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Behavioural, pharmacokinetic, and genetic evidence of a cannabidiol-oxycodone drug-drug interaction in mice

Rhianne L. Scicluna [a,b], James W.M. Kang [a,c], Nicholas A. Everett [a,b], Connie J. Badolato [a,b], Maia G. Etchart [d,e], Richard C. Kevin [d,e,f], Bianca B. Wilson [a,b], Damien C. Boorman [a,c], Rebecca S. Gordon [d,e], Lyndsey L. Anderson [d,e], Jonathon C. Arnold [a,d,e], Kevin A. Keay [a,c], Michael T. Bowen [a,b,*]

[a] The University of Sydney, Brain and Mind Centre, Sydney, NSW 2050, Australia
[b] The University of Sydney, Faculty of Science, School of Psychology, Sydney, NSW 2006, Australia
[c] The University of Sydney, School of Medical Sciences, Sydney, NSW 2006, Australia
[d] The University of Sydney, Faculty of Medicine and Health, Discipline of Pharmacology, Sydney Pharmacy School, Sydney, NSW 2006, Australia
[e] The University of Sydney, Lambert Initiative for Cannabinoid Therapeutics, Sydney, NSW 2006, Australia
[f] St Vincent's Hospital Sydney, Department of Clinical Pharmacology and Toxicology, Sydney, NSW 2010, Australia

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ABSTRACT

This study investigated the interaction between oxycodone and cannabidiol (CBD) on opioid analgesia and pharmacokinetics. We conducted in vivo behavioural studies to assess CBD's short and long-term effects on opioid analgesia and used qPCR to investigate the interaction of oxycodone and CBD at the genetic and neural level. Acute administration of CBD in male C57BL/6 J mice inhibited the metabolism of oxycodone into its less active metabolites, leading to a three-fold increase in total oxycodone exposure and a 50% increase in peak concentration. Acutely, this interaction appeared to extend the analgesic efficacy of a single oxycodone dose. In contrast, sub-chronic co-administration of CBD and oxycodone accelerated the development of tolerance to oxycodone analgesia, but not morphine. Moreover, CBD increased *Oprm1* and *Cnr1* mRNA expression in the periaqueductal gray in oxycodone, but not morphine-treated mice. This potential drug-drug interaction between oxycodone and CBD is possibly mediated by CYP2D6 and CYP3A4 enzymes, which are inhibited by CBD, and which are responsible for oxycodone, but not morphine metabolism. These findings suggest that CBD alters the analgesic efficacy of oxycodone and future translational studies are required to observe if this occurs at lower nutraceutical doses of CBD and in humans. The findings may have implications for chronic pain management and the simultaneous use of oxycodone and CBD.

1. Introduction

Concurrent opioid and cannabinoid use is common, with up to 51% of individuals receiving pharmacological treatment for opioid use disorder reporting cannabis use [1]. This pattern of co-use has been partly attributed to the proposed opioid-sparing effects of cannabinoids. Although substantial preclinical evidence supports cannabinoid-mediated opioid sparing, clinical studies have generally failed to demonstrate consistent analgesic benefit or opioid dose reduction [2–4]. Cannabidiol (CBD), has attracted increasing attention due to preclinical evidence suggesting it can modulate opioid analgesia [2,5–7] without the major psychoactive effects of delta−9 tetrahydrocannabinol (THC). One recent study showed that co-administration of CBD enhanced the analgesic efficacy of orally administered oxycodone in an operant thermal orofacial pain assay, in which rodents voluntarily exposed their faces to a cutaneous thermal stimulus to obtain a palatable reward [7]. However, human evidence remains sparse and largely observational, with correlational studies reporting associations between CBD use and reduced self-reported opioid consumption in chronic pain populations e.g. [8,9]. One recent human laboratory study examining the effect of CBD on hydromorphone, showed that CBD acutely enhanced hydromorphone analgesic and subjective effects [10]. However, it remains unclear whether these observed interactions are mediated by pharmacokinetic

* Correspondence to: Brain and Mind Centre, The University of Sydney, 94 Mallet Street, Camperdown, Sydney, NSW 2050, Australia.
E-mail address: michael.bowen@sydney.edu.au (M.T. Bowen).

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mechanisms or by other processes.

CBD has been established as a potent inhibitor of specific cytochrome P450 (CYP) enzymes, which are responsible for metabolizing approximately 90% of drugs [11–13]. CBD is therefore predicted to interact with many drugs, including opioids, antiseizure medications, antidepressants, acetaminophen, and alcohol [14], with a recent study reporting a clear DDI between CBD and the antiseizure medication clobazam [13]. Importantly, CBD has been shown to inhibit CYP3A4 and CYP2D6 enzymes [14–16], which play a vital role in the metabolism of oxycodone (Fig. 1; [17–19]), the most commonly prescribed opioid analgesic [20]. Further, simultaneous inhibition of both these enzymes with paroxetine and itraconazole (but not CYP2D6 alone) has been found to increase exposure to oral oxycodone in humans [21]. There is thus a high risk of a DDI between CBD and oxycodone that has been scarcely investigated.

Given CBD inhibits CYP3A4 and CYP2D6 [14–16], the enzymes that metabolise oxycodone [17–19], we hypothesised that there would be a DDI between CBD and oxycodone and that this would alter oxycodone analgesia and exposure. We therefore assessed whether a single co-administration of a high dose of CBD and oxycodone altered the analgesic efficacy of oxycodone in the hotplate test and its exposure in mice. We used a high CBD dose, comparable to that used in clinical trials in rare drug-resistant epilepsies, allowing a proof-of-concept test of CBD–oxycodone interactions at a dose with established human safety. To assess the potential implications of chronic simultaneous use of CBD and oxycodone, as would be most common in the context of chronic pain, we examined the effects of repeated co-administration of CBD and oxycodone on analgesia, tolerance, and *Oprm1* and *Cnr1* mRNA expression in the periaqueductal gray (PAG). The PAG was examined as it plays a key role in opioid analgesia [22] and the opioid and cannabinoid receptor systems have significant overlap in this region (they are co-localized on the same neurons in the PAG; [23]). Finally, we examined the effects of co-administration of CBD and morphine, which is metabolised primarily by phase II UDP-glucuronosyltransferases rather than CYP enzymes [24], to probe whether CBD-opioid DDIs might be more prominent in opioids metabolised by CYP3A4 and CYP2D6.

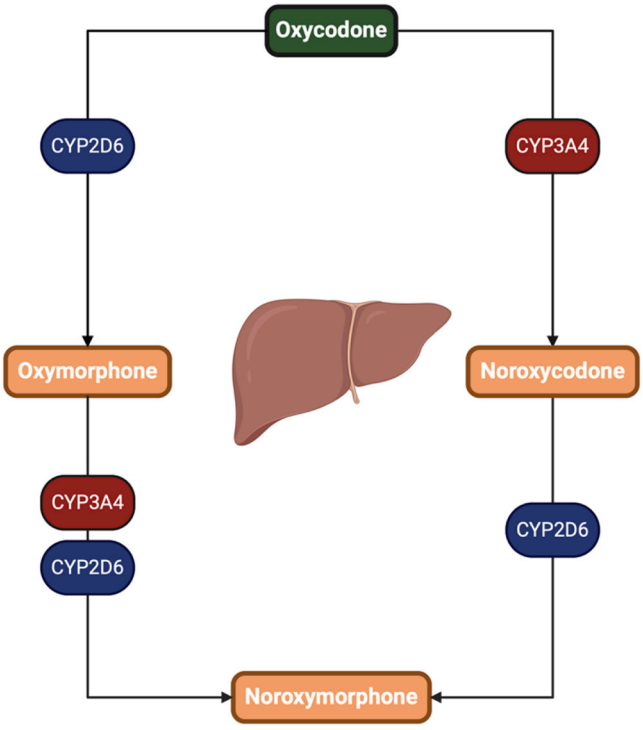

Fig. 1. Metabolism of oxycodone and metabolites by CYP450 enzymes. Oxycodone is metabolized by the CYP2D6 enzyme into oxymorphone and by the CYP3A4 enzyme into noroxycodone, with oxymorphone having analgesic effects and noroxycodone considered inactive. Although oxymorphone is twice as potent analgesic as oxycodone, it is only present in small amounts, making the clinical relevance of this metabolite questionable [25,26]. Noroxymorphone, which has a negligible role in analgesia, is formed through the metabolism of oxymorphone by CYP3A4 and CYP2D6 and noroxycodone by CYP2D6.

