., You, Z. B., Xi, Z. X., Newman, A. H., 2016. Highly Selective Dopamine D3 Receptor (D3R) Antagonists and Partial Agonists Based on Eticlopride and the D3R Crystal Structure: New Leads for Opioid Dependence Treatment. J Med Chem 59, 7634-7650.

Lejeune, F., Millan, M. J., 1995. Activation of dopamine D3 autoreceptors inhibits firing of ventral tegmental dopaminergic neurones in vivo. Eur J Pharmacol 275, R7-9.

Leone, P., Pocock, D., Wise, R. A., 1991. Morphine-dopamine interaction: ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacol Biochem Behav 39, 469-472.

Liang, J., Zheng, X., Chen, J., Li, Y., Xing, X., Bai, Y., Li, Y., 2011. Roles of BDNF, dopamine D(3) receptors, and their interactions in the expression of morphine-induced context-specific locomotor sensitization. Eur Neuropsychopharmacol 21, 825-834.

Liu, S., Scholl, L., Hoots, B., Seth, P., 2020. Nonfatal Drug and Polydrug Overdoses Treated in Emergency Departments - 29 States, 2018-2019. MMWR Morb Mortal Wkly Rep 69, 1149-1155.

Lv, Y., Hu, R. R., Jing, M., Zhao, T. Y., Wu, N., Song, R., Li, J., Hu, G., 2019. Selective dopamine D3 receptor antagonist YQA14 inhibits morphine-induced behavioral sensitization in wild type, but not in dopamine D3 receptor knockout mice. Acta Pharmacol Sin 40, 583-588.

Manvich, D. F., Petko, A. K., Branco, R. C., Foster, S. L., Porter-Stransky, K. A., Stout, K. A., Newman, A. H., Miller, G. W., Paladini, C. A., Weinshenker, D., 2019. Selective D2 and D3 receptor antagonists oppositely modulate cocaine responses in mice via distinct postsynaptic mechanisms in nucleus accumbens. Neuropsychopharmacology 44, 1445-1455.

Manzanedo, C., Aguilar, M. A., Minarro, J., 1999. The effects of dopamine D2 and D3 antagonists on spontaneous motor activity and morphine-induced hyperactivity in male mice. Psychopharmacology (Berl) 143, 82-88.

Matsui, A., Williams, J. T., 2011. Opioid-sensitive GABA inputs from rostromedial tegmental nucleus synapse onto midbrain dopamine neurons. J Neurosci 31, 17729-17735.

McBride, W. J., Murphy, J. M., Ikemoto, S., 1999. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 101, 129-152.

McGinnis, M. M., Siciliano, C. A., Jones, S. R., 2016. Dopamine D3 autoreceptor inhibition enhances cocaine potency at the dopamine transporter. J Neurochem 138, 821-829.

Mercuri, N. B., Saiardi, A., Bonci, A., Picetti, R., Calabresi, P., Bernardi, G., Borrelli, E., 1997. Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. Neuroscience 79, 323-327.

Millan, M. J., Dekeyne, A., Rivet, J. M., Dubuffet, T., Lavielle, G., Brocco, M., 2000. S33084, a novel, potent, selective, and competitive antagonist at dopamine D(3)-receptors: II. Functional and behavioral profile compared with GR218,231 and L741,626. J Pharmacol Exp Ther 293, 1063-1073.

Millan, M. J., Peglion, J. L., Vian, J., Rivet, J. M., Brocco, M., Gobert, A., Newman-Tancredi, A., Dacquet, C., Bervoets, K., Girardon, S., et al., 1995. Functional correlates of dopamine D3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)-S 14297: 1. Activation of postsynaptic D3 receptors mediates hypothermia, whereas blockade of D2 receptors elicits prolactin secretion and catalepsy. J Pharmacol Exp Ther 275, 885-898.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., Caron, M. G., 1998. Dopamine receptors: from structure to function. Physiol Rev 78, 189-225.

Moore, R. Y., Bloom, F. E., 1978. Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. Annu Rev Neurosci 1, 129-169.

Newman, A. H., Ku, T., Jordan, C. J., Bonifazi, A., Xi, Z. X., 2021. New Drugs, Old Targets: Tweaking the Dopamine System to Treat Psychostimulant Use Disorders. Annu Rev Pharmacol Toxicol 61, 609-628.

Pert, A., Sivit, C., 1977. Neuroanatomical focus for morphine and enkephalin-induced hypermotility. Nature 265, 645-647.

Phillips, A. G., LePiane, F. G., Fibiger, H. C., 1983. Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat. Life Sci 33, 2505-2511.

Pierce, R. C., Kumaresan, V., 2006. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30, 215-238.

Pontieri, F. E., Tanda, G., Di Chiara, G., 1995. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci U S A 92, 12304-12308.

