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Impact of *OPRM1* (Mu-opioid Receptor Gene) A112G Polymorphism on Dual Oxycodone and Cocaine Self-administration Behavior in a Mouse Model

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Abstract—The use of mu-opioid receptor (MOP-r) agonists such as oxycodone together with cocaine is prevalent, and deaths attributed to using these combinations have increased. *Rationale:* It is unknown if functional single nucleotide polymorphisms (SNPs), such as the OPRM1 (MOP-r gene) SNP A118G, can predispose individuals to more dual opioid and psychostimulant intake. The dual self-administration (SA) of MOP-r agonists and cocaine has not been thoroughly examined, especially with regard to neurobiological changes. *Objectives:* We examined oxycodone SA and subsequent dual oxycodone and cocaine SA in male and female A112G (A/G and G/G, heterozygote and homozygote, respectively) mice, models of human A118G carriers, versus wild-type (A/A) mice. *Methods:* Adult male and female A/G, G/G and A/A mice self-administered oxycodone (0.25 mg/kg/infusion, 4hr/session, FR 1.) for 10 consecutive days (sessions 1–10). Mice then self-administered cocaine (2 hr) following oxycodone SA (4 hr, as above) in each session for a further 10 consecutive days (sessions 11–20). Message RNA transcripts of 24 reward-related genes were examined in the dorsal striatum. *Results:* Male and female A/G and G/G mice had greater oxycodone SA than A/A mice did in the initial 10 days and in the last 10 sessions. Further, A/G and G/G mice showed greater cocaine intake than A/A mice. Dorsal striatal mRNA levels of *Pdyn*, *Fkbp5*, *Oprk1,* and *Oprm1* were altered following oxycodone and cocaine SA. *Conclusions:* These studies demonstrated that this functional genetic variation in *Oprm1* affected dual opioid and cocaine SA and altered specific gene expression in the striatum.

Key words: sequential use of oxycodone and cocaine, oxycodone, cocaine, polydrug use, A112G mice, *Pdyn, Fkbp5, Oprk1 and Oprm1* mRNA expression.

INTRODUCTION

Opioid use disorder (OUD) is a chronic relapsing disease with major biomedical and public health impacts. Concurrent use of opioids (mu-opioid receptor (MOP-r) agonists) with psychostimulants such as cocaine is prevalent (e.g., Goodwin et al., 2021; Leri et al., 2004; Roy et al., 2013). Dual exposure to MOP-r agonists and psychostimulants contributes to recent increases in overdose deaths (CDC, 2019c; CDC 2020b). Data from the Centers for Disease Control and Prevention show that the rate of opioid overdose deaths that also involved cocaine increased on average by 27% per year, from 2012 through 2018 (CDC 2020a). Dual MOP-r agonist and cocaine exposure can result in more complex or severe neurobiological changes, and these compounds can increase brain dopamine dialysate levels (e.g., Di Chiara and Imperato, 1988; Zhang et al., 2011, 2015b). In addition, exposure to MOP-r agonists causes changes in gene expression including opioid receptor or neuropeptide genes and genes related to the HPA axis (such as *Pomc, Fkbp*) (e.g., Hassan et al., 2010; Zhang et al., 2018), in brain regions including the dorsal striatum, an area associated with the transition from short-term to long-term drug self-exposure and compulsive-like patterns of drug taking (e.g., Everitt and Robbins, 2016; Porrino et al., 2004). Similarly, cocaine self-administration caused

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Abbreviations: HPA axis, Hypothalamic-pituitary-adrenal axis; IVSA, intravenous self-administration; MOP-r, Mu-opioid receptor; SA, Self administration; *OPRM1*, Human mu opioid receptor gene; OUD, Opioid use disorder; SNP, Single nucleotide polymorphism.

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alterations in opioid genes, such as *Pdyn* and *Oprm1* (e.g., (Daunais et al., 1993); (Hurd et al., 1992); (Sun et al., 2020) in the dorsal striatum.

Functional genetic polymorphisms have emerged as important factors in inter-subject variability in responsiveness to drugs of abuse in humans (Levran and Kreek, 2021). A118G is the most common single nucleotide polymorphism (SNP) in the human MOP-r (*OPRM1*) gene, found in approximately 15% of Caucasian and 40–60% of Asian populations (Bond et al., 1998; Schwantes-An et al., 2016). When studied in genetically homogeneous groups, carriers of A118G SNP (i.e., A/G or G/G) were found to be more vulnerable to heroin addiction than prototype homozygotes (A/A), in some, but not all, studies, potentially due to differences in genetic background, gene-environment interactions or phenotyping approach (e.g., Bart et al., 2004; Hasegawa et al., 2014; Kumar et al., 2011; Tan et al., 2003; Zhou et al., 2020); see also larger studies (Schwantes-An et al., 2016; Levran and Kreek, 2021; Gaddis et al., 2022).

Mice carrying an A112G (G/G and A/G) substitution are mouse models of the human A118G SNP. In these mice, a point mutation analogous to the human OPRM1 A118G SNP was created at the analogous 112 base position in the mu-opioid receptor gene (Mague et al., 2009). Dual mu-opioid agonist and cocaine self-administration have never been studied in this genetic model. There is also very limited information on how such dual self-administration overall affects the transcription of reward-related genes in the dorsal striatum.