2. Methods

2.1. Subjects

N = 206 male C57BL/6 J mice (Animal Resource Centre, Western Australia) aged 9–10 weeks and weighing 17–26 g at the start of the experiment, were housed in ventilated cages with controlled temperature (~22°C) and humidity (~55%), a 12-hour light-dark cycle, and food and water *ad libitum*. Mice were housed in ventilated cages in a temperature (~22 °C) and humidity controlled (~55%) room on a 12-hour light-dark cycle (lights on 6 am), with access to food and water *ad libitum*. All testing took place during the light cycle. Mice were given at least 10 days to acclimatise to the facility before the commencement of studies, including two handling sessions over the course of a week (for a duration of 30 s per mouse). All experimental procedures were approved by University of Sydney Animal Ethics Committee.

2.2. Drugs

Oxycodone hydrochloride (CH2 Direct, OxyNorm 50 mg/ml dissolved in physiological saline) was administered at a dose of 5 mg/kg (for the acute hot plate experiment) or 10 mg/kg IP (for the pharmacokinetic and chronic hot plate experiments). Morphine (CH2 Direct, Morphine Sulfate, 30 mg/ml dissolved in physiological saline) was administered at an equianalgesic dose of 15 mg/kg IP [27]. CBD (Linnea) was administered at a dose of 100 mg/kg IP, dissolved in DMSO, Tween 80 and saline in a ratio of 1:1:18. Mice were dosed 1 h prior to testing as this corresponds to the T*max* of CBD in a study using a similar dose of CBD IP, in similar weight mice [28]. Mice were dosed 1 h prior to testing, based off methods from our previous study [29] as this corresponds to the Tmax of CBD observed in a study using a similar IP dose and comparable mouse weights [30]. AMD3100 (Amadis Chemical, Plerixafor octahydrochloride), a CXCR4 antagonist, was administered as a positive control for chronic co-administration studies, since CXCR4 signalling contributes to the pathogenies of opioid tolerance in rodents and humans [31] and AMD3100 prevents the development of tolerance to the analgesic effect of opioids in rodents [32]. AMD3100 was administered at a dose of 10 mg/kg IP, dissolved in physiological saline. This dose was chosen based off a previous study that has demonstrated efficacy of 10 mg/kg AMD3100 IP in a nociceptive behavioural study with rats [33]. All doses were administered an injection volume of 10 mg/kg IP.

2.3. Effect of acute CBD on oxycodone metabolism and analgesic efficacy

For the experiment examining CBD effects on oxycodone metabolism, 56 mice received intraperitoneal injections of CBD and oxycodone, followed by blood collection at selected time points (see Fig. 2A). Plasma was isolated and stored at −80 °C for LC-MS/MS analysis, and PK parameters were calculated. For the experiment examining CBD for oxycodone analgesic efficacy, N = 40 mice were habituated on the hot/cold plate analgesiometer (Ugo Basile, Italy) for 5 min at 25 ±0.1 °C to avoid novelty-induced behaviours. Mice received an injection of 100 mg/kg CBD or vehicle, followed by an injection of 5 mg/kg oxycodone or saline 1 h later. At selected time points following the oxycodone injection (15 min, 30 min, 60 min, 120 min), mice were placed on the hot plate and time taken to show a nociceptive response was measured (see Fig. 2A). Nociceptive responses included a hind paw flick,

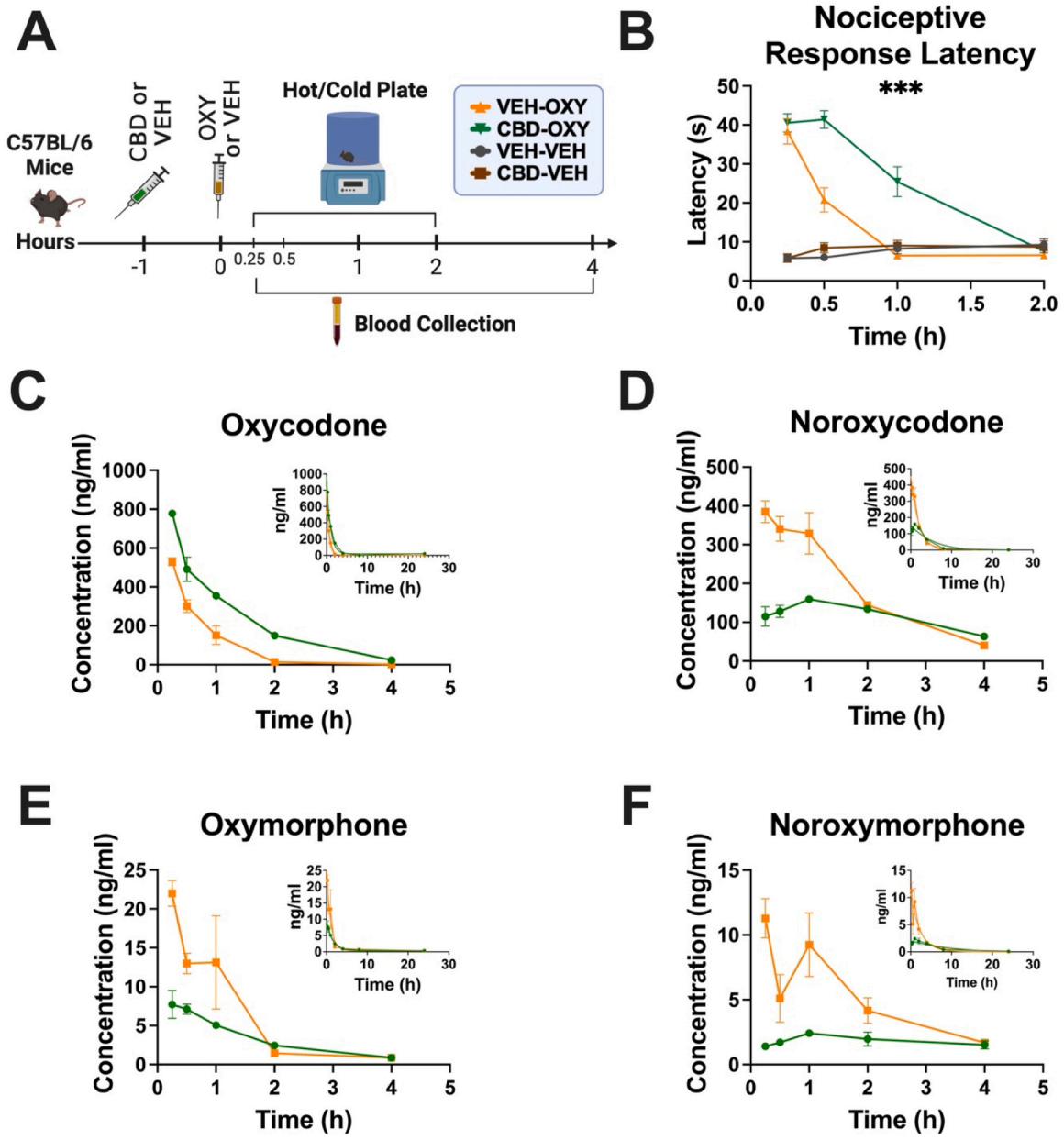

Fig. 2. Acute CBD delayed the metabolism of oxycodone into noroxycodone and oxymorphone and extended the duration of oxycodone analgesia on the hot plate. A: Experimental timeline and key for panels B-F. B: A single dose of CBD prolonged the analgesic efficacy of oxycodone on the hot plate test. C – F: Concentration-time graphs for oxycodone and metabolites. CBD treatment increased the C_{max}, $t_{1/2}$ and AUC of oxycodone, while decreasing C_{max}, prolonging $t_{1/2}$ and decreasing AUC of its metabolites. *** $p < .001$ for the trend analysis interaction for VEH-OXY vs CBD-OXY. VEH = vehicle, OXY = oxycodone, CBD = cannabidiol. Error bars represent SEM, with n = 10 per condition for B; n = 4 per condition for graphs C – F.

lick or jump. The hot plate was set at 53.4 ±0.1 °C with a cut off time of 45 s. In house piloting was used to determine the appropriate temperature to elicit a latency in oxycodone treatment mice that was sensitive to an increase or decrease in latency upon pre-treatment. Cut off times were chosen to ensure no tissue damage was inflicted. Pharmacokinetic analysis including details on sample preparation, chemicals, and reagents, and LC-MS/MS procedures are detailed in the supplementary methods.