Pritchard, L. M., Newman, A. H., McNamara, R. K., Logue, A. D., Taylor, B., Welge, J. A., Xu, M., Zhang, J., Richtand, N. M., 2007. The dopamine D3 receptor antagonist NGB 2904 increases spontaneous and amphetamine-stimulated locomotion. Pharmacol Biochem Behav 86, 718-726.

Reavill, C., Taylor, S. G., Wood, M. D., Ashmeade, T., Austin, N. E., Avenell, K. Y., Boyfield, I., Branch, C. L., Cilia, J., Coldwell, M. C., Hadley, M. S., Hunter, A. J., Jeffrey, P., Jewitt, F., Johnson, C. N., Jones, D. N., Medhurst, A. D., Middlemiss, D. N., Nash, D. J., Riley, G. J., Routledge, C., Stemp, G., Thewlis, K. M., Trail, B., Vong, A. K., Hagan, J. J., 2000. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. J Pharmacol Exp Ther 294, 1154-1165.

Reiner, D. J., Fredriksson, I., Lofaro, O. M., Bossert, J. M., Shaham, Y., 2019. Relapse to opioid seeking in rat models: behavior, pharmacology and circuits. Neuropsychopharmacology 44, 465-477.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., Kuhar, M. J., 1987. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237, 1219-1223.

Rodriguez-Arias, M., Broseta, I., Aguilar, M. A., Minarro, J., 2000. Lack of specific effects of selective D(1) and D(2) dopamine antagonists vs. risperidone on morphine-induced hyperactivity. Pharmacol Biochem Behav 66, 189-197.

Rouge-Pont, F., Usiello, A., Benoit-Marand, M., Gonon, F., Piazza, P. V., Borrelli, E., 2002. Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J Neurosci 22, 3293-3301.

Sanberg, P. R., Bunsey, M. D., Giordano, M., Norman, A. B., 1988. The catalepsy test: its ups and downs. Behav Neurosci 102, 748-759.

Schildein, S., Agmo, A., Huston, J. P., Schwarting, R. K., 1998. Intraaccumbens injections of substance P, morphine and amphetamine: effects on conditioned place preference and behavioral activity. Brain Res 790, 185-194.

Shippenberg, T. S., Bals-Kubik, R., Herz, A., 1993. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. J Pharmacol Exp Ther 265, 53-59.

Smith, J. E., Guerin, G. F., Co, C., Barr, T. S., Lane, J. D., 1985. Effects of 6-OHDA lesions of the central medial nucleus accumbens on rat intravenous morphine self-administration. Pharmacol Biochem Behav 23, 843-849.

Sokoloff, P., Le Foll, B., 2017. The dopamine D3 receptor, a quarter century later. Eur J Neurosci 45, 2-19.
Song, R., Yang, R. F., Wu, N., Su, R. B., Li, J., Peng, X. Q., Li, X., Gaal, J., Xi, Z. X., Gardner, E. L., 2012. YQA14: a novel dopamine D3 receptor antagonist that inhibits cocaine self-administration in rats and mice, but not in D3 receptor-knockout mice. Addict Biol 17, 259-273.
Sorge, R. E., Stewart, J., 2006. The effects of long-term chronic buprenorphine treatment on the locomotor and nucleus accumbens dopamine response to acute heroin and cocaine in rats. Pharmacol Biochem Behav 84, 300-305.
Spanagel, R., Herz, A., Shippenberg, T. S., 1992. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci U S A 89, 2046-2050.
Spyraki, C., Fibiger, H. C., Phillips, A. G., 1983. Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. Psychopharmacology (Berl) 79, 278-283.
Stinus, L., Koob, G. F., Ling, N., Bloom, F. E., Le Moal, M., 1980. Locomotor activation induced by infusion of endorphins into the ventral tegmental area: evidence for opiate-dopamine interactions. Proc Natl Acad Sci U S A 77, 2323-2327.
Stinus, L., Winnock, M., Kelley, A. E., 1985. Chronic neuroleptic treatment and mesolimbic dopamine denervation induce behavioural supersensitivity to opiates. Psychopharmacology (Berl) 85, 323-328.
Teitelbaum, H., Giammatteo, P., Mickley, G. A., 1979. Differential effects of localized lesions of n. accumbens on morphine- and amphetamine-induced locomotor hyperactivity in the C57BL/6J mouse. J Comp Physiol Psychol 93, 745-751.
Vaccarino, F. J., Corrigall, W. A., 1987. Effects of opiate antagonist treatment into either the periaqueductal grey or nucleus accumbens on heroin-induced locomotor activation. Brain Res Bull 19, 545-549.
Vanderschuren, L. J., Kalivas, P. W., 2000. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 151, 99-120.
Vezina, P., Kalivas, P. W., Stewart, J., 1987. Sensitization occurs to the locomotor effects of morphine and the specific mu opioid receptor agonist, DAGO, administered repeatedly to the ventral tegmental area but not to the nucleus accumbens. Brain Res 417, 51-58.
Volkow, N. D., Jones, E. B., Einstein, E. B., Wargo, E. M., 2019. Prevention and Treatment of Opioid Misuse and Addiction: A Review. JAMA Psychiatry 76, 208-216.
Wang, B., Luo, F., Ge, X., Fu, A., Han, J., 2003. Effect of 6-OHDA lesions of the dopaminergic mesolimbic system on drug priming induced reinstatement of extinguished morphine CPP in rats. Beijing Da Xue Xue Bao Yi Xue Ban 35, 449-452.
Wilcox, R. E., Bozarth, M., Levitt, R. A., 1983. Reversal of morphine-induced catalepsy by naloxone microinjections into brain regions with high opiate receptor binding: a preliminary report. Pharmacol Biochem Behav 18, 51-54.
Wise, R. A., 1989. Opiate reward: sites and substrates. Neurosci Biobehav Rev 13, 129-133.
Yamamoto, D. J., Nelson, A. M., Mandt, B. H., Larson, G. A., Rorabaugh, J. M., Ng, C. M., Barcomb, K. M., Richards, T. L., Allen, R. M., Zahniser, N. R., 2013. Rats classified as low or high cocaine locomotor responders: a unique model involving striatal dopamine transporters that predicts cocaine addiction-like behaviors. Neurosci Biobehav Rev 37, 1738-1753.