Some studies in rodents and non-human primates have modeled ‘‘speedball” self-administration (i.e., a mixture of MOP-r agonist and cocaine injected concomitantly) (e.g., Pattison et al., 2012; Mello et al., 1995). However, ‘‘speedball” is only one of the dual mu-opioid receptor agonist/cocaine usage patterns. For example, clinical studies found that heroin was used on a greater number of days than cocaine, among dual users (Leri et al., 2003; Leri et al., 2004). Also, heroin use tended to be quite regular across days, whereas cocaine use was more sporadic. Furthermore, about 70% of the heroin users also injected cocaine, but not necessarily in a concomitant manner (Leri et al., 2004). Other studies also confirm that concomitant use (‘‘speedball”) is not the only pattern of opioid/cocaine polydrug use and that sequential patterns also occur (e.g., with alternating use of heroin and cocaine) (e.g., Karamouzian et al., 2022; Roy et al., 2013).

This study therefore modeled sequential injection of oxycodone and cocaine, in two consecutive periods within daily sessions, rather than as a concomitant injection. The current study will therefore explore for the first time, how this major functional SNP can affect vulnerability to dual oxycodone and cocaine self-administration; and how chronic self-administration of oxycodone and cocaine impacts the expression of major reward-related genes in the dorsal striatum of A112G mice and their wild type littermates.

EXPERIMENTAL PROCEDURES

Mice

In-house mating of A112G heterozygous (A/G) mice (Mague et al., 2009) produced F1 offspring of each genotype (A/A, A/G and G/G of both sexes), which were used in the current experiment. Mice (11–12 week-old) were housed in groups of four and had free access to food and water in a light- (12:12 hour light/dark cycle, light on at 7:00 pm and off at 7:00 am) and temperature- (25 °C) controlled room. Animal care and experimental procedures were carried out based on the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources Commission on Life Sciences, 2011). The protocols of experiment were approved by the Institutional Animal Care and Use Committee of the Rockefeller University.

Self-Administration (SA) procedure

Catheter implantation. Mice were injected with a combination of xylazine (8.0 mg/kg i.p.) and ketamine (80 mg/kg i.p.). Incisions were made in the midscapular region and anteromedial to the foreleg following shaving and applying a 70% alcohol and iodine preparatory solution. A catheter 6 cm in length (ID: 0.31 mm, OD: 0.64 mm) (Helix Medical, Inc. CA) was passed from the dorsal to the ventral incision subcutaneously. The catheter was then guided into the right jugular vein by a 22-gauge needle and was anchored to the surrounding connective tissue with surgical silk. Subsequently, the catheter was flushed with physiological saline to avoid clotting and capped with a stopper. Antibiotic ointment was applied to the catheter exit sutures to prevent infection. Mice were group-housed up to 4 per cage after the surgery and were given 7 days of recovery before the self-administration procedure (Zhang et al., 2014; 2015b).

Intravenous self-administration chambers

The self-administration chambers (ENV-307 W; 21.6 cm × 17.8 cm × 12.7 cm; Med Associates, St. Albans, VT), which were constructed of 5.6 mm polycarbonate in the front, back and top, were located inside a larger box (Med. Associates). Each chamber contained a wall with two small holes (0.9 cm in diameter, 4.2 cm apart, and 1.5 cm from the floor of the chamber). The hole at the back was defined as active; the one in the front was inactive (i.e., a nose-poke therein was counted with no consequence). When the photocell in the active hole was triggered by a nose poke from a mouse, the infusion pump (Med Associates) delivered an infusion of 20 μl/3 sec from a 5 ml syringe. The syringe was connected to the mouse by a swivel with Tygon tubing. Both the infusion pump and syringe were located outside the chamber. There was a cue light above the active hole, illuminating during infusion. Each injection was followed by a 20-second ‘‘time-out” period during

which nose-pokes responses were recorded with no programmed consequence. All nose-pokes responses at the inactive hole were also recorded. The experiments were carried out during the dark phase of the cycle (between 8:00 a.m. and 2:00 p.m.).

Oxycodone self-administration and cocaine self-administration

Initially, a 4-hr-hour oxycodone self-administration session was conducted once a day, for 10 consecutive days. Mice were first weighed, and sterile heparinized saline (0.01 ml of 30 IU/ ml solution) was flushed through the catheter to maintain patency daily. During self-administration sessions, mice were placed into the self-administration chambers and connected to the syringes by a swivel with Tygon tubing. A nose poke through the active hole resulted in an infusion of oxycodone (0.25 mg/kg/infusion; Sigma, St. Louis, MO), under a FR1 schedule. After these initial 10 daily consecutive oxycodone SA sessions (4 h/day), there were another 10 daily sessions (i.e., sessions 11–20) with an identical 4 h oxycodone session, followed by 2 h of cocaine SA after a 5 min break (0.5 mg/kg/infusion; Sigma, St.Louis, MO), under a FR1 schedule. During sessions on days 11–20 (i.e., oxycodone and cocaine period), mice that had self-administered oxycodone during the initial 10-day period self-administered the same unit dose of oxycodone, and the experiments began at the same time each day. For the timeline of the IVSA sessions, see Fig. 1. We selected these unit doses of oxycodone and cocaine based on prior studies in the IVSA assay (e.g., Zhang et al., 2014, 2009, 2015a, 2017, 2015b, 2006). Drug infusions were controlled by a computer and Med Associates interface, taking into account changes in an animal's body weight.