2.4. Effect of sub-chronic co-administration of CBD alongside opioids on the development of tolerance opioid analgesia

Before drug administration, N = 110 mice were habituated on the hot/cold plate for 10 min at 25 ±0.1 °C to avoid novelty induced behaviours. Baseline latencies to respond to a thermal stimulus on the hot and cold plate were measured 3 times (with an intertrial interval of between 1 – 3 days) and the average of the second and third trials were used (to improve reliability of the baseline measure). Mice received a 10 ml/kg IP injection of saline, 10 mg/kg oxycodone or 15 mg/kg morphine twice daily (at 9 am and 4 pm; 7 h separating injections) for 4 days. On the 5th day, mice received a final dose of saline, oxycodone, or morphine 30 min prior to hot/cold plate testing. Mice were treated with either 100 mg/kg CBD, 1 h prior to hot/cold plate testing, 10 m/kg AMD3100, 30 min prior to hot/cold plate testing (prepared in the same solution as oxycodone), or saline, 1 h prior to hot/cold plate testing.

On day 1, 3 and 5 of drug administration, hot and cold plate latencies were measured 30 min after administration of the opioid or 1.5 h after CBD administration. For the oxycodone and morphine experiments, the hot plate was set at 55 ±0.1 °C with a cut off time of 30 s and the cold plate set at −5 ±0.1 °C with a cut of time of 60 s. In house piloting was

used to determine temperatures that yield maximum latencies on day 1 and that are sensitive to a decline overtime (due to the development of tolerance to the analgesic effects of the opioid). For the CBD control (opioid naïve) experiment, the hot plate measured 48 ±0.1 °C with a cut off time of 45 s and the cold plate measured −1 ±0.1 °C with a cut of time of 60 s. In house piloting was used to determine the appropriate temperature to elicit a latency sensitive to any nociceptive effect of CBD. Cut off times were chosen to ensure no tissue damage was inflicted. Weights were taken at 4 time points throughout the protocol.

Mice were sacrificed immediately following hot/cold plate testing by live decapitation. Brains were rapidly extracted, placed into pre-labelled tubes, and the tubes were immediately submerged in liquid nitrogen for freezing. The brains were stored at −80°C until thawed, after which the PAG was isolated by microdissection under a microscope. The isolated tissue was then returned to storage at −80°C until further analysis. Further methods for isolation of the PAG from whole brain tissue, as well as RNA extraction and complementary DNA (cDNA) synthesis, are provided in the Supplementary Methods.

Real time qPCR was performed to assess mRNA expression levels of Oprm1 and Cnr1 using Actb as the reference gene. Each qPCR experiment was carried out using a Quant Studio 6 (ThermoFisher, USA) Real-Time PCR machine. Each reaction consisted of cDNA (100 ng/reaction), SensiFAST SYBR® No-ROX Master mix (Bioline, Australia) and forward and reverse primers with a FAM reporter probe (IDT, final concentration 500 nM [primers] and 250 nM [probe]). Primer and probe (IDT, USA) sequences are provided in supplementary table 4 (S4). To assess changes in gene expression, we analysed the mean fold change of each sample using the reference gene *Actb*, according to the Pfaffl Method as previously described [34].

2.5. Statistics

The first experiment examined the effect of acute CBD on oxycodone analgesic efficacy, with treatment condition (vehicle-saline, CBD-saline, vehicle-oxycodone, and CBD-oxycodone) and time (15, 30, 60, and 120 min) as independent variables and time taken to show a nociceptive response as the dependent variable. A 4 × 4 mixed ANOVA was conducted, followed by pairwise comparisons, trend and interaction trend contrasts. Pharmacokinetic parameters were estimated from composite concentration–time profiles generated using a serial-sacrifice design, with n = 4 animals per timepoint per treatment condition. Because repeated sampling from individual mice was not feasible, blood concentrations at each timepoint were obtained from independent animals and averaged to construct group concentration–time curves. AUC_{0}–24 was calculated using the linear trapezoidal rule, and terminal half-life (t½) was estimated by log-linear regression over the terminal elimination phase. To quantify uncertainty in derived pharmacokinetic parameters, stratified bootstrap resampling (5000 iterations) was performed within each treatment × timepoint cell, and SEM and 95% confidence intervals were calculated for Cmax, AUC_{0}–24, and t½.

The next set of experiments investigated the effect of sub-chronic co-administration of CBD with oxycodone, morphine or saline on the development of tolerance. For each set of experiments, treatment condition and day (baseline, day 1, 3, and 5) were the independent variables, and the time taken to show a nociceptive response on the hot and cold plates as the dependent variable. For the oxycodone experiment, the treatment conditions were vehicle-saline, CBD-saline, vehicle-oxycodone, CBD-oxycodone, and AMD3100-oxycodone. For the morphine experiment, the treatment conditions were vehicle-oxycodone, CBD-oxycodone, vehicle-morphine, and CBD-morphine. Mixed ANOVA followed by pairwise comparisons, trend and interaction trend contrasts were conducted. For the CBD control experiment, with treatment conditions of vehicle-saline and CBD-saline, a 2 × 4 mixed ANOVA was conducted, followed by pairwise comparisons. In the PCR experiment, the effect of sub-chronic co-administration of CBD with oxycodone and morphine on OPRM1 and CNR1 mRNAs in the PAG was investigated, with treatment condition (vehicle-saline, CBD-saline, vehicle-oxycodone, CBD-oxycodone, vehicle-morphine, CBD-morphine, and AMD3100-oxycodone) as the independent variable and mRNA expression relative to the vehicle-saline group as the dependent variable. A one-way ANOVA with a Tukey's post hoc test was conducted. Data are presented as mean ±SEM. All statistical analyses were conducted using SPSS (Version 28). Significance was set at $p < 0.05$.

3. Results

To facilitate brevity and clarity of reporting, only summary statistics are presented below and in the figure legends. Detailed results from statistical analyses are provided in Tables S1 and S2.

3.1. CBD extends the duration of oxycodone analgesic efficacy

Given CBD inhibits CYP3A4 and CYP2D6 [14–16], the enzymes that metabolise oxycodone [17–19], we hypothesised there would be a DDI between CBD and oxycodone and that this would alter the analgesic effect of oxycodone. We therefore assessed whether a single co-administration of CBD and oxycodone altered the analgesic efficacy of oxycodone in the hotplate test and its exposure in mice. Administration of 100 mg/kg CBD one hour before administration of 5 mg/kg oxycodone (Fig. 2A) extended the duration of the analgesic effects of oxycodone on the hotplate test in C57BL/6 mice (Fig. 2B). Fifteen minutes after oxycodone administration all groups treated with oxycodone showed a large effect size increase in hot plate nociceptive response latencies compared to vehicle treated control mice (VEH-VEH vs: VEH-OXY 95% CI [−38.55, −26.49], $p < .001$, $d = -3.65$, 1-β >0.99; CBD-OXY 95% CI [−40.71, −28.65], $p < .001$, $d = -3.89$, 1-β >0.99). CBD had no impact on oxycodone's analgesic efficacy at this timepoint (all $p = 0.472$). CBD on its own had no analgesic effect ($p = 0.987$). A strong linear decline in oxycodone analgesic efficacy was observed over the four timepoints (0.25, 0.5, 1 and 2 h), $F(1,18) = 211.81$, $p < .001$, $\eta p^2 = .92$. This decline differed between the vehicle-treated and CBD-treated mice, as evidenced by a significant quadratic time x treatment interaction, $F(1,18) = 33.11$, $p < .001$, $\eta p^2 = .65$, indicating a more sustained analgesic response in the CBD-treated mice relative to vehicle-treated mice. In vehicle treated mice, oxycodone analgesic effects were abolished by 1 h (95% CI [−4.4, 8.06], $p = .555$, $d = 0.2$), whereas there was still a large effect size analgesic response present in CBD mice at this timepoint (VEH-VEH vs CBD-OXY 95% CI [−23.37, 10.91], $p < .001$, $d = -1.86$, 1-β = 0.98).

