# The effects of pioglitazone, a PPARγ receptor agonist, on the abuse liability of oxycodone among nondependent opioid users

**Jermaine D. Jones**[1,*], **Maria A. Sullivan**[1], **Jeanne M. Manubay**[1], **Shanthi Mogali**[1], **Verena E. Metz**[1], **Roberto Ciccocioppo**[2], and **Sandra D. Comer**[1]

[1]Division of Substance Abuse, New York Psychiatric Institute and Department of Psychiatry, College of Physicians and Surgeons of Columbia University, 1051 Riverside Drive, Unit 120, New York, New York 10032, USA

[2]Department of Experimental Medicine and Public Health, School of Pharmacy, Pharmacology Unit, University of Camerino, Via Madonna delle Carceri 9, Camerino, Macerata 62032, Italy

## Abstract

**Aims**—Activation of PPARγ by pioglitazone (PIO) has shown some efficacy in attenuating addictive-like responses in laboratory animals. The ability of PIO to alter the effects of opioids in humans has not been characterized in a controlled laboratory setting. The proposed investigation sought to examine the effects of PIO on the subjective, analgesic, physiological and cognitive effects of oxycodone (OXY).

**Methods**—During this investigation, nondependent prescription opioid abusers (N=17 completers) were maintained for 2–3 weeks on ascending daily doses of PIO (0 mg, 15 mg, 45 mg) prior to completing a laboratory session assessing the aforementioned effects of OXY [using a within-session cumulative dosing procedure (0, 10, and 20 mg, cumulative dose = 30 mg)].

**Results**—OXY produced typical mu opioid agonist effects: miosis, decreased pain perception, and decreased respiratory rate. OXY also produced dose-dependent increases in positive subjective responses. Yet, ratings such as: drug "liking," "high," and "good drug effect," were not significantly altered as a function of PIO maintenance dose.

**Discussion**—These data suggest that PIO may not be useful for reducing the abuse liability of OXY. These data were obtained with a sample of nondependent opioid users and therefore may not

*Corresponding author: Jermaine D. Jones, Ph.D., Ph: 646-774-6113, Fx: 646-774-6111, Jonesje@NYSPI.Columbia.edu, JermaineDJones@gmail.com.

**Declaration of Interests**

**Disclosures**

be applicable to dependent populations or to other opioids. Although PIO failed to alter the abuse liability of OXY, the interaction between glia and opioid receptors is not well understood so the possibility remains that medications that interact with glia in other ways may show more promise.

## Introduction

Until recently, the abuse liability of opioids was thought to be mediated primarily through neuronal processes. However, emerging evidence indicates that immunocompetent cells (glia) may contribute significantly to opioid pharmacology. Glial cells constitute over 50% of the cells in the central nervous system [CNS (1)]. Once thought to be the passive "glue" that fills the space between neurons, it is now recognized that glia play key roles in many neuronal processes and disease states (2, 3, 4, 5).

Research on the interaction between opioids and glia began over 30 years ago (6). Although this research has revealed that immune cells express opioid receptors and the mRNA to produce them, there remains vigorous debate over how opioids affect glia (7, 8, 9). Opioid use typically increases vulnerability to infection, suggesting that opioids are immunosuppressive (10). Much of the laboratory research seeking to investigate this hypothesis has examined the effects of the prototypic opioid, morphine. Several studies have shown that acute or chronic morphine exposure suppresses antibody response (11), macrophage activity (12), B-cell activity (13), T-cell activity and cytokine release (14; see also 15 & 16 for reviews).

Alternatively, it has been shown that opioids cause direct glial cell activation in a non-classical opioid receptor fashion, possibly via opioid-induced activation of a class of pattern recognition receptors referred to as toll-like receptors (17). For example, chronic morphine administration has been associated with an increase in proinflammatory cytokine protein and/or mRNA (18), glial fibrillary acid protein (19, 20), and proliferation (astrogliosis) and migration of astrocytes (21, 22, 23, 24, 25).

Although the cause of these discrepant findings is still under debate, investigators have begun to study opioid and glial interactions as a means to separate the beneficial effects of opioids (analgesia) from their detrimental effects (abuse potential). Preclinical research into the behavioral effects of opioid-glia interactions has found that selectively increasing glial (astrocyte) activity in the NAcc and intracingulate cortex results in significantly greater preference for morphine-associated cues relative to vehicle-associated cues [morphine-induced conditioned place preference (CPP)]. Investigators were able to attenuate this effect with a glial activity inhibitor (26).

As a logical extension of this work, there is a growing body of literature examining whether pharmacological modulators of glial cells may show some clinical utility to reduce the abuse potential of opioids (see reference 26 for a review). Ibudilast (AV411), minocycline, pentoxifylline and propentofylline are medications that suppress opioid-induced increases in immune factors (27, 28, 29). Furthermore, these glial modulators are hypothesized to decrease the rewarding effects of opioids. Ibudilast co-administered with morphine significantly reduced the magnitude of opioid-induced dopamine release in the NAcc (30),

and ibudilast, propentofylline, and minocycline reduce or completely block opioid-induced CPP (17, 26, 3).

Another glial modulator being investigated in this respect is the peroxisome proliferator-activated gamma receptor (PPARγ) agonist, pioglitazone (or Actos). In the central nervous system, PPARs are expressed in neurons, oligodendrocytes, and astrocytes (32, 33, 34, 35). PPARγ agonists have been shown to inhibit the expression of cytokines by monocytes/macrophages and microglia (36). Preclinical research further showed that PPARγ activation by pioglitazone (PIO) attenuated development of opioid tolerance (38) reduced heroin self-administration under a fixed-ratio and progressive-ratio schedule of reinforcement and heroin-induced increases in extracellular dopamine in the nucleus accumbens (39).

The ability of PIO to alter the effects of opioids in humans has not been characterized in controlled, clinical laboratory settings. As such, the primary aim of the current study was to examine the subjective effects of oxycodone (OXY) under maintenance on various doses of PIO [0 (placebo), 15, and 45 mg] in nondependent, prescription opioid abusers. Oxycodone was chosen because it is one of the most commonly prescribed and abused opioid analgesics (40, 41, 42). The PIO doses employed in this study are currently used clinically for the treatment of insulin resistance and type 2 diabetes (43, 44). The secondary aims of the study were to examine the influence of PIO on the analgesic, cognitive, and physiological effects of OXY. Based on preclinical evidence, we hypothesized that pioglitazone would decrease the positive subjective effects of oxycodone while potentiating its analgesic properties. If this hypothesis is supported, regulation of PPARγ may represent a new pharmacotherapeutic strategy to reduce the abuse of opioid drugs.