You, Z. B., Bi, G. H., Galaj, E., Kumar, V., Cao, J., Gadiano, A., Rais, R., Slusher, B. S., Gardner, E. L., Xi, Z. X., Newman, A. H., 2019. Dopamine D3R antagonist VK4-116 attenuates oxycodone self-administration and reinstatement without compromising its antinociceptive effects. Neuropsychopharmacology 44, 1415-1424.

You, Z. B., Gao, J. T., Bi, G. H., He, Y., Boateng, C., Cao, J., Gardner, E. L., Newman, A. H., Xi, Z. X., 2017. The novel dopamine D3 receptor antagonists/partial agonists CAB2-015 and BAK4-54 inhibit oxycodone-taking and oxycodone-seeking behavior in rats. Neuropharmacology 126, 190-199.

Zapata, A., Kivell, B., Han, Y., Javitch, J. A., Bolan, E. A., Kuraguntla, D., Jaligam, V., Oz, M., Jayanthi, L. D., Samuvel, D. J., Ramamoorthy, S., Shippenberg, T. S., 2007. Regulation of dopamine transporter function and cell surface expression by D3 dopamine receptors. J Biol Chem 282, 35842-35854.

Zhu, H., Wu, L. T., 2020. Multiple drug use disorder diagnoses among drug-involved hospitalizations in the United States: Results from the 2016 National Inpatient Sample. Drug Alcohol Depend 213, 108113.

Zocchi, A., Girlanda, E., Varnier, G., Sartori, I., Zanetti, L., Wildish, G. A., Lennon, M., Mugnaini, M., Heidbreder, C. A., 2003. Dopamine responsiveness to drugs of abuse: A shell-core investigation in the nucleus accumbens of the mouse. Synapse 50, 293-302.

Figure Legends

Fig. 1 Effects of pretreatment with PG01037 on acute morphine-induced locomotor activity. Mice were pretreated with vehicle or 0.1 – 10 mg/kg PG01037, followed 30 min later by vehicle or 5.6 – 56 mg/kg morphine. Shown are mean ± SEM total ambulations in the 120-min period following morphine administration. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, compared to vehicle at the same dose of morphine. All mice received all treatments (n = 16). “Veh” = vehicle; “PG” = PG01037. Doses on the abscissa are plotted along a log scale.

Fig. 2 Time course of changes in locomotor activity following pretreatment with PG01037 and subsequent administration of morphine. PG01037 (vehicle, 0.1 – 10 mg/kg) was administered 30 min prior to **A** 5.6 mg/kg morphine, **B** 18 mg/kg morphine, or **C** 56 mg/kg morphine. Each data point represents mean ± SEM ambulations recorded in 5-min bins. Arrows indicate time of pretreatment injection (“Veh/PG”, i.e. vehicle or 0.1 – 10 mg/kg PG01037 respectively) or time