3.2. CBD has a drug-drug interaction with oxycodone that increases oxycodone exposure

Similarly, in light of the hypothesised DDI between CBD and oxycodone, we hypothesised that CBD would alter the exposure of oxycodone in the blood. A pharmacokinetic analysis of oxycodone and its metabolites in C57BL/6 mouse plasma following administration of 100 mg/kg CBD or vehicle 1 h prior to administration of 5 mg/kg oxycodone revealed a DDI between oxycodone and CBD. CBD substantially altered the pharmacokinetic parameters of oxycodone and its three metabolites (Fig. 2C-F, Table 1). Administration of CBD did not affect the T_{max} (time taken to reach C_{max}; maximum concentration) of oxycodone but caused a 47% increase in its C_{max} (529.6–778.1 ng/ml). CBD treatment also extended the $t_{1/2}$ (half-life) of oxycodone from 22.2 to 37.8 min. The increased C_{max} and longer $t_{1/2}$ translated to an increase total oxycodone exposure by 68% (AUC 331.8 vs 1025 ng h/ml). Because CBD inhibited the metabolism of oxycodone, concentrations of metabolites were reduced. As expected, both C_{max} and AUC values decreased for all metabolites - noroxycodone (59% and 17%), oxymorphone (65% and 32%) and noroxymorphone (79% and 44%). CBD delayed noroxycodone and noroxymorphone T_{max} by 45 min (15 min to 60 min) but had no effect on oxymorphone T_{max}. CBD increased

Table 1
Effect of CBD on the pharmacokinetics of oxycodone and its metabolites noroxycodone, oxymorphone and noroxymorphine in mouse plasma samples.

	Oxycodone		Noroxycodone		Oxymorphone		Noroxymorphone	
Treatment	VEH + OXY	CBD + OXY	VEH + OXY	CBD + OXY	VEH + OXY	CBD + OXY	VEH + OXY	CBD + OXY
C_{max} (ng/ml) ± SEM (95% CI)	529.5 ± 17.1 (492, 561)	778.1 ± 15.2 (749.6, 806.5)	384.7 ± 23.3 (343.9, 436)	159.4 ± 6.9 (146.7, 173)	22 ± 1.6 (19.8, 25.5)	7.7 ± 1.2 (6.4, 11)	11.3 ± 1.3 (9.4, 14.4)	2.4 ± 0.2 (2.2, 3)
T_{max} (min)	15	15	15	60	15	15	15	60
$t_{1/2}$ (min) ± SEM (95% CI)	19.6 ± 1 (17.7, 21.8)	51.8 ± 5.5 (43.9, 65.3)	71.8 ± 2.6 (66.7, 76.7)	91.7 ± 4.6 (81.2, 99.2)	26.6 ± 1.9 (23.6, 30.8)	58.3 ± 9.1 (43.8, 79.3)	75.3 ± 13.6 (58.2, 111.4)	273.5 ± 3139.3 (137, 1344.7)
AUC 0–24 (ng h/ml) ± SEM (95% CI)	331.8 ± 32.2 (270.8, 396)	1025.1 ± 136 (831.1, 1330.8)	811.3 ± 38.7 (737.2, 889.1)	670.7 ± 21.8 (626.9, 711.2)	31.9 ± 4.2 (25.1, 40.9)	21.8 ± 1.1 (19.8, 23.9)	26.2 ± 2.4 (21.7, 31)	14.8 ± 1.4 (12.2, 17.5)

VEH = vehicle, CBD = cannabidiol, OXY = oxycodone

noroxycodone, oxymorphone and noroxymorphone $t_{1/2}$ by 158, 10 and 247 min, respectively.

3.3. CBD accelerated the development of tolerance to oxycodone analgesia

To assess the potential implications of chronic simultaneous use of CBD and oxycodone, as would be most common in the context of chronic pain, we examined the effects of repeated co-administration of CBD and oxycodone on analgesia and tolerance. Co-administration of CBD alongside twice daily oxycodone injections accelerated the development of tolerance to oxycodone analgesia on the hot and cold plate tests (Fig. 3 B and C). At baseline, there was no difference in paw withdrawal latency on the hot or cold plate tests between vehicle treated control mice and any other condition (all $p > .05$). On Day 1, all groups treated with oxycodone showed a significant increase in latency to nociceptive response on the hot and cold plate tests (with all mice reaching the maximum latency) compared to vehicle treated control mice (hot plate all 95% CI [−25.21, −22.37], $p < .001$, $d = -10.09$, 1-β >0.99; cold plate all 95% CI [−58.55, −54.39), $p < .001$, $d = -16.33$, 1-β > 0.99) and there was no difference in oxycodone's analgesic effects between the oxycodone treated conditions (all $p > .999$). CBD on its own had no analgesic effect on the hot ($p = .061$) or cold plate ($p = .900$). On the hot plate, mice treated with vehicle or CBD developed tolerance to the analgesic effects of oxycodone from day 1 to day 5, VEH $F(1,9) = 59.19$, $p < .001$, $\eta p^2 = .87$; VEH $F(1,9) = 239.97$, $p < .001$, $\eta p^2 = .96$. However, tolerance to oxycodone analgesia on the hotplate developed substantially more rapidly in mice that received CBD compared to vehicle $F(1,18) = 7.03$, $p = .016$, $\eta p^2 = 0.28$). In contrast, AMD3100, *inhibited* the development of tolerance to oxycodone on the hot plate test $F(1,9) = 4.22$, $p = .070$, $\eta p^2 = .32$. On the cold plate test, mice treated with vehicle or AMD3100 did not develop statistically significant tolerance to oxycodone analgesia (VEH $F(1,9) = 2.82$, $p = .127$, $\eta p^2 = .24$.; AMD3100 $F(1,9) = 2.72$, $p = .134$, $\eta p^2 = .23$), whereas mice treated with CBD developed significant tolerance ($F(1,9) = 23.12$, $p < .001$, $\eta p^2 = .72$; interaction $F(1,18) = 15.81$, $p < .001$, $\eta p^2 = .47$).

3.4. CBD had no effect on the development of tolerance to morphine analgesia

We examined the effects of co-administration of CBD and morphine, which is metabolised primarily by phase II UDP-glucuronosyltransferases rather than CYP enzymes [24], to determine whether CBD-opioid DDIs are specific to those opioids metabolised by CYP3A4 and CYP2D6. Co-administration of CBD alongside twice daily morphine injections had no effect on the development of tolerance to morphine analgesia on the hot or cold plate tests, and acceleration of the development of tolerance to oxycodone analgesia by CBD was replicated (Fig. 3 D and E). At baseline, there was no significant difference in paw withdrawal latency on the hot or cold plate test between any conditions [all $p > .05$]. On day 1, CBD had no effect on oxycodone analgesia ($p = .459$) on the hot plate but did appear to increase nociceptive latency to morphine (95% CI [−10.27, −1.81, $p = .006$, $d = -2.9$, 1-β = 0.54), and nociceptive latencies were longer in oxycodone versus morphine treated mice (95% CI [−10.77, −2.31], $p = .003$, $d = -1.05$, 1-β = 0.6). In contrast, CBD had no significant effect on oxycodone or morphine analgesia on the cold plate test on day 1 (all $p > .197$), and there were no differences in nociceptive latencies between oxycodone and morphine ($p = .507$). As in the previous experiment, CBD accelerated the development of tolerance to oxycodone's analgesic effects on both the hot (linear interaction $F(1,18) = 4.52$, $p = .048$, $\eta p^2 = .2$) and cold plate tests (linear interaction $F(1,18) = 10.34$, $p = .005$, $\eta p^2 = .37$; quadratic interaction $F(1,18) = 5.01$, $p = .038$, $\eta p^2 = .22$). In morphine treated mice, the linear decrease in nociceptive response latency was also greater in mice treated with CBD compared to vehicle (linear interaction $F(1,18) = 8.19$, $p = .010$, $\eta p^2 = .31$); however, since there were no differences in latencies on day 3 and day 5 (both $p > .579$) the interaction here can be attributed to CBD's enhancement of latency on day 1, rather than an acceleration of the development of tolerance. Further, CBD had no effect on the development of tolerance to morphine analgesia on the cold plate test ($p = .981$).