## Methods

### Participant Screening and Selection

Participants were recruited from the New York City metropolitan area through various print media advertisements. Respondents who met study inclusion/exclusion criteria, based upon the initial telephone interview, were scheduled to come to the New York State Psychiatric Institute for additional screening procedures. Screening consisted of both self-report and clinical interviews administered by a team of research assistants, psychologists, nurses, and physicians. Assessments were made of drug use, general health, and medical history, and multiple laboratory tests (hematology, blood chemistry panel, liver and thyroid functioning, urinalysis, and syphilis serology) were performed. Rapid urine drug screens assessed recent use of opioids, cocaine, benzodiazepines, cannabinoids, and amphetamines. Naloxone (0.2–0.8 mg) was administered intramuscularly during screening to determine if individuals were currently physiologically dependent on opioids.

Participants were required to be physically and mentally healthy recreational users of prescription opioids between the ages of 21 and 55 years. Potential participants were excluded from the study if they were seeking treatment for their drug use, had chronic pain, or had a severe Axis I psychiatric diagnosis. Potential participants were also excluded if they were physiologically dependent on opioids, alcohol or illicit drugs, with the presence of physiological withdrawal used as the principal criterion. As compensation, participants were

paid $25/day with a $25/day bonus for completing the study. All study procedures were approved by the Institutional Review Board of the New York State Psychiatric Institute (study # 6106).

### Study Design

This within-subjects investigation was placebo-controlled and conducted under single-blind conditions. Participants who passed the physiological and psychological screening were maintained on PIO (or placebo) throughout the ≈7–9 week study. For approximately 2–3 weeks participants were stabilized on each dose of PIO (0, 15, 45 mg) in ascending order. At the end of each maintenance period, the subjective, analgesic, physiological and cognitive effects of oral OXY (0 mg, 10, mg, 20 mg = cumulative dose of 30 mg) were examined. Cumulative dosing of opioids has been demonstrated to be an efficient and reliable strategy for assessing dose response within a single session. Data have also shown that cumulative opioid challenge drug dosing, conducted over time, produces reliable results with no evidence of habitation (45, 46, 47, 48, 49).

In its current clinical indications (diabetes mellitus type 2 and non-alcoholic steatohepatitis) a PIO effect typically takes 2 weeks to be observed (50). The dose order of PIO was not randomized due to the possibility of PIO carryover effects among the conditions. Although the current design also allows for the possibility of carryover effects, in this case they would have increased our likelihood of seeing a signal of PIO's effects. As the first clinical investigation into this drug for this indication, the investigators thought the latter was more important. The study was conducted on an outpatient basis with participants making 3–5 daily visits per week to the New York State Psychiatric Institute to receive study medication (administered and observed by a research nurse), provide a urine sample, and complete various study questionnaires (described below). Laboratory tests for blood glucose, liver function, and complete blood count, were performed weekly. Participants were provided with take-home PIO doses over the weekend, with pill counts and compliance assessments performed at each study visit. On the day of the lab session, participants were required to have not used alcohol recently (confirmed by breathalyzer) and provide a negative drug urine toxicology (Utox) the day of the lab session. Some exceptions were made for marijuana, because it remains detectable in the urine for such a long duration, as long as the reported use was not recent (within 2 days) based on self-report, and the participant appeared not to be acutely intoxicated. No other exceptions were made, and no lab sessions needed to be rescheduled due to the presence of other drugs of abuse.

The subjective effects of OXY (cumulative dose of 30 mg) were tested under each PIO maintenance condition. The daily maintenance dose of PIO was given on the day of the session 45 min prior to the OXY challenge doses (Table 1). Laboratory sessions typically began at 1000 hrs and took approximately 5–6 hours to complete. After the lab session, participants completed a field sobriety test and were sent home via a taxi or car service.

### Maintenance Phase Monitoring and Measures

During each outpatient visit, participants met with a research assistant and a nurse for study medication dispensing and monitoring (described below). Participants completed a number

of assessments of: general health, PIO side effects, and outpatient drug use. Participants generally completed 3–5 study visits per week. Participants were given take-home doses over the weekends, and for days that they did not appear at NYSPI for an outpatient visit. Participants were instructed to bring all of their study medication to each outpatient visit so that pill counts could be conducted by a research nurse in order to evaluate medication compliance.

Adverse medication effects were assessed at each visit using the SAFTEE (51). The SAFTEE queries participants concerning up to 30 possible adverse events (e.g., fever, gas, insomnia, palpitations, dry mouth, etc. …) and rates their: severity (1=mild, 2=moderate, 3=severe), relationship to study medication, any action taken by medical staff, and outcome. At each visit nurses also measured body weight, and drew blood weekly for blood chemistry (LFT, CBC, blood glucose, and BMP). After meeting with the nurse, participants provided a urine sample for 11-panel DrugCheck® urine dipstick analysis (w/pregnancy test). They also competed the Alcohol Use Disorders Identification Test (AUDIT-C) to assess alcohol use during the preceding week (52), a locally derived assessment of the type and frequency of other recreational drug use, and assessment of drug craving (opioids, alcohol, marijuana and tobacco) from 'Not at all' (0 mm) to 'Extremely' (100 mm).

### Laboratory Session Measures

**Subjective Effects—**Two questionnaires were used to assess subjective drug effects and opioid withdrawal symptoms. A Visual Analog Scale (VAS) was used to assess subjective and physiological drug effects such as "I feel a good drug effect" and "I feel high". Participants rated each item on the scale from 'Not at all' (0 mm) to 'Extremely' (100 mm). The second questionnaire was a modification of the Single Dose Questionnaire, which assessed for the presence of a number of potential drug effects by indicating "1" for True, or "2" for False (53, 54).