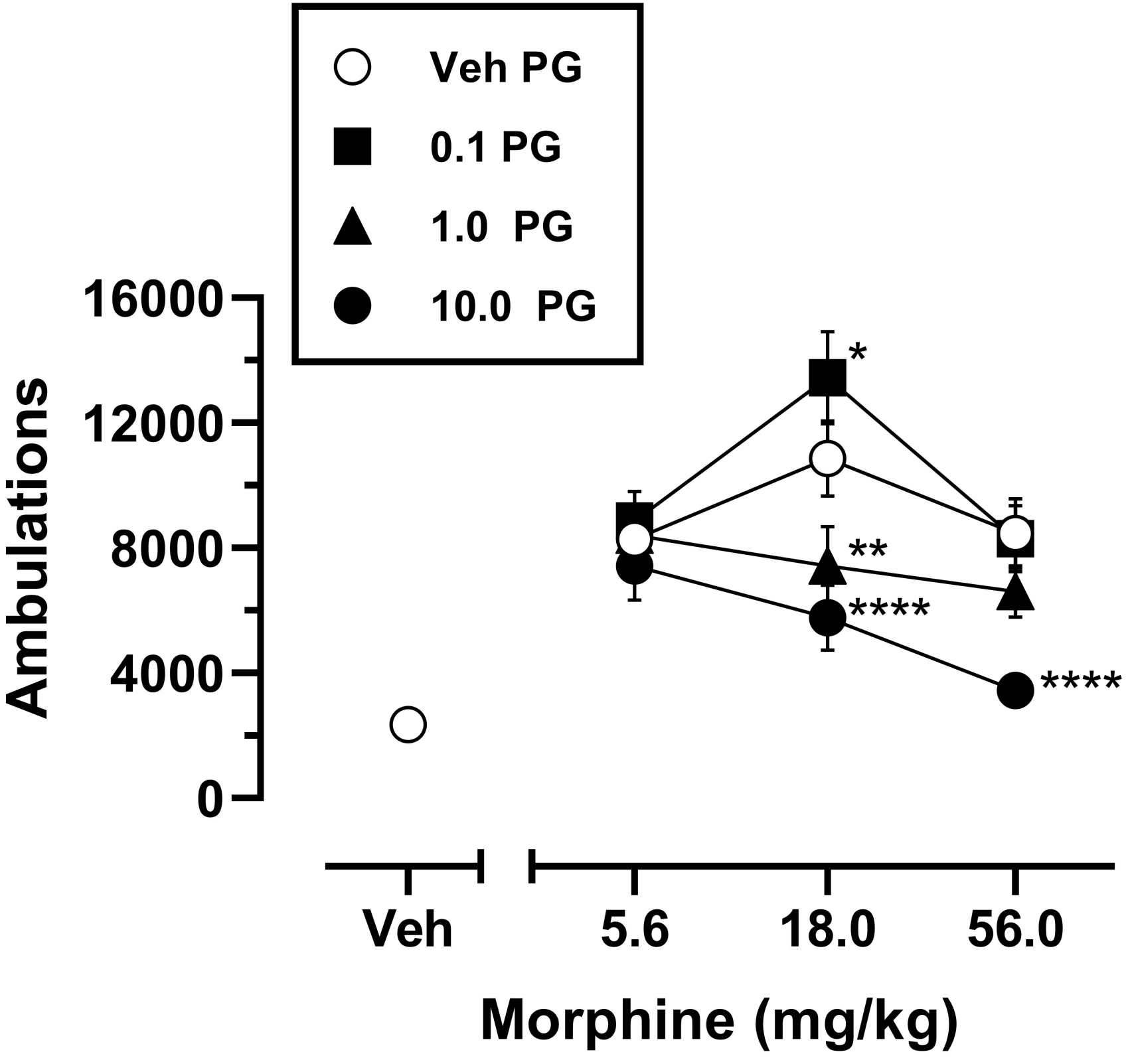
of morphine injection. All mice received all treatments (n = 16). “Veh” = vehicle; “PG” = PG01037.

