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# PKMζ alters oxycodone-taking in a dose- and sex-dependent manner

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## Abstract

Opioid use disorder involves disruptions to glutamate homeostasis and dendritic spine density in the reward system. PKMζ is an atypical isoform of protein kinase C that is expressed exclusively in neurons and plays a role in postsynaptic glutamate signaling and dendritic spine maturation. As opioid use leads to alterations in glutamate transmission and dendritic spine density, we hypothesized that PKMζ deletion would alter opioid-taking behaviors. The current study examined two doses of oxycodone self-administration in male and female mice with constitutive deletion of PKMζ compared to wildtype controls. At a dose of 0.25 mg/kg/infusion, PKMζ deletion significantly potentiated oxycodone self-administration in both male and female mice. However, increases in motivation for oxycodone, as indicated by increased breakpoint on a progressive ratio schedule, were only seen in male PKMζ knockout mice and not females. When we examined a lower dose of oxycodone, 0.125 mg/kg/infusion, PKMζ knockout led to increases in oxycodone self-administration only in female mice. Additionally, female PKMζ knockout mice exhibited higher breakpoints on a progressive ratio schedule at this dose compared to all other groups. In addition to the self-administration studies, we also examined locomotor sensitization in response to experimenter administered oxycodone. PKMζ KO decreased oxycodone induced locomotion in males and potentiated oxycodone sensitization in females. Together, these results suggest that PKMζ acts to dampen oxycodone taking in both sexes, but females may be more sensitive to its effects.



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Ethics approval and consent to participate

All procedures using experimental animals were approved by Temple's Institutional Animal Care & Use Committee.

Consent for publication

All authors read and approved the final manuscript for publication.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare no conflict of interest.

**Keywords**

Addiction; Oxycodone; PKMζ; Sex differences; Self-administration; Motivation

## 1. Introduction

Altered glutamate transmission underlies many aspects of the substance use disorder (SUD) cycle, with disruptions to glutamate homeostasis driving relapse [10,11,17]. Trafficking of glutamatergic AMPA receptors (AMPARs) specifically underlies learning and drug use [25, 26]. PKMζ, an atypical isoform of Protein Kinase C (PKC), is an AMPAR trafficking protein. It potentiates N-ethylmaleimide-sensitive factor (NSF)-mediated insertion of GluA2-containing AMPARs to the cell membrane [46]. This makes PKMζ an interesting target for studies on the synaptic plasticity underlying learning, memory, and drug use.

Early studies demonstrated a role for PKMζ in long-term potentiation (LTP). PKMζ levels increase during and after LTP induction and it was proposed to be sufficient to maintain LTP [14,22,36]. In learning and memory, PKMζ levels increase following spatial conditioning and during memory reconsolidation [4,14]. Further, an infusion of zeta inhibitory peptide (ZIP), a proposed PKMζ inhibitor, into the hippocampus prevented rats from exhibiting a previously-learned fear response [29]. Relevant to substance use, ZIP was also shown to block morphine conditioned place preference [21]. Altogether, these studies demonstrated a role for PKMζ in memory formation, memory preservation, and learning.

Recently, the specificity of ZIP has been called into question. It likely targets both PKMζ and another atypical PKC isoform, PKCi/λ. PKMζ knockout mice exhibit normal learning, memory, and LTP and ZIP still induces learning and memory deficits in these animals [18,42]. This makes studies that utilized ZIP to block PKMζ difficult to interpret. These findings could indicate PKMζ is not involved in LTP, learning, and memory. Another explanation, however, is that there are compensatory mechanisms at play in PKMζ knockout mice. As evidence of this, PKCi/λ is recruited for LTP in animals that lack PKMζ [40]. Regardless of the controversy surrounding ZIP, PKMζ does alter spine density.

There is evidence for this as PKMζ knockout increases ethanol consumption in an intermittent access paradigm in male mice [19]. We previously extended these findings to cocaine where we demonstrated PKMζ knockout potentiates cocaine-taking and seeking in both male and female mice [27]. Together, these studies indicate PKMζ works to dampen drug reward. Cocaine craving is promoted by changes in spine density within the NAc core [6]. As opioids and cocaine generally have opposing effects on spine density in the NAc [32–34], and PKMζ plays a critical role in spine maturation [13,35], we were interested in exploring how PKMζ knockout may alter opioid-taking behaviors.

Here, we examined the role of PKMζ in oxycodone-taking, motivation, and locomotor sensitization in response to oxycodone. We tested two doses of oxycodone self-administration and used a progressive ratio paradigm to further our understanding of PKMζ's role in opioid use. We found that PKMζ has a sex-specific effect at a low dose and moderate dose of oxycodone. Likewise, we found that PKMζ has a sex-specific on

oxycodone induced locomotion and sensitization. These data indicate that while PKMζ does work to blunt opioid-taking and seeking, its role in these processes is sex-specific. This highlights the possibility that while PKMζ does dampen drug reward across multiple drug classes, the sensitivity may differ across sex.

## 2. Methods

### 2.1. Subjects

The current study utilized male and female PKMζ knockout (KO) mice as described previously [42]. Heterozygous PKMζ KO mice on a C57BL/6 J background were mated resulting in mutant and wildtype littermates. After weaning, tail snips were taken and genotyped using quantitative polymerase chain reaction (qPCR). Mice (2–6 months old, age matched across group) were group housed until the start of the behavioral experiments at which point they were individually housed. All animals were housed in a temperature and humidity-controlled animal care facility with a 12 h reverse light/dark cycle (lights on a 7:00 p. m.). All procedures were approved by the Temple University Animal Care and Use Committee.