**Analgesic Effects—**The analgesic effects of OXY were evaluated with experimentally induced pain using the cold pressor test (CPT), a commonly used and well-established model for producing pain (55). Crushed ice was added to a cold tank, and warm water was placed in a warm tank. The temperature was maintained at 4°C in the cold tank (additional ice was added, if necessary) and 37°C in the warm tank. Each participant was asked first to immerse the hand in the warm tank for 2 min (to equalize baseline skin temperature across participants). Next, they were asked to immerse the same hand in the cold tank for up to 2 min. Standard instructions were read to each participant before administration of the CPT. During the cold water immersion, subjective ratings of pain were measured. Immediately following the CPT, subjective ratings of pain again were measured using the MPQ (CPT-MPQ) and the Pain Intensity/Bothersome Scales ('Not at all' (0) to 'Extremely' (10)) during which participants were asked to rate the 'Intensity' and 'Bothersomeness' of the acute pain experienced during immersion in cold (4°C) water during the CPT. Objective dependent measures included: pain threshold (time in seconds to the first report of pain) and pain tolerance (time until removal of the hand from water).

**Physiological Measures**—Miosis was assessed as a physiological indicator of mu agonist effects using a NeurOptics™ Pupillometer (Neuroptics INC. Irvine, CA) under ambient lighting conditions. For safety, a pulse oximeter continuously monitored oxygen saturation ($\%SpO_2$) during sessions, while respiration (breaths per minute), heart rate, and blood pressure (systolic and diastolic) were measured and recorded every 5 minutes. Supplemental oxygen was also provided throughout the session.

**Pharmacokinetic Measures**—Oxycodone plasma concentrations (ng/ml) were assessed throughout the lab session (Table 1). Blood (≈8 ml/sample) was collected from an intravenous catheter into tubes containing 15% EDTA and centrifuged. Blood samples collected at +165 minutes post PIO dosing were used to assess PIO levels. Plasma was separated from blood and frozen at −70°C. Frozen plasma samples were batched and transferred to the Analytical Psychopharmacology Laboratories at the Nathan Kline Institute for analysis.

### Drugs

**Pioglitazone** tablets (15 mg) were provided by the OMEROS Corporation (Seattle, WA). Each daily dose consisted of 3 capsules of active drug and/or lactose-filled placebo, depending on the final target dose (e.g. 45 mg = 3 ∘ 15 mg tablets; 15 mg = 1 ∘ 15 mg tablet + 2 placebo tablets; 0 mg = 3 placebo tablets). During weekly study visits, the 3 tablets were administered to participants by a research nurse. For weekend dosing, participants were provided with a separate bottle that contained the 3 tablets for each day. Back-up doses were also provided in this manner, in case participants missed a study visit. All drug over-encapsulation and packaging was performed by the New York State Psychiatric Institute Pharmacy.

**Oxycodone HCL** tablets (5 mg) were purchased from TYCO Healthcare (Princeton, NJ). For blinding, tablets were over-encapsulated and participants were given 4 capsules consisting of active drug and/or lactose-filled placebo.

**Naloxone HCl** (Narcan) for IM injection was obtained from the International Medication System Limited Amphastar (South Elmonte, CA).

### Statistical Analyses

Continuous and categorical demographic variables and adverse events reports were summarized descriptively. Regarding data collected during the maintenance phases, univariate analyses of variance (ANOVA) tests were employed to examine changes in drug use and drug craving as a function of PIO maintenance dose (summed across each of the 3 periods). When analyzing the data from the lab sessions, a repeated-measures ANOVA was used to compare the time course of drug effects over the various time points throughout the session for each of the PIO maintenance doses (Main effects: PIO Dose & Time). Because of the cumulative dosing procedure, this analysis allowed the investigators to evaluate the overall influence of PIO on the effects of the cumulative OXY dose (30 mg). In order to examine the effects of PIO on each individual OXY (0, 10, 30 mg) dose, planned comparisons were used to assess maximal drug effect (peak=increase or trough=decrease)

within the timeframe immediately prior to the next oxycodone administration. These planned comparisons of PIO dose failed to reveal any significant effects, and thus, for the sake of brevity were not included in this report. An α of $p<0.05$ was considered statistically significant, while $p<0.10$, was considered as trending towards significance. All data analyses were performed using SPSS version 18 (56) and SuperANOVA (57).

## Results

### Participants

Between October, 2010 and August, 2013, 32 participants were enrolled into the study. Fifteen participants either voluntarily withdrew from the study or were dropped by the investigators due to a number of factors including: elevated liver function tests ($n = 1$), vasovagal episode during the lab session ($n = 2$), becoming opioid dependent during outpatient phase ($n = 1$), pulmonary embolism ($n = 1$, determined to be unrelated to the study), difficulty interacting with staff ($n = 1$), missing too many outpatient visits ($n = 7$) and misrepresenting age or drug use history ($n = 2$). Complete data sets were obtained from 17 participants for inclusion in this analysis. The average age of the participants was 35.0 years ($\pm 9.0$), including 15 men and 2 women. The racial breakdown of the completers was as follows: 8 African-American /Black, 4 Caucasian/White, 3 Multiracial, 1 Native-American or Alaskan, 1 not reported). Ethnically, 5 of the 17 completers considered themselves to be Hispanic/Latino.

All participants had recent histories of recreational use of Rx opioids (within the last year). The majority of participants used Rx opioids on a "Weekly" basis ($n = 9$), followed by "Monthly" use ($n = 4$), "Less than Monthly" ($n = 3$) and daily ($n = 1$, opioid use may have been over reported, since naloxone challenge indicated the participant was not physiologically dependent). All participants abused Rx opioids orally, with the exception of 2 who used via oral and intranasal routes. The mean duration of recreational opioid use was 4.1 years ($\pm 3.5$). In addition to their opioid use, 70% ($n = 12$) of the participants reported occasional alcohol use (weekly-monthly, ave 4.4 drinks/month), 56% ($n = 9$) were regular marijuana users (4 daily, 2 weekly, 3 monthly), 41% ($n = 7$) were regular tobacco smokers (daily-weekly use), and 30% used stimulants (cocaine or amphetamines) sporadically (monthly or less). The use of heroin, sedatives (benzodiazepines or barbiturates), club drugs (ecstasy, GHB, ketamine) and hallucinogens (LSD or PCP) was rare among this sample.

### Maintenance Phase Measures

Prior to completing the OXY challenge testing, participants were maintained on PIO 0 mg for an average of 14.4 days ($\pm 3.1$), PIO 15 mg for 16.2 days ($\pm 2.8$) and PIO 45 mg for 15.6 days ($\pm 3.2$). Variability in dosing duration was the result of accommodation for scheduling (e.g., holidays and weekends) and participant variables (e.g., availability for scheduling sessions). The adverse events judged to be "possibly" related to the study medication and reported by multiple participants were: GI upset, gas, fatigue and drowsiness. The severity of these symptoms was rated as "mild to moderate." Adverse effects reported less regularly included chills and sweating. During the maintenance phases, mean VAS ratings for "I Want: Opioids, Alcohol, Marijuana and Tobacco," failed to significantly differ as a function of PIO

maintenance dose (Table 2). Additionally, the number of positive drug UTox samples and breathalyzer tests did not differ significantly across the three PIO maintenance phases.