We then analyzed data from mice that passed a catheter patency test, defined as loss of muscle tone within 5–10 sec after administration of 30 μl ketamine (5 mg/ml) (Fort Dodge, IA) at the end of the self-administration experiments.

RNA extraction

Mice were sacrificed 1 hour after the last self-administration session (session 20). Specifically, mice were sedated by brief exposure to CO_2 and subsequently euthanized by decapitation; brains were rapidly removed. For control, the same age-matched littermates that had stayed in home cages and were unexposed to either oxycodone or cocaine were sacrificed at the same time. The bilateral dorsal striatum from each mouse brain was dissected and homogenized in Qiazol (Qiagen, Valencia, CA) and frozen at −80 °C. Total RNA was isolated from homogenates of brain tissue using the miRNeasy kit (Qiagen, Valencia, CA). The quality and quantity of RNA from each sample were determined using the Agilent 2100 Bioanalyzer. The values of RNA integrity number (RIN) of all samples were greater than 8.

cDNA synthesis

cDNA was synthesized from each sample of the dorsal striatum using the first strand synthesis kit (Qiagen, Valencia, CA). Five hundred ng of RNA from each sample of the dorsal striatum was used for reverse transcription. cDNAs were diluted 1:10 for reverse transcription polymerase chain reaction (RT-PCR) analysis.

Custom RT2 Profiler™ PCR array

The custom RT2 Profiler™ PCR Array (AAPA3800-1: CLAM45539, Qiagen) used for the present study measures the expression of 24 genes related to reward, drugs of abuse, and stress responsivity (see Table 1). This array was based on prior studies in this laboratory and others, focusing on genes shown to be responsive to exposure to MOP-r agonists or cocaine, and genes involved in the hypothalamic–pituitary–adrenal (HPA) stress axis (e.g., Piechota et al., 2010; Zhang et al., 2018, 2014, 2012). The profiler array contains five house-keeping genes (glyceraldehyde 3-phosphate dehydrogenase, Gapdh; β-glucuronidase, Gusb; heat shock protein 90 alpha (cytosolic), class B member 1, Hsp90ab1; peptidylprolyl isomerase A, Ppia; TATA-binding protein, Tbp). Real-time PCR was carried out by the SYBR Green detection method.

Each real-time PCR reaction had a total volume of 10 μl and comprised cDNA diluted in 2 × SuperArray RT2 Real-Time™ SYBR Green PCR Master Mix (Qiagen) and water. The real-time PCR reactions were performed in a QuantStudio 6 Flex Detection System (Applied Biosystems, Foster City, CA) with the following program: 10 min at 95 °C (15 s at 95 °C and 1 min at 60 °C) × 40 cycles, 15 s at 95 °C, 15 s at 60 °C, and 15 s at 95 °C. The QuantStudio 6 Flex Detection System was also used to calculate the Ct value for each well. Any sample with a cycle threshold (Ct) greater than 35, was not included in the final data analysis. All data was normalized to the five reference "house-keeping" genes and reported as $2^{-\Delta Ct}$ where ΔCt was the cycle threshold of the mRNA of interest, minus the cycle threshold of the "house-keeping" genes.

Statistical analysis

Self-administration measured as the number of nose pokes in the active hole in each self-administration session during the initial 10 sessions (oxycodone SA, sessions 1–10), and the subsequent 10 sessions (oxycodone and cocaine SA, sessions 11–20) was assessed by a four-way analysis of variance (ANOVA), Genotype (A/A, A/G,

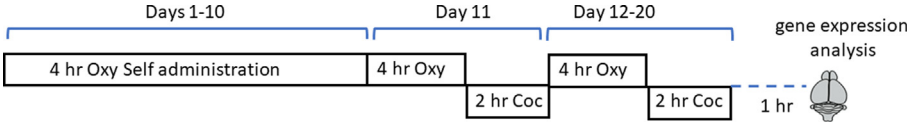

Fig. 1. Timeline of daily oxycodone and cocaine self-administration sessions.

Table 1. Custom RT^2 Profiler™ PCR Array genes and significant changes in dorsal striatal mRNA levels following oxycodone and cocaine (drug) SA versus control: Main effect of drug.

Gene	Protein	*P* value	*P*adj	Direction of Change Drug vs Control
Oprm1	Mu opioid receptor	0.00056	0.0085	↑
Oprk1	Kappa opioid receptor	0.00077	0.0085	↑
Pdyn	Preprodynorphin	0.000001	0.000012	↑
Fkbp5	FK506 binding protein 5	0.000001	0.000012	↑
Htr7	Serotonin receptor 7	0.035000	NS	↓
Oprd1	Delta opioid receptor		NS	
Pomc	Proopiomelanocortin		NS	
Penk	Preproenkephalin		NS	
Oprl1	Opioid-related nociceptin receptor		NS	
Pnoc	Prepronociceptin		NS	
Avpr1b	Arginine vasopressin receptor 1B		NS	
Avp	Arginine vasopressin		NS	
Crhr1	Corticotropin releasing hormone receptor 1		NS	
Crh	Corticotropin releasing hormone		NS	
Nr3c1	Glucocorticoid receptor		NS	
Nr3c2	Mineralocorticoid receptor		NS	
Mc2r	Melanocortin receptor 2		NS	
Cnr1	Cannabinoid receptor 1		NS	
Cnr2	Cannabinoid receptor 2		NS	
Galr1	Galanin receptor		NS	
Gal	Galanin		NS	
Htr2a	Serotonin receptor 2A		NS	
Oxtr	Oxytocin receptor		NS	
Oxt	Oxytocin		NS	
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase		NS	
Hsp90ab1	Heat shock protein 90 alpha (cytosolic), class B member 1		NS	
Tbp	TATA box binding protein		NS	
Gusb	Glucuronidase, beta		NS	
Ppia	Peptidylprolyl isomerase A (cyclophilin A)		NS	

G/G) × Sex (Male, Female) × Nose Poke (Active, Inactive) × Sessions (1–10 or 11–20).