### 2.2. Drugs

Oxycodone was obtained from the National Institutes of Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9 % saline.

### 2.3. Jugular catheterization surgery

Prior to surgery, mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was placed into the right jugular vein and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a backmount platform (Instech Laboratories, Inc.) that secured the placement. Catheters were flushed daily with 0.1 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline. Mice recovered in their home cage 3 days prior to the start of our experiments.

### 2.4. Operant sucrose training

Following catheterization, mice were trained to perform an operant response for sucrose pellets. The mice were placed in operant chambers (Med-Associates) and trained to press a lever to receive a sucrose pellet. Mice performed 2 days of fixed ratio 1 (FR1) responding where only the active lever was available. A compound cue stimulus consisting of a cue light above the active lever, a 2900 Hz tone, and house light off was concurrent with each pellet administration, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Mice were allowed to self-administer a maximum of 50 pellets per 60 min operant session. The mice were food restricted to approximately 90 % of their free feeding weight throughout the course of the operant training. They returned to ad libitum feeding 6 days into oxycodone self-administration.

### 2.5. Oxycodone self-administration

Oxycodone self-administration was measured over 2 h sessions (7 days per week) in the same chamber used for operant sucrose training. Mice were trained to self-administer oxycodone for 3 days using an active and inactive wheel. Following the training phase, the active and inactive wheels were replaced with levers and mice continued oxycodone self-administration for 12 days on a fixed ratio 1 (FR1) schedule. Throughout all of oxycodone self-administration, responding on the active manipulandum delivered an intravenous oxycodone injection (0.25 or 0.125 mg/kg/infusion) paired with the same cues as food training. Following the self-administration phase, mice were tested for 1 day on a progressive ratio (PR) schedule, where the response requirement for each infusion increased until the subject did not fulfill the requirement. The response requirement was defined as R(i)=[5e0.15i-5] and the session ended if the animal took longer than 30 min to meet the requirement. The breakpoint is defined as the final ratio completed.

### 2.6. Oxycodone sensitization

Mice were tested for 5 consecutive days in a white plastic arena (35 × 35 × 24 cm) under red light conditions (8.4–9.5 lx). Each day animals were first placed in the chamber for 30 min to habituate. Directly following habituation, mice were given an injection of oxycodone (5 mg/kg, i.p.) and placed back in the chamber. Locomotor activity was recorded for an additional 60 min following the injection. Total distance travelled was tracked during habituation and testing using the ANY-Maze Video Tracking System (Stoelting Co., Version 7.4).

### 2.7. Data analysis

All analyses were performed using GraphPad Prism 10 software (GraphPad Software). Self-administration data were analyzed using two-way ANOVA with Sidak's post hoc tests or linear mixed-effects models [41] as appropriate. Oxycodone behavioral sensitization data were analyzed using a repeated measures three-way (raw locomotor activity) and two-way ANOVAs (following area under the curve calculations). Statistical significance for all tests was set at $\alpha = 0.05$.

## 3. Results

### 3.1. PKMζ knockout potentiates 0.25 mg/kg/inf oxycodone self-administration in both sexes

Wildtype and PKMζ knockout mice self-administered 0.25 mg/kg/infusion oxycodone for 12 days. Both male and female PKMζ knockout mice earned more infusions [effect of genotype; females: F(1,41)=5.64, $p$=.022, Fig. 1A; males: $F$(1,42)=18.4, $p$<.001; Fig. 1B]. Males responded more for oxycodone [effect of genotype: males: $F$(1,42)=7.81, $p$=.008; Fig. 1D] and had significantly lower inactive presses [effect of genotype; $F$(1,42)=5.80, $p$=.021; Fig 1F] than wildtype conspecifics. There was no effect of PKMζ knockout in females on either active responding [effect of genotype; $F$(1.41)=3.61, $p$=.065; Fig 1C] or inactive responding [effect of genotype; $F$(1,41)=0.33, $p$=.57; Fig 1E].

### 3.2. PKMζ knockout alters motivation for 0.25 mg/kg/inf oxycodone in males and females

We next examined whether PKMζ knockout affects motivation to obtain oxycodone as measured by a progressive ratio paradigm. At this dose of oxycodone, we found a main effect of genotype, indicating that PKMζ knockout led to higher breakpoints [$F(1,30)=7.70$, $p=.009$].

### 3.3. PKMζ knockout potentiates 0.125 mg/kg/inf oxycodone self-administration exclusively in female animals

Wildtype and PKMζ knockout mice self-administered 0.125 mg/kg/inf oxycodone for 12 days. Female PKMζ knockout animals earned more infusions [effect of genotype; $F(1,16)=7.23$, $p=.016$; Fig. 3A] and responded significantly more for oxycodone [effect of genotype; $F(1,16)=9.039$, $p=.008$; Fig. 3C] than wildtype conspecifics. In contrast, while male PKMζ knockout animals exhibit a trend towards a higher number of infusions [$F(1,15)=4.28$, $p=.056$; Fig. 3B], they did not exhibit a higher number of active responses compared to wildtype conspecifics [$F(1,15)=1.013$, $p=.33$, Fig. 2D]. There was no significant effect of genotype on inactive lever responding [females: $F(1,16)=4.18$, $p=.057$; Fig. 3E; males: $F(1,15)=0.037$, $p=.85$; Fig. 3F].