## Lab Session Measures

**Positive Subjective Effects**—VAS assessments of positive subjective effects including: "Liked the Choice (Figure 1: Upper Panel)," "High," "Good Effect," "Quality," "Stimulated," and amount participants "Would Pay" for the drug, increased as a function of Time (p's < 0.001). For all measures, the maximal drug effect was observed between 180 and 210 minutes (i.e. ratings increased as a function of increasing OXY dose). For none of these measures was there a significant main effect of PIO dose, and no PIO X Time interaction. This same pattern of results was found for the ARCI measures of: "I Feel:" "High," "Relaxed," and "Drunken," (Time, p's < 0.05), and again with the more general indicators of drug effect, VAS "Potent," and "Sedated" (p's < 0.001).

**Negative Subjective Effects**—VAS ratings of "Irritable" significantly increased later in the session (Time, p< 0.01). In contrast, VAS ratings of overall "Bad Drug Effect" were minimal throughout the laboratory session, although the main effect of Time did approach significance (p = 0.07, shown in Figure 1: Lower Panel). ARCI ratings of "I Feel Nervous" did not significantly vary as a function of Time or PIO dose. None of these ratings varied as a function of PIO maintenance dose and no interaction was found. Significant drug effects were found on subjective measures of gastrointestinal (GI) upset. ARCI measures of "Turning Stomach," and VAS assessment of "Nauseous" increased as the session progressed (Time, p's < 0.001). Again, no effect of PIO or interaction was found on these measures.

**Other Drug Effects**—Assessments of: "Mellow," "Restless," and "Sleepy," increased as a function of Time (p < 0.001, p <0.01, & p < 0.001; respectively) but there were no significant main effects of PIO dose, and no PIO X Time interactions. There were no significant findings (Time, PIO, PIO X Time) on assessments of: "Alert," "Anxious," "Depressed," "Energetic," "Gooseflesh," "Muscle Pain," or "Talkative."

**Experimental pain/CPT**—ANOVA revealed that as the laboratory session progressed (and the OXY dose increased) there were significant increases in the analgesic effects of oxycodone. Active doses of oxycodone doubled latency of participants to withdraw their hand from 4° water (p < 0.001, Figure 2: Upper Panel) and latency to report feeling pain (p < 0.001), while significantly decreasing self-reported "Bothersomeness" of the pain (p < 0.01). These ratings did not vary as a function of PIO maintenance dose.

**Physiological Effects**—The average pupil diameter, heart rate and breaths per minute decreased significantly as the session progressed (Time: p < 0.01, p < 0.05, p < 0.01, respectively). However, no influence of PIO, or PIO x Time interactions were found.

**Pharmacokinetic Measures**—Mean plasma oxycodone levels significantly increased as function of Time (p < 0.001), but no effects of PIO or interactions were found (Figure 2, Lower Panel). Analysis of plasma samples also revealed that significantly higher levels of

plasma PIO were achieved following active PIO maintenance periods vs placebo periods (Shown in Table 2, p < 0.01).

## Discussion

The current study sought to determine whether pioglitazone, a PPARγ agonist and glial modulator, would alter the effects of oxycodone. In our sample of non-dependent recreational prescription opioid users, oral OXY produced typical mu opioid agonist effects, including miosis, decreased pain perception and respiratory rate, and mild cognitive impairment. Oxycodone also produced dose-dependent increases in reports of positive subjective effects with relatively minimal aversive effects. This subjective profile (which included mild GI upset) is similar to what has been previously reported by comparable participant samples (59, 60, 61). These data, once again, demonstrate the abuse potential common to most opioid analgesics, exemplifying the need to develop ways to alter these effects in order to deter recreational use that often leads to abuse and dependence (62). Unfortunately, our data revealed that an approximately 2-week period of PIO maintenance had no observable influence on the subjective, cognitive, analgesic, or physiological effects of OXY. Moreover, drug craving and recreational drug use did not vary as a function of PIO during the outpatient periods in between the testing sessions.

Pioglitazone dosing parameters (duration and mg amounts) were based on its clinical utility for treating diabetes, though these may not be related to its effects on glia (63). Although robust plasma concentrations of PIO were achieved, the lack of an observable PIO x OXY interaction may have been due to an inability of our dosing parameters to reduce levels of inflammatory markers. Unfortunately, the investigators did not include a measure of glial activation to test this hypothesis. The most relevant measurements of inflammatory markers would come from cerebrospinal fluid (CSF) but performing spinal taps on our participants would have significantly increased the risks associated with the study, and would have not been well-tolerated (64). Although measurements of inflammatory markers can also be found in plasma samples, previous research has demonstrated that plasma levels of inflammatory markers cannot be used to identify relative changes in the CSF (65).

The results of this study may also have varied if an opioid-dependent sample had been employed. According to the theory of opioid-induced glial activation and neuroinflammation; chronic opioid (ab)users may have higher tonic levels of glial activity upon which PIO could act (17, 29, 66). The use of a nondependent sample may also explain why the results of the current study are discrepant with preclinical studies on the efficacy of PIO for this purpose (39). Animal models may reflect a more severe state of opioid dependence.

In light of the present data, we must also consider the possibility that the opioid effects studies here may not be modulated by its actions on glial cells. As previously mentioned, there is an alternative body of literature arguing that opioids are immunosuppressive. According to this dissenting opinion, the logic behind the proposed clinical utility of an opioid + glia inhibitor combination is flawed. Therefore, the lack of an interaction observed in the current study would be consistent with this line of research (16, 67). Due to the

primary limitation of this study (i.e., lack of a measure of glial activity), what the current data adds to this debate is limited.

The primary limitation of this study (i.e., lack of a measure of glial activity) reflects the difficulty in performing this type of animal-to-human translational research. Specifically, no viable tools are available in clinical settings to confirm the mechanism by which glial modulators, such as ibudilast, minocycline, or pioglitazone, may be producing their effects. Although, within the current parameters, PIO failed to alter the subjective effects of OXY, it may be more effective in treating other forms of drug dependences. PPAR agonists have been shown to decrease ethanol consumption, ethanol-seeking and reduce withdrawal severity and susceptibility to stress-induced relapse in rodents (68, 69, 70).