3.5. Effect of CBD alone on nociception on the hot plate and cold plate

To examine if the observed effects of combining CBD and oxycodone result from their interaction rather than an additive effect of each compound individually, we assessed the impact of CBD alone on nociception. Five days of CBD administration had minimal effect on nociceptive response latencies on the hot or cold plate tests (Fig. 3 F and G). CBD or vehicle was administered twice daily for 4 days, followed by a single administration on day 5. Hot and cold plate nociceptive response latencies were measured at baseline and on days 1, 3 and 5. There was no effect of day or CBD treatment on hot plate nociceptive response latency (both $p > .238$). However, there was an interaction between treatment condition and day ($p = .015$), such that CBD treated rats had a longer hot plate nociceptive response latency on day 5 compared to vehicle ($p = .012$), but there was no difference on at baseline, day 1 or day 3 (all $p > .156$). There was no effect of day or CBD treatment on cold plate response latency (all $p > .080$).

3.6. Chronic co-administration of CBD and oxycodone increased *Oprm1* and *Cnr1* mRNA expression in the PAG

We examined the effects of repeated co-administration of CBD and oxycodone on *Oprm1* and *Cnr1* mRNA expression in the PAG, as this region plays a key role in opioid analgesia [22] and the opioid and cannabinoid receptor systems have significant overlap in this region [23]. Relative real time RT-PCR analysis was used to assess the changes in gene expression in the PAG following co-administration of CBD with morphine or oxycodone. As shown in Figs. 4A and 4B, CBD co-administration with oxycodone selectively increased expression of *Oprm1* mRNA (~ 2-fold; $p < 0.05$) and *Cnr1* mRNA (~ 2-fold; $p < 0.01$).

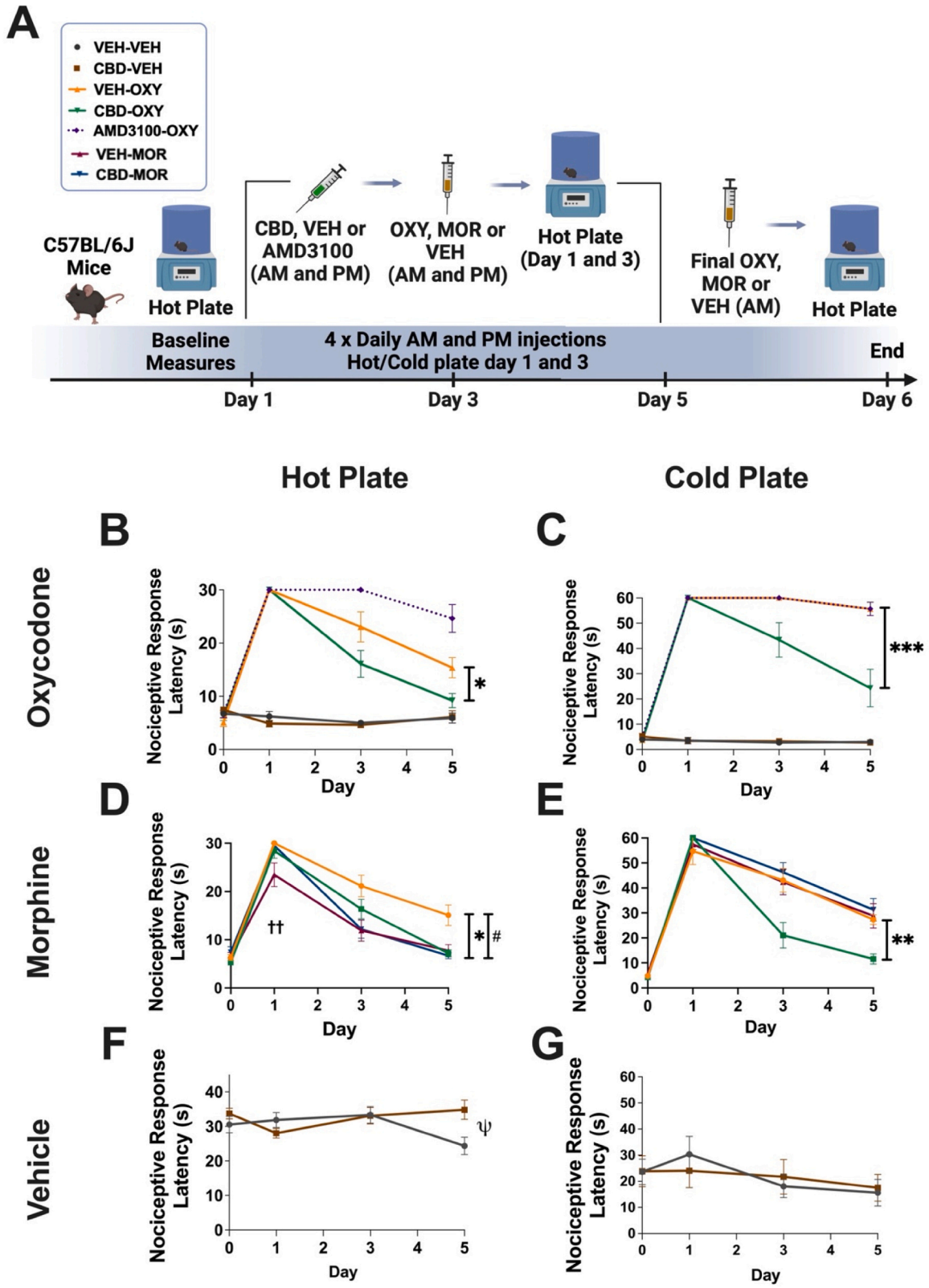

Fig. 3. Chronic co-administration of CBD accelerated the development of tolerance to the analgesic effects of oxycodone, but not morphine. A: Timeline of events and key for panels B-G. B: CBD accelerated the development of tolerance to the analgesic effects of oxycodone on the hot plate and AMD3100 prevented the development oxycodone tolerance. C: On the cold plate test, mice treated with vehicle or AMD3100 did not develop tolerance to oxycodone analgesia, whereas mice treated with CBD developed tolerance D-E: CBD accelerated the development of tolerance to the analgesic effects of oxycodone, but not morphine on the hot and cold plate. F-G: There was no effect of day or CBD treatment on hot or cold plate response latency. *$p < .05$, **$p < .01$, **$p < .01$ for the trend analysis interaction for VEH-OXY vs CBD-OXY.$^{\#}p < .01$ for the trend analysis interaction for VEH-MOR vs CBD-MOR. $^{\dagger\dagger}p < 05$, VEH-MOR vs; VEH-OXY and CBD-MOR. $p < .05$, VEH vs CBD. VEH = vehicle, OXY = oxycodone, MOR = morphine, CBD = cannabidiol. Error bars represent SEM, with n = 10 per condition.

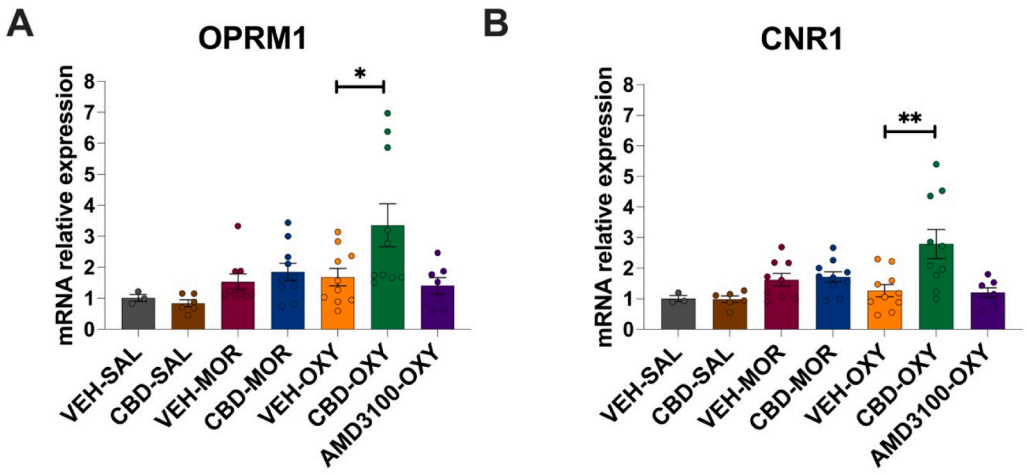

Fig. 4.. CBD co-administration alongside oxycodone, but not morphine or saline, increased Oprm1 and Cnr1 mRNA expression in the PAG of mice. A: Co-administration of CBD with oxycodone, but not morphine or saline, increased *Oprm1* mRNA expression in the PAG. B: Co-administration of CBD with oxycodone, but not morphine, increased *Cnr1* mRNA expression in the PAG. *p < .05 and **p < .01. Error bars represent SEM.