### 3.4. PKMζ knockout increases motivation for 0.125 mg/kg/inf oxycodone exclusively in female animals

When we examined responding for this dose of oxycodone on a progressive ratio schedule, we found that PKMζ deletion led to an increase in breakpoint in female mice but not males [effect of genotype: $F(1,31)=8.56$, $p=.006$; effect of sex: $F(1,31)=2.35$, $p=.14$, interaction: $F(1,31)= 6.46$, $p=.016$; Sidak post-hoc: wildtype females vs. PKMζ KO females, $t(31)=4.02$, $p<.0007$; Fig. 4].

### 3.5. PKMζ knockout alters oxycodone induced locomotion and sensitization in a sex-specific manner

When we examined the impact of PKMζ deletion on the locomotor response to a novel environment during the habituation phase of the sensitization experiment, we did not see any effect of genotype or sex on locomotor activity on day 1 of habituation, prior to any oxycodone administration (3-way ANOVA: effect of genotype: $F(1,24)=0.0003$, $p=.99$; effect of sex: $F(1,24)=3.36$, $p=.08$). Examining the area under the curve over the five daily habituation sessions, we did not see any impact of genotype over time in either the male mice or the female mice [males: 2-way ANOVA: effect of genotype: $F(1,60)= 0.03$, $p=.87$; Fig. 5A; females: 2-way ANOVA: effect of genotype: $F(1,60)=2.10$, $p=.15$; Fig. 5B].

We next assessed oxycodone-induced locomotor activity by measuring distance traveled in an open field for 60 min. Data was binned in 5-minute intervals. On days 1 and 2, along with the main effect of time, we found a significant time x genotype interaction [Day1, Fig 5C: effect of time: $F(2.298,55.15)=40.9$, $p<.0001$; time x genotype interaction: $F(11,264)=2.04$, $p=.025$; Day 2, Fig. 5D: effect of time: $F(3.080,73.92)= 44.1$, $p<.0001$; time x genotype interaction: $F(11,264)= 2.77$, $p=.002$]. On day 3, we detected an effect of time, sex and significant interactions between time and genotype, time and sex, and genotype and sex [Day 3, Fig. 5E: effect of time: $F(3.433,82.38)=31.2$, $p<.0001$, effect of

sex: $F(1,24)=7.18$, $p=.013$; time x genotype interaction: $F(11,264)=3.89$, $p<.0001$; time x sex interaction: $F(11,264)=2.46$, $p=.006$; genotype x sex interaction: $F(1,24)=4.27$, $p=.049$]. On day 4, there was only a main effect of time on distance travelled [Day 4, Fig. 5F: effect of time: $F(2.834,68.02)=24.6$, $p<.0001$]. Finally, on day 5 we detected significant effects of time along with genotype by sex and time x genotype x sex interactions [Day 5, Fig. 5G: effect of time: $F(2.456,58.95)=9.97$, $p<.0001$; genotype x sex interaction: $F(1,24)= 9.66$, $p=.0048$; time x genotype x sex interaction: $F(11,264)=2.31$, $p=.010$].

To further characterize these complicated interactions, we examined the area under the curve during the 60 min following oxycodone injection over the course of the 5 daily injections. We found that PKMζ deletion led to an overall decrease in oxycodone-induced locomotor activity in male mice [effect of genotype: $F(1,60)=22.44$, $p<.001$; Fig. 5H]. In contrast, PKMζ deletion in females potentiated the locomotor response to oxycodone over time [effect of genotype: $F(1,60)= 9.16$, $p=.004$; effect of day: $F(4,60)=0.32$, $p=.87$; interaction: $F(4,60)= 1.69$, $p=.17$; Fig. 5I].

## 4. Discussion

Glutamatergic AMPAR activity underlies much of the SUD cycle [25, 26]. PKMζ, an atypical isoform of Protein Kinase C, potentiates NSF-mediated insertion of GluA2-containing AMPARs to the cell membrane [30]. This made PKMζ a popular target for studies on synaptic plasticity, mainly LTP [14,22,36]. Despite some controversy surrounding the role of PKMζ in synaptic plasticity and behavior [18,40,42], PKMζ can alter dendritic spine density and is involved in cocaine and ethanol-taking behaviors [19,27,35]. As cocaine and opioids alter spine density in opposing manners, we were interested in whether PKMζ knockout would affect opioid self-administration in a different manner than we previously found with cocaine [33]. In these experiments we found biological sex can influence the effect of PKMζ knockout on oxycodone-taking and motivation. Our results indicate PKMζ works to dampen drug reward across multiple drug classes and PKMζ may play a role in sex differences in the dose response for oxycodone.

### 4.1. PKMζ blunts oxycodone-taking and motivation for oxycodone in both sexes at a moderate dose of oxycodone

In our first experiment, we found PKMζ knockout potentiates oxycodone self-administration and motivation in both sexes at a dose of 0.25 mg/kg/inf. We first examined this dose as it is a moderate dose of oxycodone that is readily self-administered by mice [47]. Our data show significant potentiation in both the number of infusions earned for both sexes and the number of active responses in male PKMζ knockout animals compared to wildtype controls across 12 days of self-administration. This is in line with previous data showing PKMζ works to dampen ethanol- and cocaine consumption [19,27]. We do not see an increase in the number of responses on the inactive lever, indicating potentiated responding for oxycodone is not due to increases in perseverative responding. PKMζ knockout animals also showed a significant increase in motivation for oxycodone, as indicated by their increased breakpoint on the progressive ratio schedule. Although not statistically significant, it is

noteworthy that this effect of genotype on breakpoint was most likely driven by male animals.