Although PIO failed to alter the abuse liability of OXY under the current parameters, the interaction between glia and opioid receptors is not well understood. Other glial modulators acting through different mechanisms may show more promise. The prevalence and serious adverse consequences of opioid abuse make it imperative that we continue to investigate novel interventions such as this.

## Acknowledgments

## References

1. Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B. Neocortical glial cell numbers in human brains. Neurobiol Aging. 2008; 29:1754–62. [PubMed: 17544173]

2. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19: 312–18. [PubMed: 8843599]

3. Nakajima K, Kohsaka S. Functional roles of microglia in the brain. Neurosci Res. 1993; 17:187–203. [PubMed: 8233123]

4. Stoll G, Jander S. The role of microglia and macrophages in the pathophysiology of the CNS. Prog Neurobiol. 1999; 58: 233–47. [PubMed: 10341362]

5. Verkhratsky A, Kettenman H. Calcium signaling in glial cells. Trends Neurosci. 1996; 19: 346–52. [PubMed: 8843604]

6. Wybran J, Appelboom T, Famaey JP, Govaerts A. Suggestive evidence for receptors for morphine and methionine–enkephalin on normal human blood T lymphocytes. J Immunol. 1979; 123:1068–70. [PubMed: 224107]

7. Carr DJ, Rogers TJ, Weber RJ. The relevance of opioids and opioid receptors on immunocompetence and immune homeostasis. Proc Soc Exp Biol Med. 1996; 213:248–57. [PubMed: 8985308]

8. Gomez-Flores, R., Weber, RJ. Opioids, opioid receptors, and the immune system. In: Plotnikoff, NP.Faith, RE.Murgo, AJ., Good, RA., editors. Cytokines, stress and immunity. CRC Press; Boca Raton: p. 281-314.

9. Mellon RD, Bayer BM. Evidence for central opioid receptors in the immunomodulatory effects of morphine: review of potential mechanism(s) of action. J Neuroimmunol. 1998; 83: 19–28. [PubMed: 9610669]

10. Alonzo NC, Bayer BM. Opioids immunology and host defenses of intravenous drug abusers. Infect Dis Clinic N Am. 2002; 16: 553–69.

11. Bussiere JL, Adler MW, Rogers TJ, Eisenstein TK. Cytokine reversal of morphine-induced suppression of the antibody response. J Pharmacol Exp Ther. 1993; 264: 591–97. [PubMed: 8437110]

12. Pacifici R, di Carlo S, Bacosi A, Zuccaro P. Macrophage functions in drugs of abuse-treated mice. Int J Immunopharmacol. 1993; 15: 711–16. [PubMed: 8407054]

13. Zhang EY, Xiong J, Parker BL, Chen AY, Fields PE, Ma X, et al. Depletion and recovery of lymphoid subsets following morphine administration. Br J Pharmacol. 2011; 164: 1829–44. [PubMed: 21557737]

14. Bhargava HN, Thomas PT, Thorat S, House RV. Effects of morphine tolerance and abstinence on cellular immune function. Brain Res. 1994; 642: 1–10. [PubMed: 8032870]

15. Eisenstein, TK., Hilburger, ME., Lawrence, DMP. Immunomodulation by morphine and other opioids. In: Friedman, H.Klein, TW., Specter, S., editors. Drugs of abuse, immunity and infections. CRC Press; Boca Raton: 1996. p. 103-120.

16. Eisenstein TK, Rahim RT, Feng P, Thingalaya NK, Meissler JJ. Effects of opioid tolerance and withdrawal on the immune system. J Neuroimmune Pharmacol. 2006; 1: 237–49. [PubMed: 18040801]

17. Hutchinson MR, Lewis SS, Coats BD, et al. Reduction of opioid withdrawal and potentiation of acute opioid analgesia by systemic AV411 (ibudilast). Brain Behav Immun. 2009; 23: 240–50. [PubMed: 18938237]

18. Johnston IN, Milligan ED, Wieseler-Frank J, Frank MG, Zapata V, Campisi J, et al. A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine. J Neurosci. 2004; 24: 7353–65. [PubMed: 15317861]

19. Eng LF. Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. J Neuroimmunol. 1985; 8: 203–14. [PubMed: 2409105]

20. Marie-Claire C, Courtin C, Roques BP, Noble F. Cytoskeletal genes regulation by chronic morphine treatment in rat striatum. Neuropsychopharmacol. 2004; 29: 2208–15.

21. Beitner-Johnson D, Guitart X, Nestler EJ. Glial fibrillary acidic protein and the mesolimbic dopamine system: regulation by chronic morphine and Lewis-Fischer strain differences in the rat ventral tegmental area. J Neurochem. 1993; 61:1766–73. [PubMed: 8228992]

22. Garrido E, Perez-Garcia C, Alguacil LF, Diez-Fernandez C. The alpha2-adrenoceptor antagonist yohimbine reduces glial fibrillary acidic protein upregulation induced by chronic morphine administration. Neurosci Lett. 2005; 383: 141–4. [PubMed: 15936527]

23. Song P, Zhao ZQ. The involvement of glial cells in the development of morphine tolerance. Neurosci Res. 2001; 39: 281–6. [PubMed: 11248367]

24. Hutchinson MR, Watkins LR. Why is neuroimmunopharmacology crucial for the future of addiction research? Neuropharmacolo. 2014; 76: 218–27.

25. Watkins LR, Hutchinson MR, Johnston IN, Maier SF. Glia: novel counter-regulators of opioid analgesia. Trends Neurosci. 2005; 28: 661–9. [PubMed: 16246435]

26. Narita M, Miyatake M, Narita M, et al. Direct evidence of astrocytic modulation in the development of rewarding effects induced by drugs of abuse. Neuropsychopharmacol. 2006; 31:2476–88.

27. Cooper ZD, Jones JD, Comer SD. Glial Inhibitors: A novel pharmacological approach to modulating the behavioral effects of abused substances. Expert Opin Investig Drugs. 2012; 21: 169–78.

28. Ledeboer A, Sloane EM, Milligan ED, et al. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain. 2005; 115: 71–83. [PubMed: 15836971]

29. Sweitzer, S., De Leo, J. Methylxanthines, Handbook of Experimental Pharmacology. Vol. 200. Berlin: Springer-Verlag; 2011. Propentofylline: glial modulation, neuroprotection, and alleviation of chronic pain.