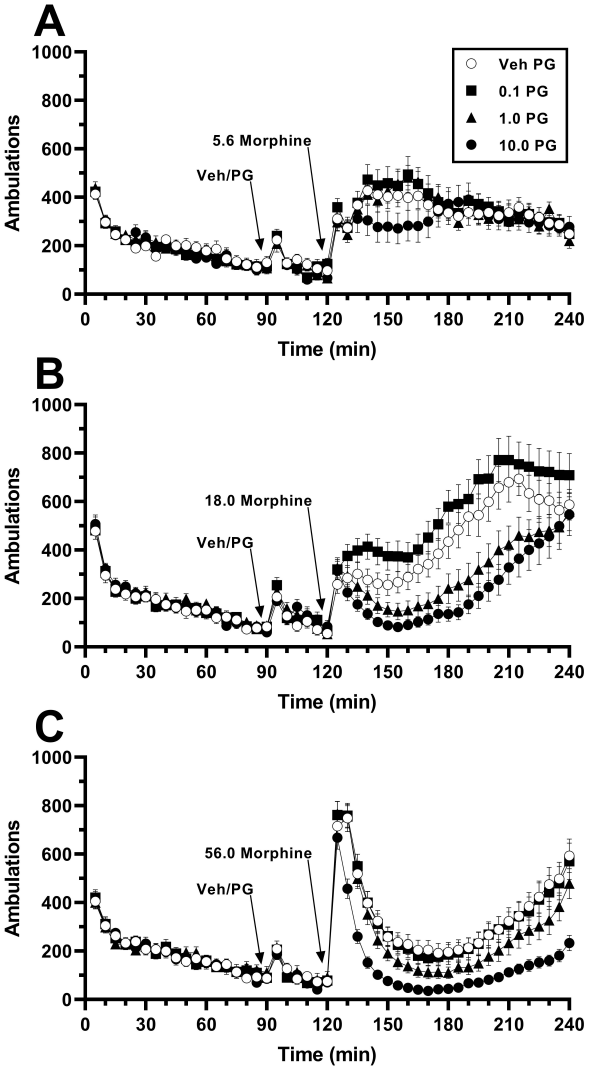
Fig. 3 Effects of pretreatment with PG01037 on morphine-induced locomotor sensitization. Mice received the combination of either vehicle + 56 mg/kg morphine or 10 mg/kg PG01037 + 56 mg/kg morphine daily for 5 days. One week later (day 12), all mice received vehicle pretreatment prior to a challenge with 56 mg/kg morphine. Shown are mean ± SEM total number of ambulations in the 120-min period following injection of 56 mg/kg morphine, which was administered 30 min after PG01037 pretreatment. ## $p < 0.01$, #### $p < 0.0001$, significant difference compared to Day 1 (collapsed across PG01037 pretreatment doses). * $p < 0.05$, main effect of pretreatment dose (collapsed across days 1-5). n = 8/group. “N.S.” = not significant; “Veh” = vehicle; “PG” = PG01037.

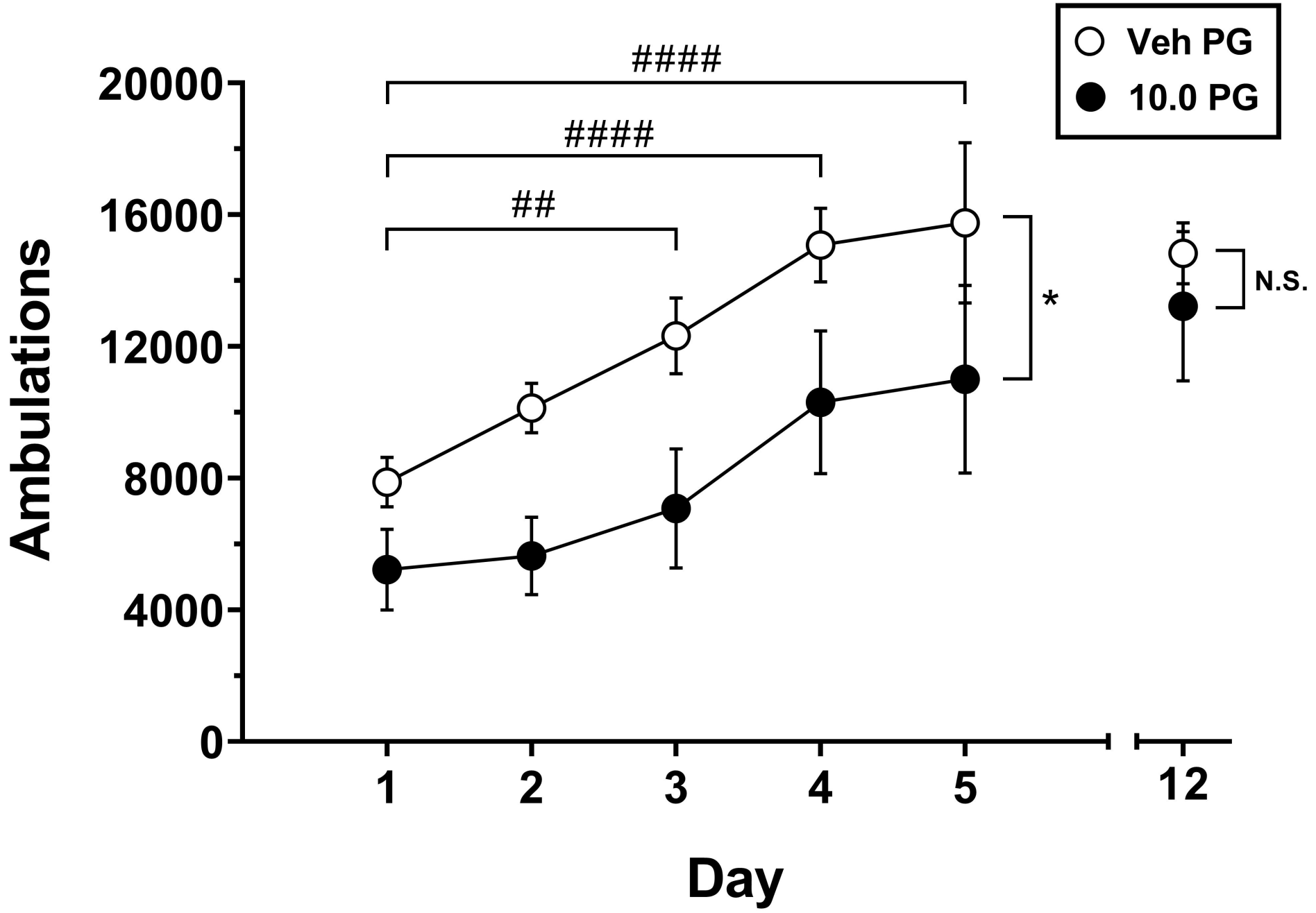
Fig. 4 Time course of locomotor activity following pretreatment with PG01037 and subsequent administration of 56 mg/kg morphine during induction days 1-5 of sensitization, and challenge test with morphine alone one week later. Experimental details are as described for Figure 3. Shown are mean ± SEM ambulations recorded in 5-min bins on **A** induction day 1, **B** induction day 2, **C** induction day 3, **D** induction day 4, and **E** induction day 5 of sensitization induction, or **F** challenge day. Arrows indicate time of pretreatment injection (“Veh/PG”, i.e. vehicle or 10 mg/kg PG01037 respectively) or time of morphine injection. n = 8/group. “Veh” = vehicle; “PG” = PG01037.