PKMζ potentiates insertion of GluA2 AMPARs to the cell membrane. Previous data show disrupting GluA2 trafficking through mechanisms other than PKMζ alters cocaine reinstatement [5,9,44,45]. Though there are fewer studies examining opioid self-administration, opioid exposure does alter GluA2 trafficking [12]. Here, our findings further the data that disrupting GluA2 trafficking alters drug-taking behaviors by demonstrating a role for PKMζ in opioid self-administration. Further, we found the effect of PKMζ knockout on opioid-taking is present in both sexes. The results from our first experiment highlight the ubiquitous role of GluA2 trafficking in drug use in both males and females. In combination with previous studies, these data specifically further the finding that GluA2 trafficking via PKMζ broadly works to dampen drug reward in both sexes.

In addition to its role in GluA2 AMPAR insertion, PKMζ may also play a role in spine morphology. PKMζ induces a maturation of dendritic spines in cultured cortical neurons [35]. Though the role of PKMζ in spine density is still being explored, this highlights the possibility knocking out PKMζ would lead to less stable spines. As opiates, including oxycodone, lead to decreased dendritic spine density in the nucleus accumbens [33], prefrontal cortex [33,38], and hippocampus [43], PKMζ knockout could potentiate this spine loss. However, cocaine and opioids affect spine density in the NAc in opposing manners [7,8,16, 20,28,32–34,39] and we found the same behavioral phenotype in PKMζ knockout animals across both drugs. This indicates that while PKMζ may play a role in spine maturation, its role in drug-taking may not be due explicitly to its role in spine density.

### 4.2. PKMζ plays a sex-specific role in oxycodone-taking and motivation at a low dose of oxycodone

When examining responding for a lower dose of oxycodone (0.125 mg/kg/infusion), we found PKMζ knockout potentiates oxycodone self-administration and motivation exclusively in female animals. While we did not see any significant effect of sex on oxycodone-taking at the moderate dose, there are known dose-dependent sex differences in drug-taking behaviors [3]. We chose this lower dose as it may elicit more responding and capture group differences better than higher doses [47]. We conclude PKMζ knockout blunts opioid reward in both sexes at multiple doses. Nonetheless, we do not see any effects of genotype on either the number of infusions earned or active responding in the male animals. Our data indicate there is a sex-specific effect of PKMζ knockout on low dose oxycodone self-administration.

We saw a similar effect with PKMζ knockout enhancing the final breakpoint in female animals at the 0.125 mg/kg/inf dose and male animals at the 0.25 mg/kg/inf dose. Our current data indicate there is a role for PKMζ in motivation to acquire oxycodone that varies based on dose. Biological sex can modulate behavioral responses to different doses of the same drug. Females acquire drug-taking at lower doses and when given a choice will choose a higher dose of cocaine than males [3]. Thus, varying the dose may elicit effects of sex not previously seen at other doses. PKMζ can also be modulated by biological sex. There are sex differences in expression within the hippocampus and NAc following drug

exposure [2,27]. This indicates that while many behaviors involving PKMζ may present in a similar manner, PKMζ activity may not explicitly be the same between the sexes. Previous studies demonstrate this, where constitutive PKMζ knockout potentiates cocaine-taking in both sexes but site-specific knockout in the NAc potentiates cocaine-taking exclusively in males [27]. Altogether, these data indicate that PKMζ does work to dampen drug reward, but its role is not explicitly the same in males and females.

Oxycodone suppresses glutamatergic input in the hippocampus of male rats [24]. In addition to there being region specific differences between the sexes, perhaps PKMζ plays a differential homeostatic role in maintaining excitatory balance within learning and reward regions by stabilizing synapses. There is additional evidence that the effects of opioids on glutamatergic synapses may be sex specific. Chronic exposure to oxycodone was found to have differential effects on synaptic protein gene expression in the hippocampus of male and female rats [31], including kinase expression. Other reward related regions may also show sex specific alterations in synaptic proteins after opiate administration. Our results show a sex-specific effect of oxycodone at the lower dose. This may be due to specific synaptic protein changes that occur after oxycodone self-administration. These mechanisms may compensate for lack of PKMζ in males but not females at a lower dose of oxycodone. A previously proposed mechanism suggests that PKCi/λ may compensate for LTP deficits in the absence of PKMζ [23]. This was only studied in male mice and further work is needed to investigate whether these compensatory mechanisms are the same in female animals.

### 4.3. PKMζ plays a sex-specific role in oxycodone-induced locomotion

In our final experiment, we examined oxycodone-induced locomotion to see if PKMζ influences either the acute locomotor response to oxycodone or locomotor sensitization over time. While the current dose of oxycodone did not produce behavioral sensitization in our wildtype mice, we did detect effects of PKMζ on oxycodone-induced locomotion. While in male mice, PKMζ deletion led to a decrease in oxycodone-induced locomotor behavior, in female mice we see increased sensitivity. This supports our hypothesis that PKMζ deletion may potentiate the effects of oxycodone at lower doses in females.