4. Discussion

To the best of our knowledge, this is the first study to demonstrate inhibition of oxycodone metabolism by CBD. Acute CBD delayed oxycodone metabolism into noroxycodone, oxymorphone and noroxymorphone, resulting in a three-fold increase in total oxycodone exposure and ~50% increase in peak oxycodone concentration, which extended the analgesic efficacy of oxycodone following a single co-administration of oxycodone and CBD. This is consistent with prior studies showing that orally administered CBD enhances the analgesic effects of orally administered oxycodone in rats [7] and that CBD may exert opioid-sparing effects [2,35]. In contrast, sub-chronic co-administration of CBD and oxycodone accelerated the development of tolerance to oxycodone analgesia, but not morphine. As expected, administration of the positive control CXCR4 antagonist, AMD3100, prevented the development of tolerance to the analgesic effects of oxycodone, consistent with previous studies [36–38], demonstrating the model we used is sensitive to detect both positive and negative effects on opioid tolerance. CBD increased *Oprm1* and *Cnr1* mRNA expression in the PAG in oxycodone, but not morphine treated mice. Given morphine is not metabolized by the CYP2D6 and CYP3A4 enzymes involved in oxycodone metabolism, CBD inhibits these enzymes [12,14–16], and inhibition of both these enzymes in humans substantially increases exposure to oral oxycodone [21], one possible explanation is a DDI between CBD and oxycodone, arising from inhibition of CYP2D6- and CYP3A4-mediated oxycodone metabolism. This is consistent with evidence that inhibition of these enzymes in humans substantially increases exposure to oral oxycodone [23]. The apparent DDI between sub-chronic CBD and oxycodone may result from increased oxycodone exposure, resulting in elongation of oxycodone effects acutely, causing changes in receptor mRNA expression and more rapid development of tolerance following repeated co-administration.

To ensure the relevance of our findings to humans, we used doses of CBD and oxycodone that are comparable to those used in clinical trials and practice. CBD was administered IP at a dose of 100 mg/kg, which approximates doses used in clinical trials in rare drug-resistant epilepsies (oral 20 mg/kg) after allometric scaling and accounting for the low oral bioavailability of CBD [13]. The doses of oxycodone used (5 – 10 mg/kg IP), equate to a human equivalent dose of 0.407–0.813 mg/kg, or 25–50 mg in a 60 kg human, based on allometric scaling [39]. Extended-release oxycodone tablets range from 10 mg – 160 mg (Package Insert; [40]). Further, plasma exposure levels observed in the current study (529.6 ng h/ml) are consistent with exposure achieved following the administration of 40 mg controlled release oxycodone tablets (423.1 ng hr/ml; 40). Therefore, the oxycodone doses and exposures achieved are clinically relevant, whereas the CBD dose represents a high-exposure scenario aligned with prescription doses in epilepsy, rather than low-dose nutraceutical use. This design allowed a proof-of-concept test of CBD-oxycodone interactions at an exposure with established human safety, but it limits direct extrapolation to lower doses commonly used in the community. Future work should include multiple CBD doses (e.g. 1–100 mg/kg) with concurrent measurement of CBD and oxycodone plasma concentrations to define the exposure-response relationship.

Given CBD increased clinically relevant plasma levels of oxycodone, it is possible that CBD may also increase the risk of adverse effects associated with oxycodone. These could include the onset of side effects usually associated with higher doses of oxycodone, such as respiratory depression, at lower doses. A future study could examine whether CBD increases the respiratory depressant effects of oxycodone in mice across a range of CBD and oxycodone doses. Further, increased oxycodone exposure could raise concerns about effects on abuse liability. However, some studies demonstrate that CBD reduces the abuse liability of oxycodone by decreasing self-administration in rats [41,42] and others demonstrate neutral effects on abuse liability [43]. One possibility is that CBD increases plasma and brain concentrations of oxycodone, allowing for lower dosing schedules while maintaining brain exposure. Another potential consequence of increased oxycodone exposure relates to the development of opioid tolerance. If CBD alters oxycodone pharmacokinetics, repeated co-administration could influence the rate at which tolerance develops and, consequently, the need for dose escalation to maintain analgesic efficacy. In clinical settings, greater opioid dose escalation is associated with increased risk of opioid dependence, opioid use disorder, and overdose, which may be relevant for chronic pain patients using CBD alongside prescribed opioids.

MORs in the PAG are critically involved in opioid analgesia [44]. Most opioids have been shown to downregulate MOR expression [45] and downregulation of MOR expression, MOR desensitisation, as well as other changes in MOR receptor function, play a role in the development of tolerance to opioids [46,47]. As such, the increased MOR mRNA expression in the PAG following CBD-oxycodone co-administration observed in the present study may be a compensatory response to receptor internalisation or recycling, or some other functional decrease in MOR activity, resulting from chronic oxycodone exposure. Consistent with this interpretation, high quantities of oxycodone self-administration have been shown to cause a downregulation of striatal MOR protein accompanied by an upregulation in *Oprm1* mRNA expression [48]. Our findings suggest that a similar phenomenon may

exist in the PAG, which could play an important role in the development of tolerance to oxycodone analgesia and the acceleration of the development of tolerance when CBD is co-administered with oxycodone. However, as this interpretation is based on mRNA data alone, future studies should confirm these changes at the protein level and assess whether alterations in receptor binding or signalling accompany the observed transcriptional effects.

CBD co-administration with oxycodone also increased *Cnr1* mRNA levels in the PAG. The opioid and cannabinoid receptor systems have significant overlap: they are co-localized on the same neurons in the PAG, form heterodimers, and share intracellular signalling mechanisms [23,49,50]. Further, opioid administration triggers the release of endocannabinoids and cannabinoids trigger the release of opioid peptides [51,52]. Consistent with our findings, repeated MOR agonism has been shown to increase *Cnr1* mRNA, protein, and density in multiple brain regions in rats [53–56]. Since CBD only impacted *Oprm1* and *Cnr1* mRNA expression when co-administered with oxycodone, and not when co-administered with morphine or administered alone, this further supports the pharmacokinetic, behavioural and neural interactions reported between CBD and oxycodone being mediated by CYP3A4 and CYP2D6 metabolism. While the absence of significant changes in the morphine group supports the specificity of this interaction, broader gene expression profiling including other opioid- and cannabinoid-related genes would provide deeper mechanistic insight and should be pursued in future studies.

The present findings are not without limitations and emphasise multiple avenues for future investigation to elucidate the mechanisms underlying the CBD-oxycodone interaction and its broader implications. Although we hypothesize that the CBD–oxycodone interaction is mediated through inhibition of CYP2D6 and CYP3A4, this mechanism remains speculative in the absence of direct enzyme activity data. Future in vitro or ex vivo studies should evaluate CBD's effects on CYP2D6 and CYP3A4 activity in the mouse model and include established CYP inhibitors as controls to explore whether the observed pharmacokinetic interactions are enzyme mediated. Further, it should be noted that oxycodone, morphine and CBD can influence locomotor activity, which may confound pain response latencies [57–59]. While locomotion was not assessed in the present study, previous work from our group indicates that CBD does not affect locomotor activity at the dose used, in the context of opioid withdrawal [33], which is consistent with what's observed in the literature [60]. It is therefore unlikely that the observed effects were driven by CBD-induced alterations in motor activity, although potential opioid-related effects on locomotion cannot be excluded. Future work should incorporate locomotor assays (e.g. open field) alongside nociceptive tests to dissociate analgesic from motor effects.

Additionally, in the morphine-treated group, tolerance appears to develop rapidly, with response latencies returning to near baseline levels by day 3, therefore it is possible that a floor effect that may obscure the detection of accelerated tolerance. Future research should assess tolerance more frequently - potentially multiple times daily from day 1 to day 4 - given the rapid onset of tolerance observed. Further, testing with a lower morphine dose or using a lower temperature on the hot plate test with a higher maximum latency could improve sensitivity. Future research might also explore the impact of CBD on other pain modalities, such as mechanical pain assessed via the von Frey test and investigate its effect on opioid analgesia within the context of chronic pain, specifically using a spared nerve injury model. Finally, the study exclusively used male C57BL/6 J mice, which limits the generalizability of the findings. Sex differences in opioid metabolism and CYP enzyme activity, pain sensitivity, and cannabinoid effects are well documented [61–63]. Future studies should include both sexes to determine whether the observed CBD–opioid interactions are sex-dependent and to enhance the translational relevance of these findings.