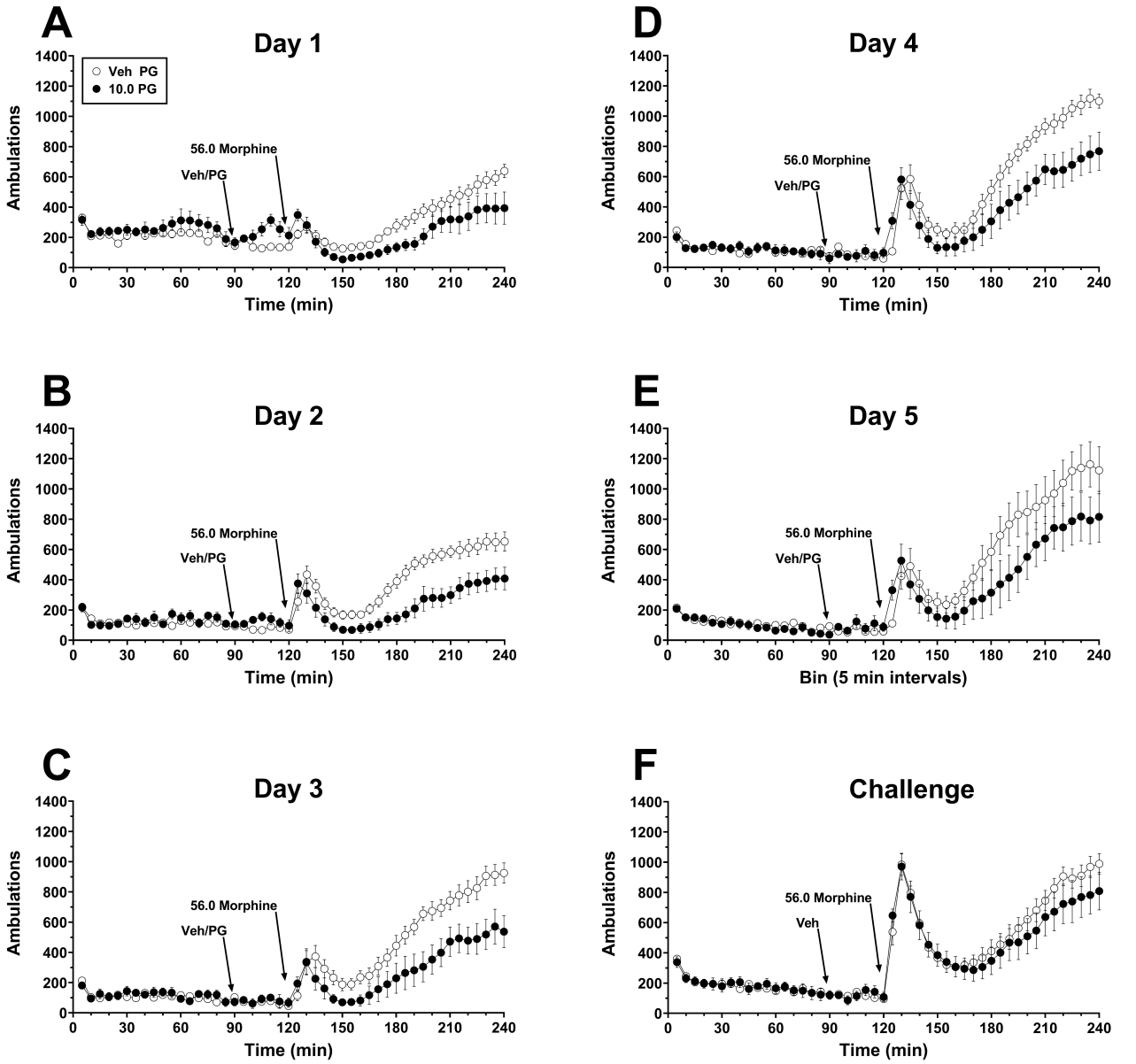
Fig. 5 Thermal nociception in mice following pretreatment with PG01037 alone or in combination with morphine. **A** Mice were administered PG01037 (vehicle, 0.1 – 10 mg/kg) and thermal nociception was assessed over 120 min following PG01037 injection. **B** Mice were administered PG01037 (vehicle, 0.1 – 10 mg/kg) 30 min prior to 18 mg/kg morphine, and thermal nociception was assessed over 120 min following morphine injection. *** $p < 0.001$, **** $p < 0.0001$, compared to vehicle at the same dose of morphine. n = 8/group. "Veh" = vehicle; "PG" = PG01037.