In the gene expression study, the analysis focused on the effects of genotype, sex, and drugs on the expression of the 24 genes in the dorsal striatum. Normality was examined with the Shapiro-Wilk test and followed by a visual examination of sample data distribution. Normalized RT-PCR gene expression was analyzed by three-way ANOVA with the main effects of genotype, sex, and drug condition, as well as their interaction. The Benjamini-Hochberg method (false discovery rate set at 10%) was used to identify differences in gene expression remaining significant after correction for multiple testing (see Table 1). Genes showing significant effects are reported in Table 1.

RESULTS

Oxycodone self-administration behavior of male and female A/A, A/G and G/G mice across the initial 10 consecutive daily sessions (Days 1–10: Oxycodone only)

A four-way analysis of variance (ANOVA) was used to examine the nose-poke responses in the initial 10-day oxycodone self-administration behavior of male and female A/A, A/G and G/G mice: Genotype (A/A, A/G, G/G) × Sex (Male, Female) × Nose Poke (Active, Inactive) × Sessions (1–10). There was a significant Genotype × Nose Poke × Session interaction, F (18, 720) = 4.57, $p < 0.000001$. There was also a significant Genotype × Nose Poke interaction, F (2, 80) = 19.64, $p < 0.000001$, a significant Genotype × Sessions interaction, F (18, 720) = 2.40, $p < 0.001$ and a significant Nose Poke × Session interaction, F (9, 720) = 48.99, $p < 0.000001$. There was a significant main effect of Genotype, F (2, 80) = 14.01, $p < 0.00001$. Newman-Keuls *post hoc* tests showed that both A/G and G/G mice emitted more active nose pokes for oxycodone than A/A mice ($p < 0.05$ and $p < 0.0005$, respectively). In addition, G/G mice emitted more active nose pokes than A/G mice, $p < 0.005$. There was a significant main effect of Nose Poke, F (1, 80) = 267.60, $p < 0.000001$ and a significant main effect of Sessions, F (9, 720) = 30.66, $p < 0.000001$. No significant main effect of Sex was found, F < 1.0. See Fig. 2(A and B).

Oxycodone self-administration behavior of male and female A/A, A/G and G/G mice across 10 consecutive daily sessions with sequential cocaine SA (Days 11–20)

After the initial 10 days of oxycodone SA, mice underwent an identical 10-day self-exposure period to oxycodone, followed by 2 hours of cocaine self-administration. A four-way ANOVA was used to examine the nose-poke behavior at the active and inactive holes: Genotype (A/A, A/G, G/G) × Sex (Male, Female) × Nose Poke

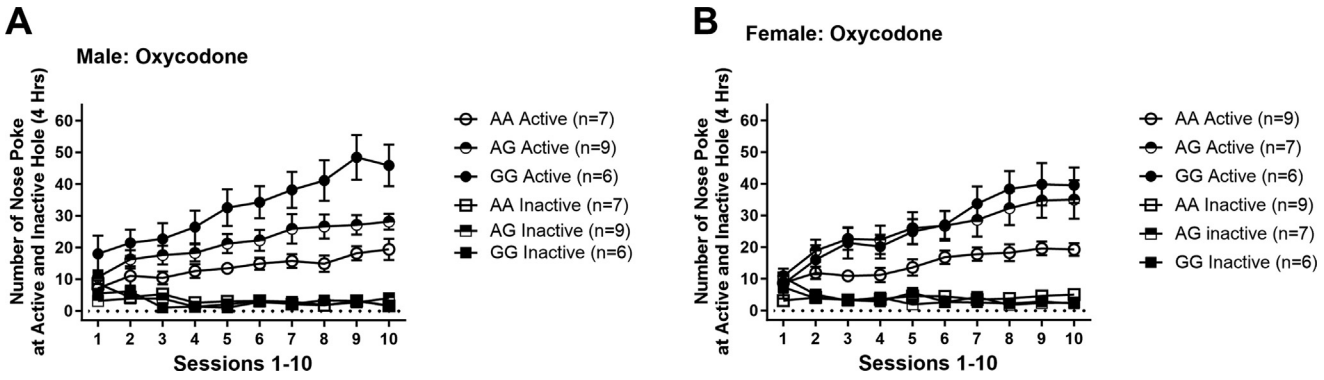

Fig. 2. Oxycodone self-administration behavior of A/A, A/G, G/G male **(A)** and female **(B)** mice (n = 6–9) across initial 10-day sessions. Nose pokes at the active hole increased across the 10 sessions in both male and female of A/A, A/G, G/G mice, with A/G and G/G mice showing higher levels of oxycodone intake (±SEM). Nose pokes at the inactive hole did not escalate across the 10 daily sessions, or differ among genotypes.