30. Tawfik VL, Nutile-McMenemy N, Lacroix-Fralish ML, Deleo JA. Efficacy of propentofylline, a glial modulating agent, on existing mechanical allodynia following peripheral nerve injury. Brain Behav Immun. 2007; 21:238–46. [PubMed: 16949251]

31. Bland ST, Hutchinson MR, Maier SF, et al. The glial activation inhibitor AV411 reduces morphine-induced nucleus accumbens dopamine release. Brain Behav Immun. 2009; 23: 492–7. [PubMed: 19486648]

32. Hutchinson MR, Bland ST, Johnson KW, Rice KC, Maier SF, Watkins LR. Opioid-induced glial activation: mechanisms of activation and implications for opioid analgesia, dependence and reward. Scientific World J. 2007; 7: 98–111.

33. Gelinas DS, Lambermon MH, McLaurin J. Ciglitazone increases basal cytokine expression in the central nervous system of adult rats. Brain Res. 2005; 1034: 139–46. [PubMed: 15713265]

34. Moreno S, Farioli-Vecchioli S, Ceru MP. Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. Neurosci. 2004; 123:131–145.

35. Sarruf DA, Yu F, Nguyen HT, Williams DL, Printz RL, Niswender KD, Schwartz MW. Expression of peroxisome proliferator-activated receptor-gamma in key neuronal subsets regulating glucose metabolism and energy homeostasis. Endocrinol. 2009; 150: 707–12.

36. Woods JW, Tanen M, Figueroa DJ, Biswas C, Zycband E, Moller DE, Austin CP, Berger JP. Localization of PPARdelta in murine central nervous system: expression in oligodendrocytes and neurons. Brain Res. 2003; 975: 10–21. [PubMed: 12763589]

37. Kielian T, Drew PD. Effects of PPARc agonists on central nervous system inflammation. J Neurosci Res. 2003; 71:315–25. [PubMed: 12526021]

38. de Guglielmo G, Kallupi M, Scuppa G, Stopponi S. Demopulos G Gaitanaris G Ciccocioppo R. Analgesic tolerance to morphine is regulated by PPARγ. Br J Pharmacol. 2014; 171: 5407–16. [PubMed: 25048682]

39. de Guglielmo G, Melis M, De Luca MA, Kallupi M, Li HW, Niswender K, Giordano A. PPARγ Activation Attenuates Opioid Consumption and Modulates Mesolimbic Dopamine Transmission. Neuropsychopharmacol. 2015; 40: 927–37.

40. Cone EJ, Fant RV, Rohay JM. Oxycodone involvement in drug abuse deaths: a DAWN-based classification scheme applied to an oxycodone postmortem database containing over 1000 cases. J Anal Toxicol. 2003; 27: 57–67. [PubMed: 12669998]

41. Dhalla IA, Mamdani MM, Sivilotti ML. Prescribing of opioid analgesics and related mortality before and after the introduction of long-acting oxycodone. CMAJ. 2009; 181: 891–96. [PubMed: 19969578]

42. Katz N, Fernandez K, Chang A, et al. Internet-based survey of nonmedical prescription opioid use in the United States. Clin J Pain. 2008; 24: 528–35. [PubMed: 18574362]

43. Chang F, Jaber LA, Berlie HD, O'Connell MB. Evolution of peroxisome proliferator-activated receptor agonists. Ann Pharmacother. 2007; 41: 973–83. [PubMed: 17519293]

44. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPAR gamma. Annu Rev Biochem. 2008; 77: 289–312. [PubMed: 18518822]

45. Begelow G, Preston KL, Schmittner J, Dong Q, Gastfriend DR. Opioid Challenge Evaluation of Blockade by Extended-Release Naltrexone in Opioid-Abusing Adults: Dose-Effects and Time-Course. Drug Alcohol Depend. 2012; 123(1–3):57–65. [PubMed: 22079773]

46. Walker DJ, Zacny JP. Subjective, psychomotor, and physiological effects of cumulative doses of opioid mu agonists in healthy volunteers. J Pharmacol Exp Ther. 1999; 289(3):1454–64. [PubMed: 10336539]

47. Walker DJ, Zacny JP, Galva KE, Lichtor JL. Subjective, psychomotor, and physiological effects of cumulative doses of mixed-action opioids in healthy volunteers. Psychopharmacology (Berl). 2001; 155(4):362–371. [PubMed: 11441425]

48. Strain EC, Walsh SL, Bigelow GE. Blockade of hydromorphone effects by buprenorphine/naloxone and buprenorphine. Psychopharmacology (Berl). 2002; 159(2):161–6. [PubMed: 11862344]

49. Walsh SL, Sullivan JT, Preston KL, Garner JE, Bigelow GE. Effects of naltrexone on response to intravenous cocaine, hydromorphone and their combination in humans. J Pharmacol Exp Ther. 1996; 279: 524–38. [PubMed: 8930154]

50. Berger J, Wagner JA. Physiological and therapeutic roles of peroxisome proliferator-activated receptors. Technol Ther. 2002; 4: 163–74.

51. Levine J, Scholer NR. Strategies for analyzing side effect data from Safetee: a workshop held Fall 1985 in Rockville, Maryland. Psychopharmacol Bull. 1986; 22: 343–57. [PubMed: 3774930]

52. Bush K, Kivlahan DR, McDonell MB, et al. The AUDIT Alcohol Consumption Questions (AUDIT-C): An effective brief screening test for problem drinking. Arch Internal Med. 1998:1789–95. [PubMed: 9738608]

53. Fraser HF, Van Horn GD, Martin WR, Wolbach AB, Isbell H. Methods for evaluating addiction liability. (A) "Attitude" of opiate addicts toward opiate-like drugs. (B) a short-term "direct" addiction test. J Pharmacol Exp Ther. 1961; 133: 371–87. [PubMed: 13701509]

54. Martin WR, Fraser HF. A comparative study of physiological and subjective effects of heroin and morphine administered intravenously in post-addicts. J Pharmacol Exp Ther. 1961; 133: 388–99. [PubMed: 13767429]

55. Zacny JP, McKay MA, Toledano AY, Marks S, Young CJ, Klock PA, et al. The effects of a cold-water immersion stressor on the reinforcing and subjective effects of fentanyl in healthy volunteers. Drug Alcohol Depend. 1996; 42: 133–42. [PubMed: 8889412]

56. SPSS I. SPSS 18.0.0 for windows. Chicago, Illinois: 2009.

57. Gagnon J, Roth JM, Carroll M, Haycock KA, Plamondon J, Feldman DS, Simpson J. Superanova accessible general linear modeling. Yale J Biolo Med. 1990; 63: 191–2.