Taken together, our study suggests that there may be a significant DDI between oxycodone and CBD. This DDI between oxycodone and CBD may have implications for pain management and for the safety and tolerability of these two substances when used in combination. Given CBD is widely available [64], oxycodone is a commonly prescribed opioid [20], and CBD and opioids are increasingly being used in combination [1], there may be need for caution when using these drugs in combination. Future studies are required to more comprehensively examine the interaction between CBD and oxycodone in preclinical models and in humans.

Author contributions

Michael T. Bowen and Rhianne L. Scicluna conceived of and designed the studies, analysed the data, prepared the full draft of the manuscript, and editing of subsequent iterations. Rhianne L. Scicluna lead the execution of behavioural experiments, tissue collection, and significantly contributed to data generation across all figures, in addition to drafting the manuscript. Michael T. Bowen provided research funding and facilities for the research project. Connie J. Badolato, Bianca B. Wilson and Nicholas A. Everett assisted with the behavioural experiments, while Damien C. Boorman and Everett provided additional advisory insights. Maia G. Etchart and Rebecca S. Gordon handled tissue processing for pharmacokinetic analysis, with Richard C. Kevin leading this segment and contributing to the manuscript methodology and results. James W. M. Kang and Kevin A. Keay provided expertise and conducted the quantitative PCR (qPCR) analyses, contributing to the methodology and results section. Lyndsey L. Anderson and Jonathon C. Arnold, and Kevin A. Keay offered senior advisory roles in the design and execution of the experiments.

CRediT authorship contribution statement

Lyndsey L. Anderson: Writing – review & editing, Methodology, Conceptualization. **Jonathon C. Arnold:** Supervision, Methodology, Conceptualization. **Rhianne L. Scicluna:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kevin A. Keay:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **James W.M. Kang:** Methodology, Formal analysis, Data curation. **Michael T. Bowen:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Rebecca S. Gordon:** Methodology. **Bianca B. Wilson:** Investigation. **Damien C. Boorman:** Conceptualization. **Nicholas A. Everett:** Writing – review & editing, Supervision, Methodology, Data curation. **Connie J. Badolato:** Project administration, Data curation. **Maia G. Etchart:** Methodology, Investigation. **Richard C. Kevin:** Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

In accordance with *Pharmacological Research*'s requirements for transparency and to address potential conflicts of interest, we hereby disclose the external affiliations and roles of certain authors alongside their financial support, while affirming the absence of any influence on the research presented in this manuscript. In addition to his academic role, Professor Michael Bowen is co-founder and Chief Scientific Officer of Kinoxis Therapeutics Pty Ltd, an Australian-based company developing novel small molecule treatments for brain disorders, including the treatment of opioid withdrawal and Professor Michael Bowen receives research funding from Kinoxis Therapeutics. Dr Nicholas Everett is Head of Behavioural Neuroscience at Kinoxis Therapeutics and conducts research to develop treatments for opioid withdrawal. Professor Jonathon Arnold is Deputy Academic Director of the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically-funded research program at the University of Sydney. Prof Arnold has served as an expert witness in various medicolegal cases involving cannabis. He has received consulting fees from the Medicinal Cannabis Industry Australia

(MCIA), Haleon and Creo Ingredients Inc. Prof Arnold reports grants from the Australian National Health and Medical Research Council (NHMRC) and grants from Lambert Initiative for Cannabinoid Therapeutics. He reports the patents WO2019227167 and WO2019071302 issued. None of this work by any of the authors involves the research presented in this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2026.119277.

Data availability

Data will be made available on request.