(Active, Inactive) × Sessions (11–20). There was a significant Genotype × Nose Poke interaction, F (2, 80) = 23.53, p < 0.000001. There was a significant main effect of Genotype, F (2, 80) = 21.33, p < 0.000001. Newman-Keuls *post hoc* tests showed that both A/G and G/G mice nose poked more for oxycodone than A/A mice, p < 0.05 and p < 0.0005, respectively. In addition, G/G mice nose poked greater amounts of oxycodone than A/G mice, p < 0.005. There was also a significant main effect of Nose Poke, F (1, 80) = 270.84, p < 0.000001, and a significant main effect of Session, F (9, 720) = 3.29, p < 0.001. No significant main effect of Sex was found, F < 1.0. See Fig. 3(A and B).

Cocaine self-administration behavior of male and female A/A, A/G and G/G mice across 10 consecutive daily sessions after oxycodone SA (Days 11–20)

A four-way ANOVA was used to analyze the nose-poke behavior at the active and inactive holes: Genotype (A/A, A/G, G/G) × Sex (Male, Female) × Nose Poke (Active, Inactive) × Sessions (11–20). There was a significant Genotype × Nose Poke interaction, F (2, 80) = 21.99, p < 0.000001, and a significant Nose Poke × Session interaction, F (9, 720) = 3.83, p < 0.0001. There was a significant main effect of Genotype, F (2, 80) = 17.84, p < 0.000001. Newman-Keuls *post hoc* tests showed that both A/G and G/G mice nose poked more for cocaine than A/A mice, p < 0.0005 and p < 0.0005, respectively. There was no difference between A/G and G/G. There was also a main effect of Nose Poke, F (1, 80) = 718.68, p < 0.000001, and a main effect of Session, F (9,720) = 6.22, p < 0.000001. There was no significant effect of Sex, F (1,104) < 1. See Fig. 4(A and B).

Main effect of oxycodone and cocaine SA on dorsal striatal gene expression versus home cage controls

Note: "Drug" indicates 10 days of oxycodone intravenous SA followed by 10 days of oxycodone and cocaine intravenous SA, as described in Methods (Fig. 1), mice were euthanized, and brains were harvested 1 hour after the 20th daily session.

There were significant Genotype differences in two genes: *Oprd1* and *Pdyn* and significant Sex differences in three genes: *Pdyn, Pnoc,* and *Avpr1b;* however none of these retained significance after multiple correction. There were significant Drug-induced differences in the expression of five genes*: Pdyn, Fkbp5, Htr7, Oprk1, and Oprm1* (Table 1). Specifically, the mRNA levels of the

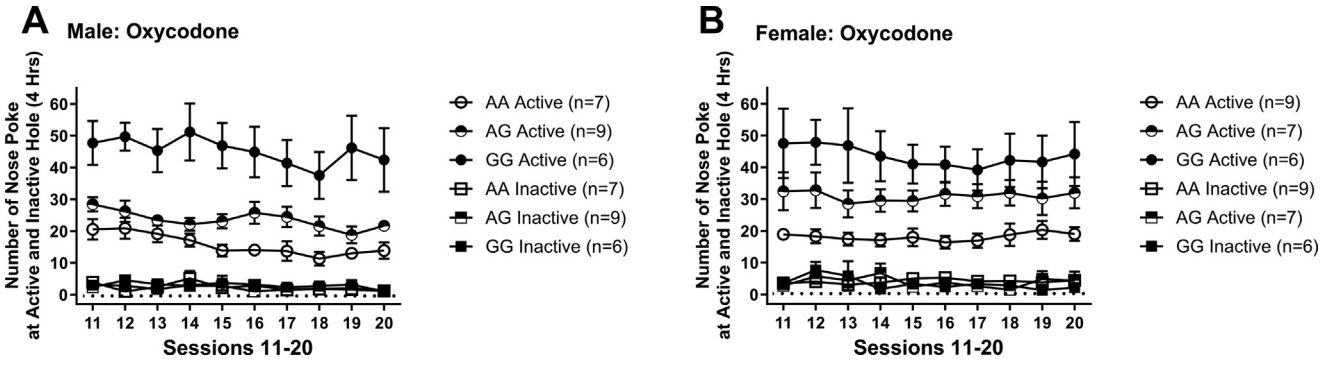

Fig. 3. Oxycodone self-administration behavior of A/A, A/G and G/G male **(A)** and female **(B)** mice (n = 6–9) in the sessions on days 11–20, when combined with cocaine. Nose pokes at the active hole did not increase across the 10-day sessions in both sexes and in all genotypes, with G/G mice showing the highest levels of oxycodone SA and A/A mice showing the lowest level of oxycodone SA (±SEM). Nose pokes at the inactive hole did not escalate across the 10 daily sessions, or differ among genotypes.