58. Walsh SL, Nuzzo PA, Lofwall MR, Holtman JR Jr. The relative abuse liability of oral oxycodone, hydrocodone and hydro-morphine assessed in prescription opioid abusers. Drug Alcohol Depend. 2008; 98: 191–202. [PubMed: 18606504]

59. Zacny JP, Gutierrez S. Characterizing the subjective, psychomotor, and physiological effects of oral oxycodone in non-drug-abusing volunteers. Psychopharmacol. 2003; 170: 242–54.

60. Zacny JP, Gutierrez S. Within-subject comparison of the psychopharmacological profiles of oral hydrocodone and oxycodone combination products in non-drug-abusing volunteers. Drug Alc Depend. 2009; 101: 107–14.

61. Wightman R, Perrone J, Portelli I, Nelson L. Likeability and abuse liability of commonly prescribed opioids. J Med Toxicol. 2012; 8: 335–40. [PubMed: 22992943]

62. Gillies PS, Dunn CJ. Pioglitazone. Drugs. 2000; 60: 333–43. [PubMed: 10983737]

63. Banks RE. Measurement of cytokines in clinical samples using immunoassays: problems and pitfalls. Crit Rev Clin Lab Sci. 2000; 37: 131–82. [PubMed: 10811142]

64. Hopkins SJ, McMahon CJ, Singh N, Galea J, Hoadley M, Scarth S, Patel H, Vail A, Hulme S, Rothwell NJ, King AT, Tyrrell PJ. Cerebrospinal fluid and plasma cytokines after subarachnoid haemorrhage: CSF interleukin-6 may be an early marker of infection. J Neuroinflammation. 2012; 9: 255–64. [PubMed: 23176037]

65. Watkins LR, Hutchinson MR, Rice KC, Maier SF. The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. Trends Pharmacol Sci. 2009; 30: 581–91. [PubMed: 19762094]

66. Eisenstein TK. Opioids and the immune system: what is their mechanism of action? Br J Pharmacol. 2011; 164: 1826–8. [PubMed: 21627636]

67. Stopponi S, de Guglielmo G, Somaini L, Cippitelli A, Cannella N, Kallupi M, Ubaldi M, Heilig M, Demopulos G, Gaitanaris G, Ciccocioppo R. Activation of PPARγ by pioglitazone potentiates the effects of naltrexone on alcohol drinking and relapse in msP rats. Alcohol Clin Exp Res. 2013; 37: 1351–60. [PubMed: 23550625]

68. Stopponi S, Somaini L, Cippitelli A, Cannella N, Braconi S, Kallupi M, Ruggeri B, Heilig M, Demopulos G, Gaitanaris G, Massi M, Ciccocioppo R. Activation of nuclear PPARγ receptors by

the antidiabetic agent pioglitazone suppresses alcohol drinking and relapse to alcohol seeking. Biol Psychiatry. 2011; 69: 642–9. [PubMed: 21276964]