References

[1] T. Rosic, et al., The association between cannabis use and outcome in pharmacological treatment for opioid use disorder, Harm Reduct. J. 18 (2021) 24.
[2] C.H.A. Jesus, et al., Cannabidiol enhances the antinociceptive effects of morphine and attenuates opioid-induced tolerance in the chronic constriction injury model, Behav. Brain Res 435 (2022) 114076.
[3] S. Nielsen, et al., Opioid-sparing effect of cannabinoids: a systematic review and meta-analysis, Neuropsychopharmacology 42 (2017) 1752–1765.
[4] A. Noori, et al., Opioid-sparing effects of medical cannabis or cannabinoids for chronic pain: a systematic review and meta-analysis of randomised and observational studies, BMJ Open 11 (2021) e047717.
[5] M. Rodríguez-Muñoz, Y. Onetti, E. Cortés-Montero, J. Garzón, P. Sánchez-Blázquez, Cannabidiol enhances morphine antinociception, diminishes NMDA-mediated seizures and reduces stroke damage via the sigma 1 receptor, Mol. Brain 11 (2018) 51.
[6] H. Neelakantan, et al., Distinct interactions of cannabidiol and morphine in three nociceptive behavioral models in mice, Behav. Pharm. 26 (2015) 304–314.
[7] A.C. Brice-Tutt, et al., Cannabidiol interactions with oxycodone analgesia in an operant orofacial cutaneous thermal pain assay following oral administration in rats, Pharmacol. Biochem. Behav. 250 (2025) 173968.
[8] J.M. Schilling, et al., Cannabidiol as a treatment for chronic pain: a survey of patients' perspectives and attitudes, J. Pain. Res 14 (2021) 1241–1250.
[9] A. Capano, R. Weaver, E. Burkman, Evaluation of the effects of CBD hemp extract on opioid use and quality of life indicators in chronic pain patients: a prospective cohort study, Post. Med 132 (2020) 56–61.
[10] C.L. Bergeria, et al., A within-subject, double-blind, placebo-controlled randomized evaluation of the combined effects of cannabidiol and hydromorphone in a human laboratory pain model, Pain 166 (2025) e175–e184.
[11] T. Lynch, A. Price, The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects, Am. Fam. Physician 76 (2007) 391–396.
[12] P.T. Doohan, L.D. Oldfield, J.C. Arnold, L.L. Anderson, Cannabinoid interactions with cytochrome P450 drug metabolism: a full-spectrum characterization, Aaps J. 23 (2021) 91.
[13] L.L. Anderson, et al., Coadministered cannabidiol and clobazam: preclinical evidence for both pharmacodynamic and pharmacokinetic interactions, Epilepsia 60 (2019) 2224–2234.
[14] P. Balachandran, M. Elsohly, K.P. Hill, Cannabidiol interactions with medications, illicit substances, and alcohol: a comprehensive review, J. Gen. Intern. Med. JGIM 36 (2021) 2074–2084.
[15] S. Bansal, N. Maharao, M.F. Paine, J.D. Unadkat, Predicting the potential for cannabinoids to precipitate pharmacokinetic drug interactions via reversible inhibition or inactivation of major cytochromes P450, Drug Metab. Dispos. Biol. fate Chem. 48 (2020) 1008–1017.
[16] S. Nasrin, et al., Inhibition of UDP-glucuronosyltransferase enzymes by major cannabinoids and their metabolites, Drug Metab. Dispos. 49 (2021) 1081–1089.
[17] R. Huddart, M. Clarke, R.B. Altman, T.E. Klein, PharmGKB summary: oxycodone pathway, pharmacokinetics, Pharm. Genom. 28 (2018) 230–237.
[18] M. Kinnunen, P. Piirainen, H. Kokki, P. Lammi, M. Kokki, Updated clinical pharmacokinetics and pharmacodynamics of oxycodone, Clin. Pharmacokinet. 58 (2019) 705–725.
[19] B. Lalovic, et al., Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites, Clin. Pharmacol. Ther. 79 (2006) 461–479.
[20] M.V. Kiang, K. Humphreys, M.R. Cullen, S. Basu, Opioid prescribing patterns among medical providers in the United States, 2003-17: retrospective, observational study, l6968-l6968, BMJ (Online) 368 (2020). l6968-l6968.
[21] J. Grönlund, et al., Exposure to oral oxycodone is increased by concomitant inhibition of CYP2D6 and 3A4 pathways, but not by inhibition of CYP2D6 alone, Br. J. Clin. Pharm. 70 (2010) 78–87.
[22] H. Zhang, et al., The contribution of periaqueductal gray in the regulation of physiological and pathological behaviors, Front Neurosci. 18 (2024) 1380171.
[23] A.R. Wilson-Poe, M.M. Morgan, S.A. Aicher, D.M. Hegarty, Distribution of CB1 cannabinoid receptors and their relationship with mu-opioid receptors in the rat periaqueductal gray, Neuroscience 213 (2012) 191–200.
[24] H.S. Smith, Opioid metabolism, Mayo Clin. Proc. 84 (2009) 613–624.
[25] B. Lalovic, et al., Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites, Clin. Pharm. Ther. 79 (2006) 461–479.
[26] B. Lalovic, B. Phillips, L.L. Risler, W. Howald, D.D. Shen, Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes, Drug Metab. Dispos. 32 (2004) 447–454.
[27] S. Nielsen, L. Degenhardt, B. Hoban, N. Gisev, Comparing opioids: A guide to estimating oral morphine equivalents (OME) in research. Technical Report No. 329 Sydney: National Drug and Alcohol Research Centre, University of NSW, 2014.
[28] S. Deiana, et al., Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ 9 -tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour, Psychopharmacology 219 (2012) 859–873.
[29] R.L. Scicluna, et al., Cannabidiol reduced the severity of gastrointestinal symptoms of opioid withdrawal in male and female mice, Cannabis Cannabinoid Res. (2022), https://doi.org/10.1089/can.2022.0036.
[30] S. Deiana, et al., Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ⁹-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour, Psychopharmacol. (Berl.) 219 (2012) 859–873.
[31] C.P. Lin, et al., CXCL12/CXCR4 signaling contributes to the pathogenesis of opioid tolerance: a translational study, Anesth. Analg. 124 (2017) 972–979.
[32] S. Inan, et al., Coadministration of chemokine receptor antagonists with morphine potentiates morphine's analgesic effect on incisional pain in rats, J. Pharm. Exp. Ther. 367 (2018) 433–441.
[33] N.M. Wilson, H. Jung, M.S. Ripsch, R.J. Miller, F.A. White, CXCR4 signaling mediates morphine-induced tactile hyperalgesia, Brain Behav. Immun. 25 (2011) 565–573.
[34] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res 29 (2001) e45.
[35] B. Le Foll, Opioid-sparing effects of cannabinoids: myth or reality? Prog. Neuro-Psychopharmacol. Biol. Psychiatry 106 (2021) 110065.
[36] C.-P. Lin, et al., CXCL12/CXCR4 signaling contributes to the pathogenesis of opioid tolerance: a translational study, Anesth. Analg. 124 (2017) 972–979.
[37] X. Chen, et al., The effect of gp120 on morphine's antinociceptive and neurophysiological actions, Brain Behav. Immun. 25 (2011) 1434–1443.
[38] S. Inan et al., Chemokine receptor antagonists enhance the antinociceptive activity of oxycodone and meperidine on incisional pain in rats (2019), 10.1016/j.bja.2019.01.021.
[39] A.B. Nair, S. Jacob, A simple practice guide for dose conversion between animals and human, J. Basic Clin. Pharm. 7 (2016) 27–31.
[40] FDA (Oxycodone HCL Controlled-Release Tablets FDA Package Insert in ⟨https://www.fda.gov/media/131026/download⟩.
[41] A.W. Bruijnzeel, et al., Cannabidiol reduces oxycodone self-administration while preserving its analgesic efficacy in a rat model of neuropathic pain, 2025.2004.2021.649778, bioRxiv (2025), https://doi.org/10.1101/2025.04.21.649778, 2025.2004.2021.649778.
[42] J.D. Nguyen, Y. Grant, C. Yang, A. Gutierrez, M.A. Taffe, Oxycodone self-administration in female rats is enhanced by Δ(9)-tetrahydrocannabinol, but not by cannabidiol, in a progressive ratio procedure, bioRxiv (2023), https://doi.org/10.1101/2023.10.26.564282.
[43] H.M. Harris, W. Gul, M.A. ElSohly, K.J. Sufka, Differential effects of cannabidiol and a novel cannabidiol analog on oxycodone place preference and analgesia in mice: an opioid abuse deterrent with analgesic properties, Cannabis Cannabinoid Res 7 (2022) 804–813.
[44] H. Pathan, J. Williams, Basic opioid pharmacology: an update, Br. J. Pain. 6 (2012) 11–16.
[45] L. Li, J. Chen, Y.Q. Li, The downregulation of opioid receptors and neuropathic pain, Int J. Mol. Sci. 24 (2023).
[46] J. Zhou, et al., Molecular mechanisms of opioid tolerance: from opioid receptors to inflammatory mediators (Review), Exp. Ther. Med 22 (2021) 1004.
[47] S.L. Borgland, Acute opioid receptor desensitization and tolerance: is there a link? Clin. Exp. Pharm. Physiol. 28 (2001) 147–154.
[48] C.A. Blackwood, et al., Molecular adaptations in the rat dorsal striatum and hippocampus following abstinence-induced incubation of drug seeking after escalated oxycodone self-administration, Mol. Neurobiol. 56 (2019) 3603–3615.
[49] K. Befort, Interactions of the opioid and cannabinoid systems in reward: insights from knockout studies, Front Pharm. 6 (2015) 6.
[50] M.J. Christie, Opioid and cannabinoid receptors: friends with benefits or just close friends? Br. J. Pharm. 148 (2006) 385–386.
[51] D. Parolaro, et al., Cellular mechanisms underlying the interaction between cannabinoid and opioid system, Curr. Drug Targets 11 (2010) 393–405.
[52] A. Mohammadkhani, S.L. Borgland, Cellular and behavioral basis of cannabinioid and opioid interactions: implications for opioid dependence and withdrawal, J. Neurosci. Res. 100 (2022) 278–296.
[53] L. Jin, et al., Expression and localization of cannabinoid receptor 1 in rats' brain treated with acute and repeated morphine, Acta Neurobiol. Exp. 74 (2014) 288–297.
[54] T. Rubino, L. Tizzoni, D. Viganò, P. Massi, D. Parolaro, Modulation of rat brain cannabinoid receptors after chronic morphine treatment, Neuroreport 8 (1997) 3219–3223.

[55] L. Fattore, et al., Bidirectional regulation of mu-opioid and CB1-cannabinoid receptor in rats self-administering heroin or WIN 55,212-2, Eur. J. Neurosci. 25 (2007) 2191–2200.
[56] S. González, et al., Behavioral and molecular changes elicited by acute administration of SR141716 to Delta9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence, Drug Alcohol Depend. 74 (2004) 159–170.
[57] V. Iyer, T.J. Woodward, R. Pacheco, A.G. Hohmann, A limited access oral oxycodone paradigm produces physical dependence and mesocorticolimbic region-dependent increases in DeltaFosB expression without preference, Neuropharmacology 205 (2022) 108925.
[58] Y.-l Liu, et al., Effects of l-tetrahydropalmatine on locomotor sensitization to oxycodone in mice, Acta Pharmacol. Sin. 26 (2005) 533–538.
[59] K. Niikura, A. Ho, M.J. Kreek, Y. Zhang, Oxycodone-induced conditioned place preference and sensitization of locomotor activity in adolescent and adult mice, Pharm. Biochem Behav. 110 (2013) 112–116.
[60] F. Calapai, et al., Effects of cannabidiol on locomotor activity, Life 12 (2022).
[61] N. Arguelles, J. Richards, A.A. El-Sherbeni, S. Miksys, R.F. Tyndale, Sex, estrous cycle, and hormone regulation of CYP2D in the brain alters oxycodone metabolism and analgesia, Biochem Pharm. 198 (2022) 114949.
[62] D.J. Waxman, M.G. Holloway, Sex differences in the expression of hepatic drug metabolizing enzymes, Mol. Pharm. 76 (2009) 215–228.
[63] G.S. Lopes, et al., Sex differences in associations between CYP2D6 phenotypes and response to opioid analgesics, Pharmgenomics Pers. Med. 13 (2020) 71–79.
[64] G.V. Reserach (2022) Cannabidiol Market Size, Share & Trends Analysis Report By Source Type (Hemp, Marijuana), By Sales Type (B2B, B2C), By End-use (Medical, Personal Use), By Region, And Segment Forecasts, 2023 - 2030.