## 5. Conclusion

Here, we found PKMζ knockout potentiates oxycodone-taking in a dose- and sex-dependent manner. Additionally, PKMζ has sex-specific effects on oxycodone sensitization. This adds to the literature demonstrating PKMζ works to dampen drug-taking across multiple drug classes. Additionally, this work suggests that PKMζ plays a differential role in homeostatic mechanisms between the sexes where females may be more sensitive to opioid induced changes in excitatory transmission. These experiments highlight the importance of including both sexes in biomedical research. Recent evidence indicates men and women do not always respond to SUD treatments in the same manner [1,15,23,37]. Further investigation into the neural mechanisms driving SUD in both sexes could aid in the development of more effective pharmacotherapies for males and females.

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## Data availability

Data will be made available on request.

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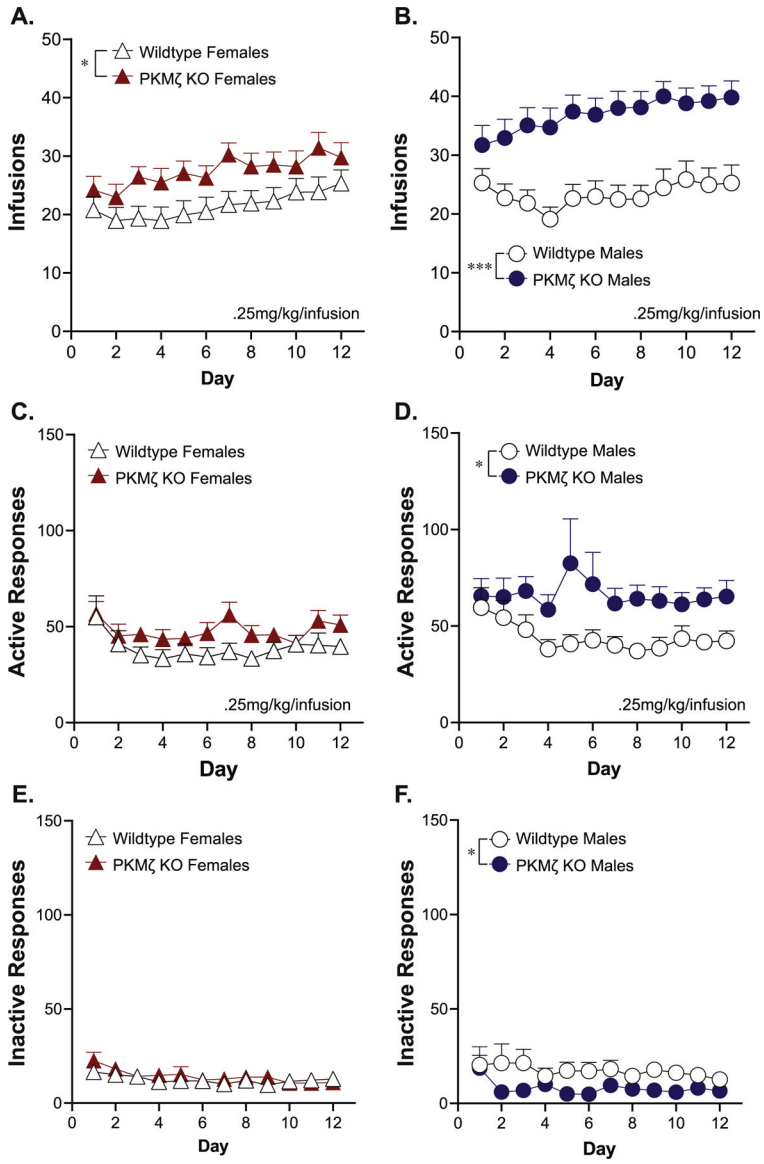


**Fig. 1. PKMζ knockout potentiates 0.25 mg/kg/inf oxycodone self-administration in both sexes.**

Constitutive PKMζ deletion leads to an increase in the number of oxycodone infusions earned in both females ($n = 20$) (A) and males ($n = 20$) (B) in comparison to female ($n = 23$) and male wildtype ($n = 24$) counterparts. Male PKMζ knockout mice also exhibit an increase in the number of active responses across 12-days of oxycodone self-administration (D), effect that is not seen in female PKMζ knockout mice (C). While there were no significant effects of PKMζ knockout on inactive responding in females (E), there was a decrease in inactive responding in male knockout mice (F). *$p<.05$, ***$p<.001$ main effect of genotype.