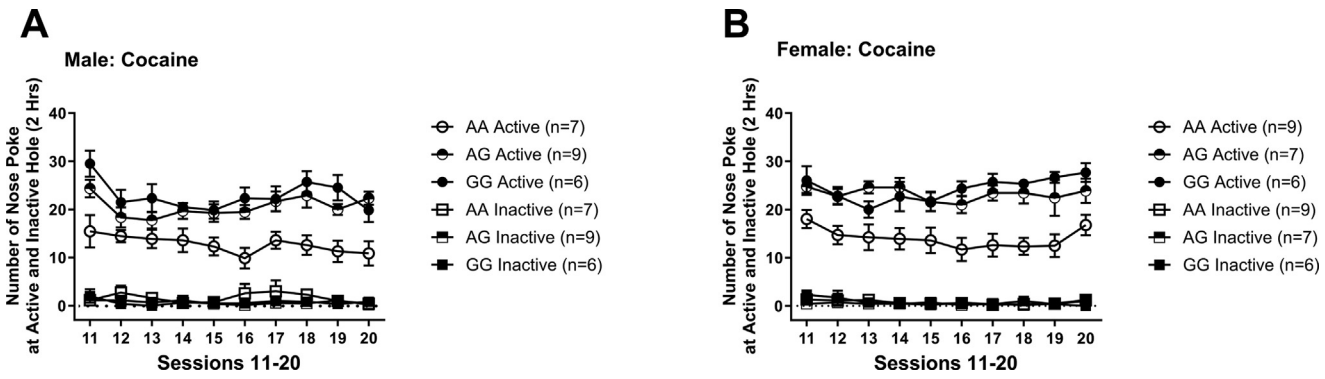

Fig. 4. Cocaine self-administration behavior of A/A, A/G and G/G male **(A)** and female **(B)** mice (n = 6–9) in the sessions on days 11–20, when combined with oxycodone. Nose pokes at the active hole did not increase across the 10-day sessions in both sexes and in all genotypes, with A/G and G/G mice showing higher levels of cocaine SA than A/A mice (±SEM). Nose pokes at the inactive hole did not escalate across the 10 daily sessions, or differ among genotypes.

aforementioned genes were increased after drug SA, compared to controls. Following correction for multiple testing, four of these genes (*Pdyn, Fkbp5, Oprk1* and *Oprm1*) retained significance (Fig. 5). There were also (a) a significant Drug × Sex interactions in three genes: *Htr7, Nr3c2, Opmr1*; (b) a significant Genotype × Sex interactions in four genes: *Penk, Htr2a, Nr3c1, Cnr1*; (c) a significant Genotype × Drug interaction in one gene: *Oxtr*; and (d) a significant Genotype × Sex × Drug interaction in the expression of only one gene: *Nr3c2*. Following correction for multiple testing, none of these interactions retained significance (Table 1).

DISCUSSION

This study examined sequential self-administration of a frequently abused prescription opioid oxycodone and the psychostimulant cocaine, in a mouse model of the major *OPRM1* functional SNP A118G. We found that in addition to self-administering greater amounts of oxycodone during the initial 10 sessions (Zhang et al., 2015b; Collins et al., 2020; Zhang et al., 2020), male and female G/G and A/G mice also showed increased cocaine-intake when this was combined sequentially with oxycodone self-administration, compared to wild type A/A mice. This confirms our prior studies with heroin and oxycodone self-administration in this mouse model (Zhang et al., 2015b), and expands this with the examination of dual self-administration of cocaine and oxycodone. To our knowledge, these are also the first studies in which cocaine SA was studied in A112G mice.

The dual use of mu-opioid agonists and cocaine is relatively prevalent among persons with substance use disorders. Prior studies have examined primarily self-administration behavior that used mixtures of a psychomotor stimulant and a mu-opioid receptor agonist (typically referred to as ‘‘speedball”) in monkeys and rodents (e.g., Martin et al., 2008; Mello et al., 1995; Rowlett and Woolverton, 1997; Schulze et al., 2002; Woolverton et al., 2008). However, such concomitant ‘‘speedball” use is not the only pattern of MOP-r agonist/cocaine co-exposure in humans (e.g., Leri et al., 2004; Piechota et al., 2010; Roy et al., 2013). Therefore, the current study examined a sequential model of oxycodone and cocaine self-administration. In this study, we modeled the initial use of a MOP-r agonist, where mice were allowed to first self-administer oxycodone for 10 consecutive days. Mice then continued to self-administer oxycodone for a further 10 days, followed by daily cocaine self-administration. Therefore, mice first acquired oxycodone self-administration, mimicking the onset of an opioid use disorder, followed by the use of both opioid and cocaine, mimicking polydrug use (e.g., Leri et al., 2004; Roy et al., 2013; Stewart et al., 2014).

We found for the first time that both heterozygote A/G and homozygote G/G mice responded more for oxycodone than A/A mice did, suggesting that even one allele of this *Oprm1* variant alters sensitivity to mu-opioid agonist intake. Greater oxycodone self-administration in the homozygote G/G mice than A/A wild type mice agrees with our earlier studies with heroin and oxycodone (Zhang et al., 2015b; Zhang et al., 2020). Intravenous opioid SA by heterozygote A/G mice has not been studied in this or another OPRM1 A118G mouse model (Ramchandani et al., 2011). Studies in A/G heterozygotes are also relevant to the human condition, since heterozygotes are considerably more frequent than G/G homozygotes, based on allelic frequency (e.g., Bart et al., 2005; Ducat et al., 2013; Schwantes-An et al., 2016; Wand et al., 2002).