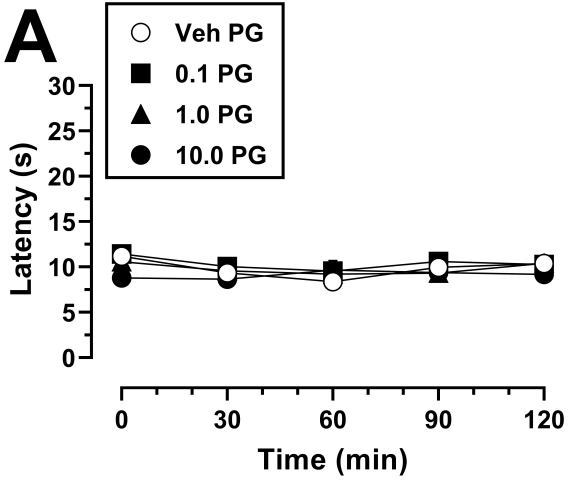
Fig. 6 Catalepsy following administration of PG01037 alone or in combination with morphine. Mice were pretreated with PG01037 (vehicle or 10 mg/kg) followed 30 min later by administration of vehicle or 56 mg/kg morphine, or 3 mg/kg risperidone followed 30 min later by administration of vehicle morphine. Each data point represents mean ± SEM latency in seconds to withdraw a paw in the bar test. Latencies were measured at 0, 15, 30, 60, and 120 min relative to the second injection. The dotted line represents the 60-s maximal time allowed for paw withdrawal. All mice received all treatments (n = 8). "Veh" = vehicle; "PG" = PG01037; "Risp" = risperidone; "Morph" = morphine.

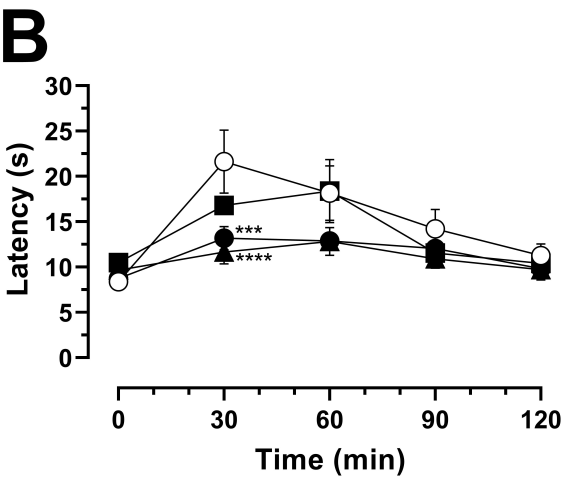

Veh PG
0.1 PG
1.0 PG
10.0 PG
Ambulations
16000
12000
8000
4000
0
*
**
****
****
Veh
5.6
18.0
56.0
Morphine (mg/kg)