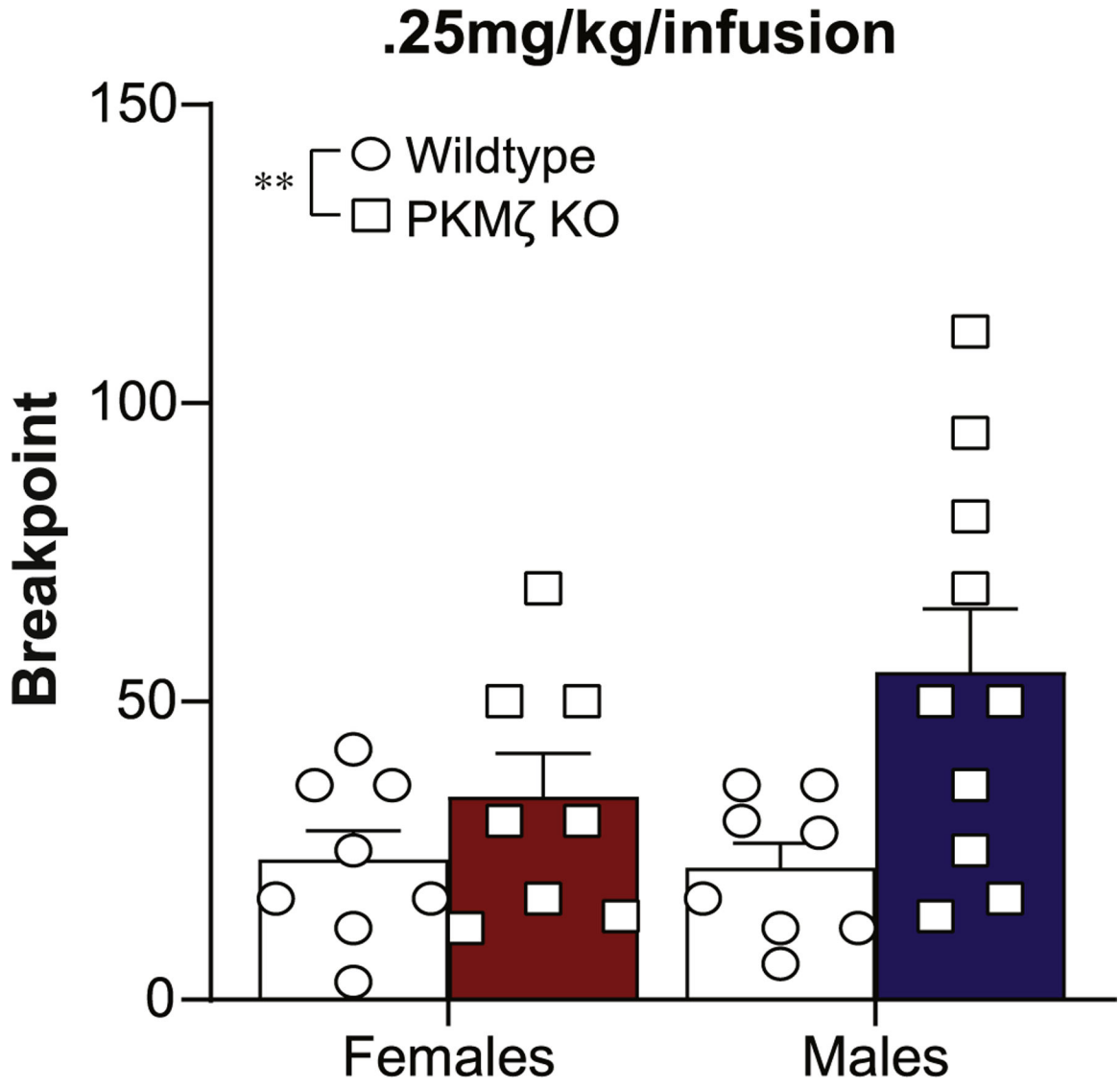


**Fig. 2. PKMζ knockout potentiates the final breakpoint in a progressive ratio paradigm at 0.25 mg/kg/inf oxycodone in males and females.**

Constitutive PKMζ deletion leads to an increase in breakpoint for oxycodone at the 0.25 mg/kg/inf dose in males ($n = 10$) and females ($n = 8$) in comparison to male ($n = 8$) and female ($n = 8$) wildtype counterparts $^{**}p<.01$ main effect of genotype.