Further, both A/G and G/G mice continued to self-administer more oxycodone than A/A mice did during the sessions with sequential self-administration of oxycodone with cocaine (i.e., Days 11–20). MOP-r agonists and cocaine each increase extracellular dopamine levels (Di Chiara and Imperato, 1988), although they do so through different mechanisms. Mu-opioid agonists act by inhibiting GABAergic neurons and disinhibiting dopamine neurons in the midbrain, resulting in increases in dopamine release (e.g., Johnson and North, 1992; Pert et al., 1976). By contrast, cocaine acts directly as a dopamine reuptake inhibitor (e.g., Kilty et al., 1991; Shimada et al., 1991). Our earlier study found that G/G mice showed higher striatal dopamine levels compared with A/A mice following the administration of oxycodone (Zhang et al., 2015b). The effects of cocaine

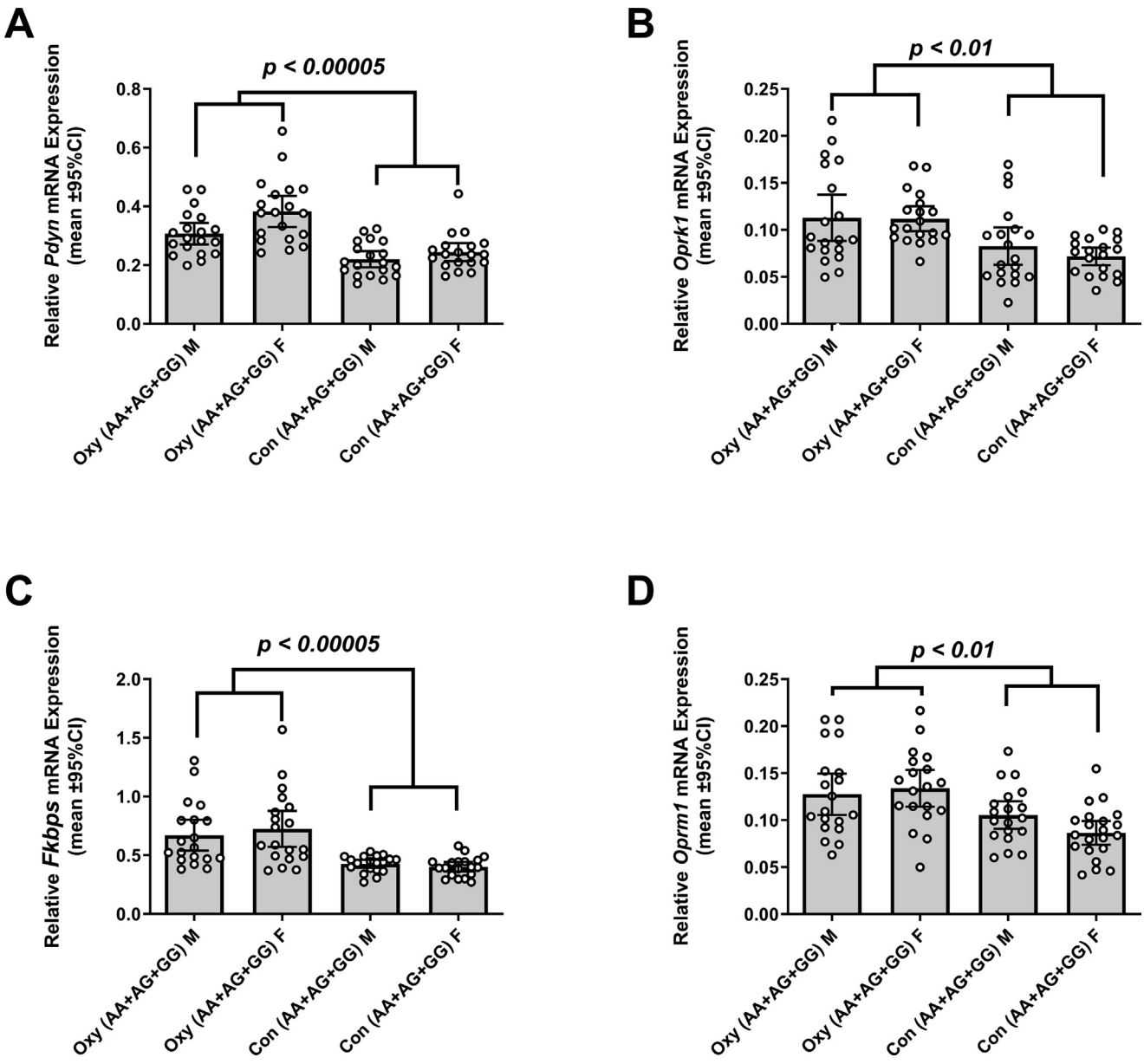

Fig. 5. Increases in the relative mRNA levels of *Pdyn* **(A)**, *Oprk1* **(B)**, *Fkbp5* **(C)** and *Oprm*1(D) (±SEM) in the dorsal striatum of male or female A/A, A/G and G/G mice combined after the oxycodone and cocaine SA session vs controls on Day 20.

on dopamine release in the A112G model are unknown. Of interest, daily exposure to cocaine on days 11–20 did not cause a further escalation of oxycodone intake, compared to days 1–10 (i.e., with oxycodone IVSA alone) (see also Schulze et al., 2002).

Furthermore, A/G and G/G mice self-administered more cocaine than A/A mice did, when available sequentially after oxycodone in the same sessions. This finding suggests that the 112G-allele also changes sensitivity to cocaine IVSA. Future studies would have to determine whether A/G and G/G mice would also self-administer more cocaine without prior exposure to a MOP-r agonist.