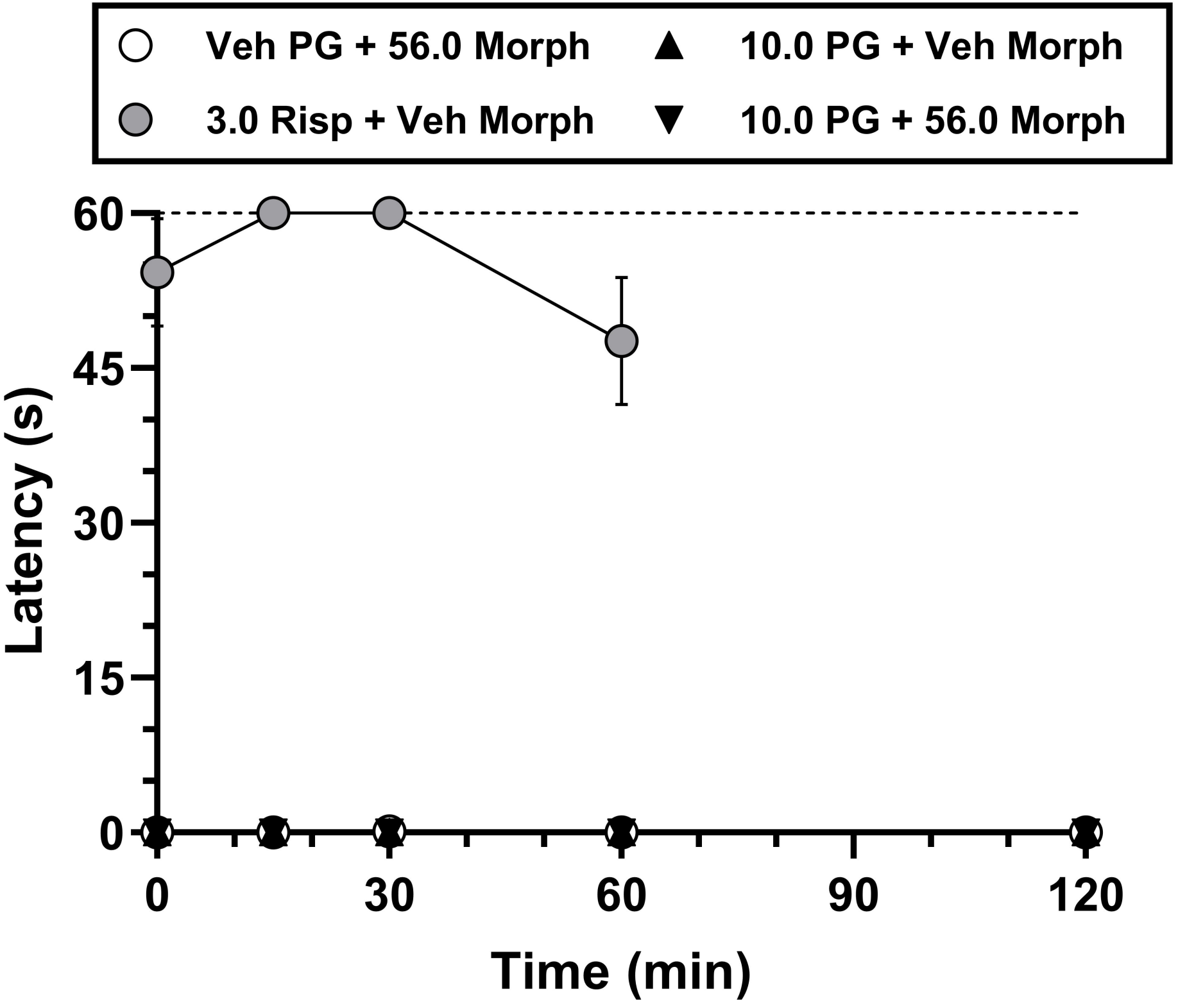

A
Ambulations
Time (min)
Veh PG
0.1 PG
1.0 PG
10.0 PG
5.6 Morphine
Veh/PG
B
Ambulations
Time (min)
18.0 Morphine
Veh/PG
C
Ambulations
Time (min)
56.0 Morphine
Veh/PG


Veh PG
10.0 PG
####
####
##
*
N.S.
Ambulations
20000
16000
12000
8000
4000
0
1
2
3
4
5
12
Day


A
Day 1
Veh PG
10.0 PG
56.0 Morphine
Veh/PG
Ambulations
Time (min)
B
Day 2
56.0 Morphine
Veh/PG
Ambulations
Time (min)
C
Day 3
56.0 Morphine
Veh/PG
Ambulations
Time (min)
D
Day 4
56.0 Morphine
Veh/PG
Ambulations
Time (min)
E
Day 5
56.0 Morphine
Veh/PG
Ambulations
Bin (5 min intervals)
F
Challenge
56.0 Morphine
Veh
Ambulations
Time (min)


A
Veh PG
0.1 PG
1.0 PG
10.0 PG
Latency (s)
30
25
20
15
10
5
0
0
30
60
90
120
Time (min)


B
Latency (s)
30
25
20
15
10
5
0
***
****
0
30
60
90
120
Time (min)


Veh PG + 56.0 Morph
10.0 PG + Veh Morph
3.0 Risp + Veh Morph
10.0 PG + 56.0 Morph
Latency (s)
60
45
30
15
0
0
30
60
90
120
Time (min)