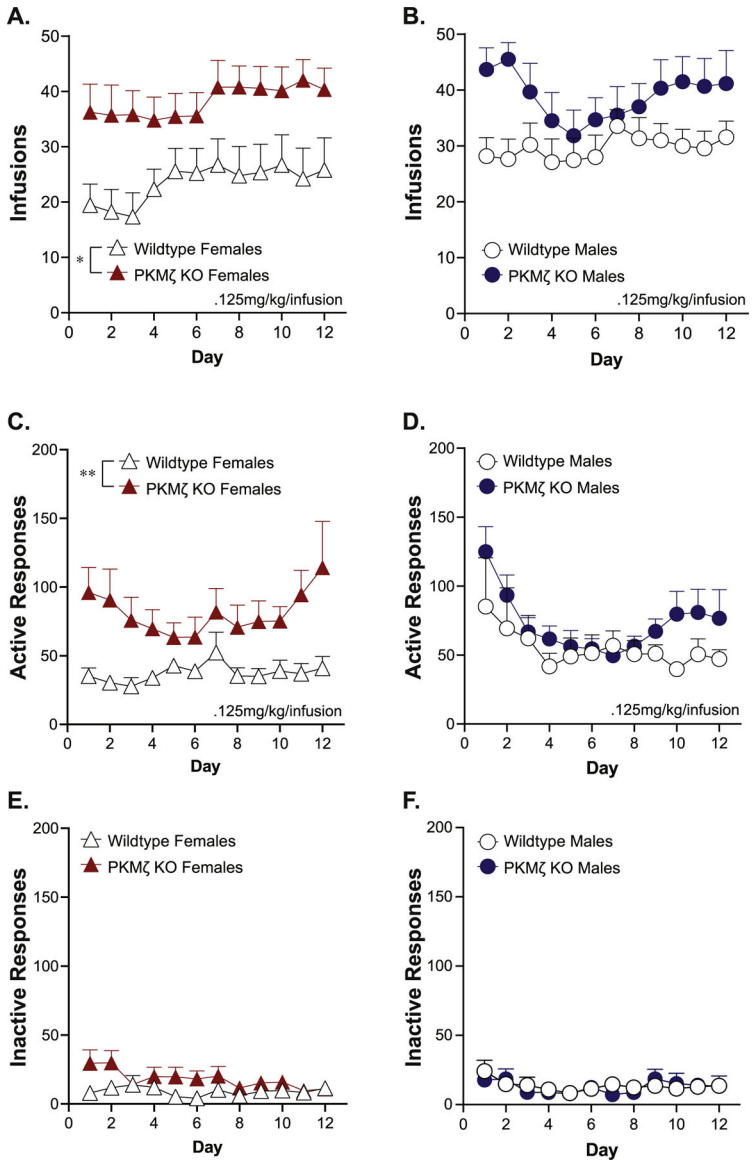


**Fig. 3. PKMζ knockout potentiates 0.125 mg/kg/inf oxycodone self-administration exclusively in female animals.**

Constitutive PKMζ deletion leads to an increase in the number of oxycodone infusions earned in females ($n = 9$) (A) but not males ($n = 6$) (B) in comparison to female ($n = 9$) and male ($n = 11$) wildtype counterparts. Female PKMζ knockout mice also exhibit an increase in the number of active responses across 12-days of oxycodone self-administration (C), effect that is not seen in male PKMζ knockout mice (D). There were no significant effects of PKMζ knockout on inactive responding in either females (E) or males (F). *$p<.05$, **$p<.01$ main effect of genotype.

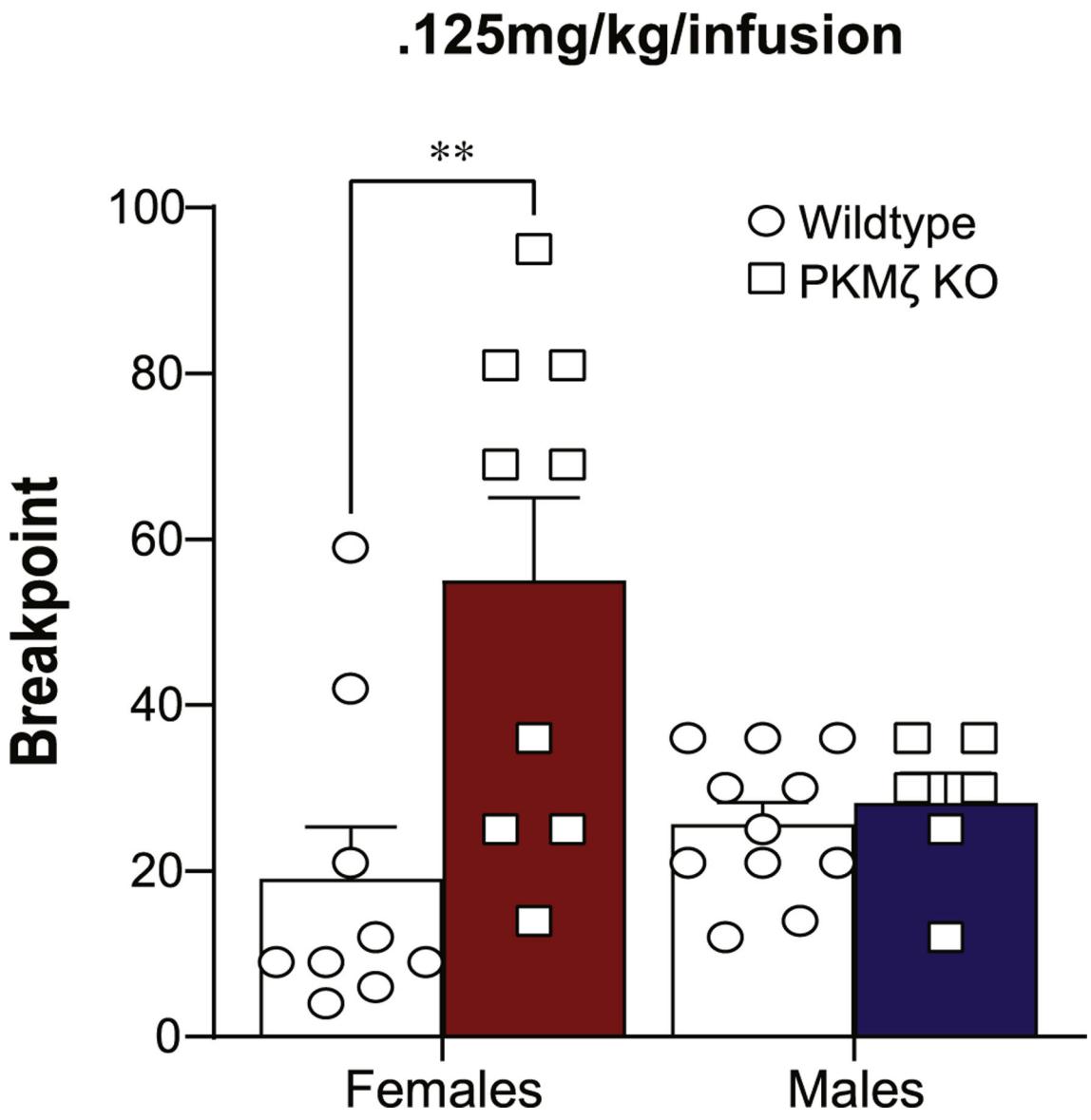


**Fig. 4. PKMζ knockout potentiates the final breakpoint in a progressive ratio at 0.125 mg/kg/inf paradigm exclusively in female animals.**

Constitutive PKMζ deletion leads to an increase in the breakpoint for oxycodone at the 0.125 mg/kg/inf dose in female, but not male, mice. ** $p<.01$, pairwise comparison wildtype females vs. PKMζ knockout females due to significant interaction effect.