Some, but not all, clinical studies found that A118G SNP carriers showed greater vulnerability to opioid use disorder compared with non-carriers when studied in specific cohorts with little genetic admixture (e.g., Ahmed et al., 2018; Bart et al., 2004; Deb et al., 2010). Importantly, large genome-wide association studies in European Americans overall detected the significance of this SNP, but as a protective factor, when the population also included a considerable number of opioid-exposed controls (Zhou et al., 2020).

Alterations in gene expression, because of genotype differences or long-term drug exposure, can potentially contribute to changes in drug- and stress-mediated behaviors (e.g., Kreek et al., 2005; Kreek et al., 2012; McClung et al., 2005; Nestler, 2005). In the current study, we examined the expression levels of 24 genes that are regulated by exposure to MOP-r agonists, cocaine, or stress, including components of the hypothalamic-pituitary adrenal axis, which is dysregulated in opioid

use disorders (e.g., Zhang et al., 2018; Zhang et al., 2014; Koob and Kreek, 2007). Furthermore, these selected genes encompassed the four major opioid receptor types and their cognate neuropeptides. We focused on the dorsal striatum, because this brain region is involved in regulating reward, habit learning, and compulsive-like behavior, especially after repeated exposure to drugs of abuse (e.g., Belin and Everitt, 2008; Ito et al., 2002; Porrino et al., 2007).

After correction for multiple comparisons, mRNA levels of *Pdyn* (encoding for dynorphin, the endogenous ligands of kappa-opioid receptors) and *Oprk1* (encoding for kappa opioid receptor) in the striatum of A/A, A/G, and G/G mice increased following combination of oxycodone and cocaine self-administration. The endogenous kappa opioid receptor system is known to modulate dopaminergic and MOP-r systems in the brain. In the striatum, dynorphin peptides are localized in the majority of GABAergic medium spiny neurons (Gerfen, 1988). Both MOP-r agonists and cocaine alone can increase striatal dopamine levels, and levels of *Pdyn* mRNA were increased in the dorsal striatum in A/A, A/G, and G/G mice under the drug condition, compared to those of the controls. Earlier studies found that chronic cocaine administration resulted in increases in *Pdyn* mRNA levels within the caudate putamen (e.g., Hurd et al., 1992; Spangler et al., 1993; Daunais et al., 1993), and tissue levels of dynorphin peptide also increased in this brain region (Sivam, 1989). The increases in *Pdyn and Oprk1* mRNA levels found in the current study indicated that sequential chronic oxycodone and cocaine self-administration led to a compensatory increase in the activity of the kappa opioid system.

The mRNA levels of the *Fkbp5* gene showed a significant increase in response to drug exposure (oxycodone/cocaine) in this study compared to controls. The protein encoded by this gene plays a role in immune regulation and basic cellular processes involving protein folding and trafficking, as well as regulation of glucocorticoid receptor (NR3C2) function, thus related to the HPA stress axis (Ising et al., 2008; Hassan et al., 2010). It was proposed that *Fkbp5* mediates the abuse potential of mu-opioid agonists, and is a key regulator of the development of opioid tolerance and dependence (Homayoun et al., 2003; McClung et al., 2005). *Fkbp5* gene expression level in the whole brain was up-regulated in Sprague-Dawley rats following oxycodone administration 15 mg/kg i.p. every 12 h for 8 days (Hassan et al., 2010). Furthermore, two *Fkbp5* SNPs were associated with heroin addiction in humans (Levran et al., 2014).

In the dorsal striatum, the basal mRNA level of *Oprm1* was previously shown to be lower in the G/G mice than in the A/A mice (Mague et al., 2009; Collins et al., 2018). We found here that dual self-administration of oxycodone and cocaine increased striatal *Oprm1* mRNA levels. Some earlier studies found that cocaine administrations alone increased brain *Oprm1* mRNA levels (e.g., Azaryan et al., 1996; Collins et al., 2018; Mague et al., 2009; Zhou et al., 2007; Azaryan et al., 1998). Based on the design of this study, it is not clear whether the observed increases in *Oprm1* mRNA levels are due to exposure to cocaine, oxycodone, or their combination.

LIMITATIONS AND DESIGN CONSIDERATIONS

This study modeled sequential administration of a MOP-r agonist and cocaine in the same session, as opposed to "speedball"; both of these major patterns of use can occur in persons with dual opioid and cocaine use disorders (e.g., Leri et al., 2004; Roy et al., 2013). As mentioned above, dorsal striata were obtained on Day 20th, after mice had self-administered both oxycodone and cocaine. Therefore, future studies would have to determine whether the specific pattern of mRNA changes observed here were due to oxycodone or cocaine self-administration, or their combination.

This study examined oxycodone as a MOP-r agonist, based on prior experience with this widely misused or abused prescription opioid, in this IVSA model (e.g., Zhang et al., 2018; Zhang et al., 2006). Future comparative studies could determine if any of these findings differ with other MOP-r agonists, including fentanyl, the cause of major increases in overdoses in the last several years (Ciccarone, 2021; Brown et al., 2022).

The present study demonstrates that both homozygote (G/G) and heterozygote (A/G) male and female mice of the common functional *Oprm1* polymorphism A118G escalated oxycodone SA more than the wild type (A/A) mice during initial exposure to oxycodone exposure. Furthermore, the G/G and A/G mice also displayed greater cocaine intake when combined sequentially after oxycodone SA. Overall, the behavioral and neurobiological changes found in the current study provide initial insight into the genetic mechanisms that may underlie poly-drug self-exposure to MOP-r agonists and cocaine.

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