69. Ferguson LB, Most D, Blednov YA, Harris RA. PPAR agonists regulate brain gene expression: relationship to their effects on ethanol consumption. Neuropharmacolo. 2014; 86: 397–407.
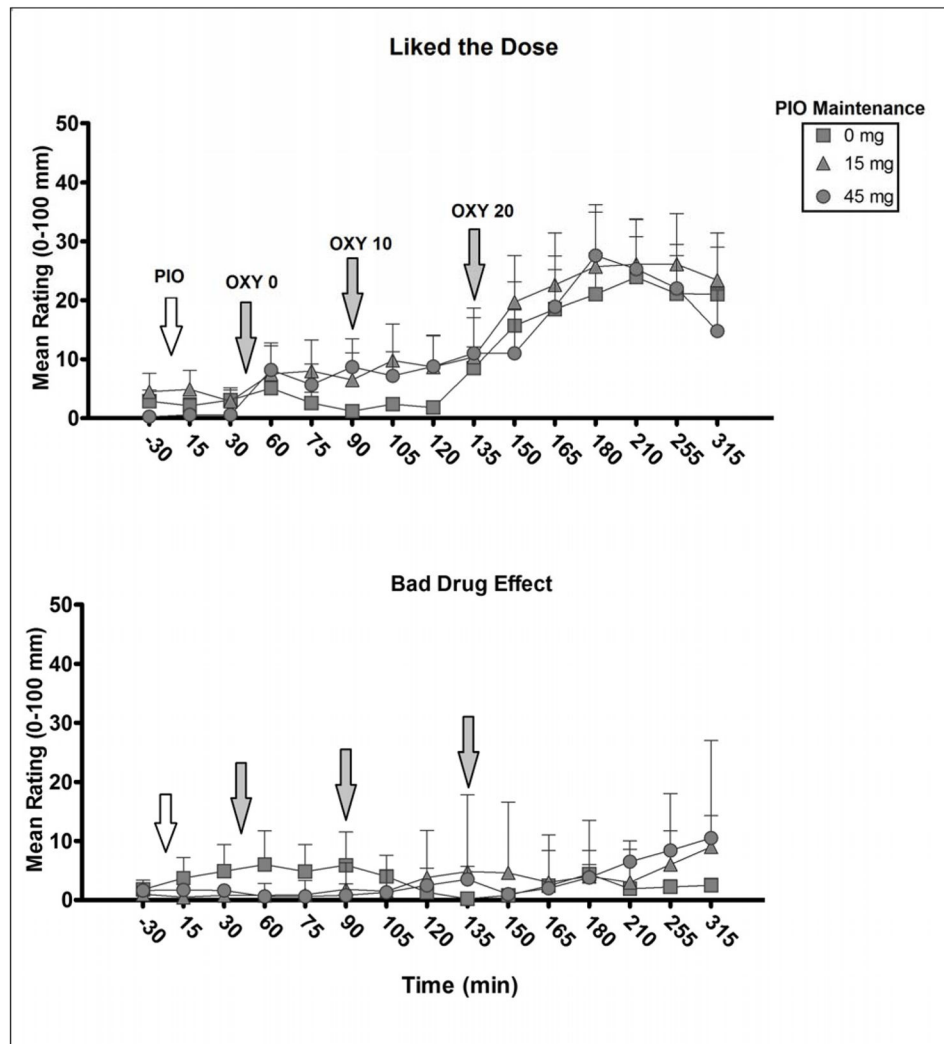
**Figure 1.**
Mean (± SEM) Visual Analog Scale (VAS) ratings of "Liked the Drug" and "Bad" drug effect, shown as a function of each preceding PIO maintenance condition (n=17).
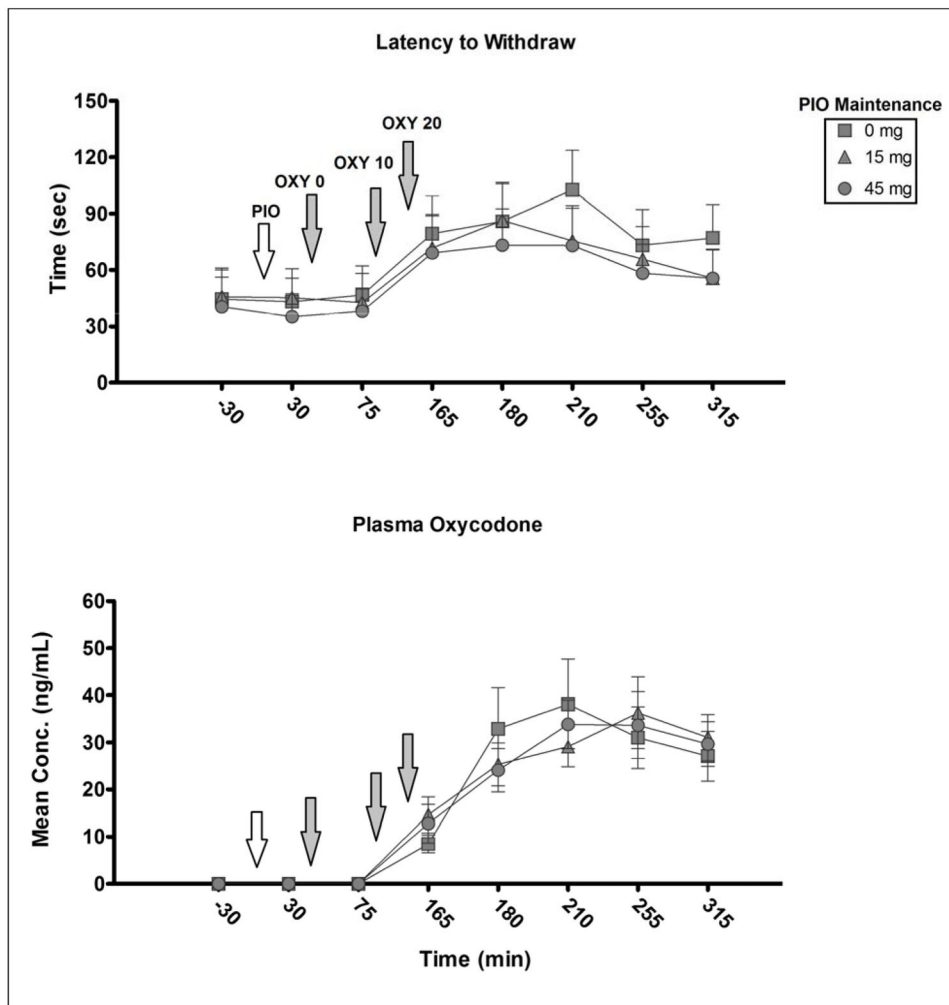
**Figure 2.**
Mean (± SEM) amount of time participants (n=17) kept their hand immersed in cold water
(4° C), and plasma oxycodone concentration (ng/ml), shown as a function of each preceding
PIO maintenance condition.

**Table 1**

**Laboratory Session Events**

Time points at which physiological, plasma, performance and subjective assessments were made throughout the testing session.

| Time (min) | Event |
|---|---|
| −30 | Begin continuous physiological monitoring, pupils, subjective effects, CPT, performance battery |
| **0** | **Pioglitazone or Placebo** |
| 15 | Subjective effects, pupils, |
| 30 | Subjective effects, pupils, blood, CPT |
| **45** | **OXY 0 mg, PO** |
| 60 | Subjective effects, pupils, |
| 75 | Subjective effects, pupils, blood, CPT |
| **90** | **OXY 10 mg, PO:** Subjective effects, pupils |
| 105 | Subjective effects, pupils, |
| 120 | Subjective effects, pupils, blood |
| **135** | **OXY 20 mg, PO (cumulative dose of 30 mg):** Subjective effects, pupils |
| 150 | Subjective effects, pupils, |
| 165 | Subjective effects, pupils, blood, CPT |
| 180 | Subjective effects, pupils, blood, CPT, |
| 195 | Performance battery |
| 210 | Subjective effects, pupils, CPT |
| 255 | Subjective effects, pupils, performance battery, CPT |
| 315 | Subjective effects, pupils, blood, CPT, performance battery |

**Table 2**

**Mean Maintenance Phase Measures**

[a]

Results of various study measures collected during the 3 PIO maintenance periods leading to laboratory testing.

| | PIO 0 mg | PIO 15 mg | PIO 45 mg | | |
|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | Mean (SD) | F Value | Sig. |
| **I Want Rx Ops (0–100)** | 6.4 (9.7) | 3.9 (8.6) | 4.2 (9.3) | 1.4 | 0.9 |
| **I Want Marijuana** | 12.8 (28.2) | 12.3 (21.1) | 9.4 (15.9) | 1.1 | 0.85 |
| **I Want Alcohol** | 5.1 (24.4) | 4.5 (12.5) | 3.3 (10.3) | 0.79 | 0.78 |
| **I Want Tobacco** | 11.9 (27.8) | 13.4 (23.0) | 9.5 (16.9) | 0.66 | 0.74 |
| **# of Opioid + UTox [b]** | 0.71 (0.88) | 1.1 (0.5) | 1.3 (0.8) | 0.74 | 0.47 |
| **# of Cocaine + UTox [b]** | 2.0 (1.9) | 3.6 (2.2) | 3.8 (1.9) | 0.32 | 0.22 |
| **Plasma PIO [c] (ng/ml)** | 0.0 (0.0) | 514 (257) | 1155 (514) | **9.65** | **0.001** |

[a]
Only positive samples for cocaine, opioids, oxycodone and THC were found. THC is unreported because the drug's persistence in urine samples significantly confounds estimating separate positive samples.

[b]
Mean taken only from those participants who tested positive.

[c]
Obtained during the lab session following each maintenance/stabilization phase.