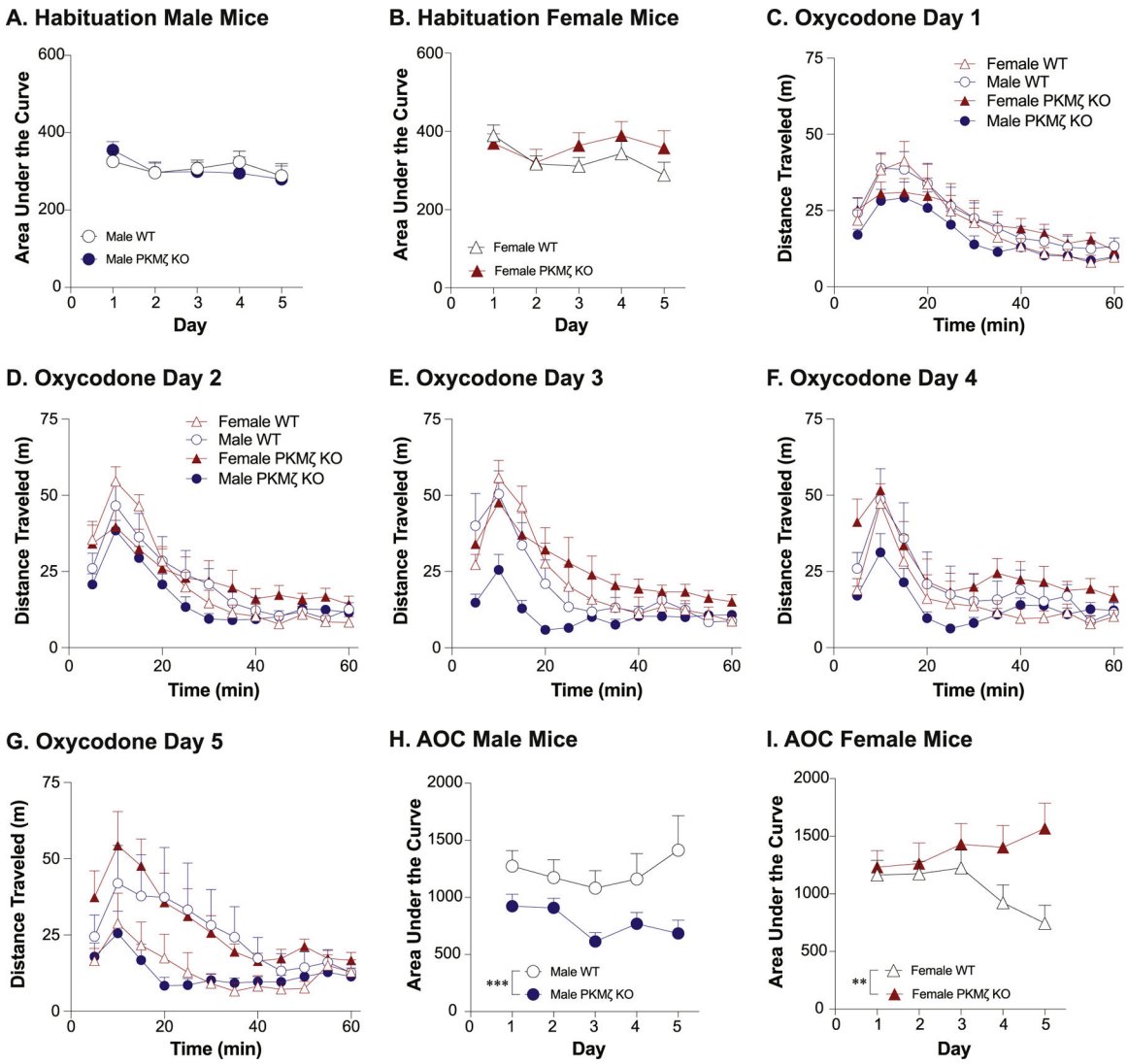


**Fig. 5. PKMζ knockout alters oxycodone-induced locomotion differentially in male and female mice.**

Constitutive PKMζ deletion does not alter the locomotor response to the testing chamber during the habituation phase prior to oxycodone injection in either male (A) or female (B) mice ($n$ = 6–8/group). For 60 min following each daily oxycodone injections (5 mg/kg, i.p.) we measured distance travelled in meters (m) and binned the data in 5-minute intervals (C-G). The influence of genotype on oxycodone-induced locomotor behavior varied by sex, an effect that is more clearly illustrated when we examined area under the curve across the five days. Male PKMζ knockout mice exhibit a decrease in oxycodone-induced locomotion across all five days (H, ***$p$<.001, effect of genotype). In contrast, female PKMζ knockout mice exhibit greater oxycodone-induced locomotion, particularly during the final two days of injections, where the wildtype mice appear to exhibit habituation (I, **$p$<.01, effect of genotype).