Current Topics in Pharmacology
Vol. 26, 2022

Original Article

# Oxytocin and nalmefene combination reduced excessive alcohol drinking and 'relapse' in both male and female mice

**Yan Zhou*, David C. Zhou and Mary Jeanne Kreek**

Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

## ABSTRACT

In recent clinical studies, intranasal oxytocin (OX) was found to reduce alcohol craving and decrease withdrawal symptoms in alcohol-dependent patients with high anxiety, suggesting that neuropeptide OX has potential for the development as an anti-alcoholism treatment. In rodent models, oxytocin receptor (OXTR) activation by central or systemic OX administration reduces alcohol consumption and cue-induced alcohol seeking. An interaction between mu-opioid receptors (MOR) and OX/OXTR is well established: beta-endorphin decreases OX neuronal activity. Recently we reported that co-administration of OX with MOR antagonist naltrexone profoundly decreased excessive and relapse-like alcohol drinking in mice. Here we further explored whether nalmefene, a clinically used MOR antagonist with partial kappa-opioid receptor agonist, could improve the effect of OX on alcohol intake. We found that OX at a subeffective dose 0.03 mg/kg (effective dose 0.1 mg/kg) combined with nalmefene at a subeffective dose 0.125 mg/kg (effective dose 0.5 mg/kg) reduced excessive alcohol intake in both male and female mice in a 3-week intermittent-access alcohol drinking model. Similarly, relapse-like alcohol drinking after 1-week abstinence in an alcohol deprivation effect (ADE) model was effectively prevented by the OX and nalmefene combination at subeffective doses without sex difference. Our study suggests that OX and nalmefene combination offers a novel approach in alcoholism treatment.

**KEYWORDS:** nalmefene, oxytocin, combined therapy, excessive alcohol drinking, alcohol deprivation effect.

## 1. INTRODUCTION

There are high expression levels of neuropeptide oxytocin (OX) and its G protein-coupled receptor oxytocin receptor (OXTR) in many brain structures, including the mesolimbic regions, hypothalamus and extended amygdala [1, 2]. In both humans and rodents, chronic alcohol exposure or withdrawal profoundly altered the brain OX/OXTR levels or activity: (A) OX levels were persistently decreased in the hypothalamus of rats, mice, and humans [3-7]; and (B) in contrast, there are increases in OXTR at both binding and mRNA levels in several brain regions of the rats (prefrontal cortex, striatum, amygdala, hippocampus) and in similar brain post-mortem regions of human alcoholics [4]. Therefore, after chronic alcohol exposure and then in the negative affective state during withdrawal, there seems a relative 'OX deficiency' with an increased OXTR activity that may be the part of homeostatic neuroadaptations of the OX/OXTR circuits [8, 9].

Consistently, neuropharmacological studies have provided supportive evidence as OX reduces alcohol rewarding effect [10] and alcohol consumption in mice and rats [8, 11-14], and blocks alcohol seeking in the alcohol-dependent rats [4, 15, 16].

*Corresponding author: zhouya@rockefeller.edu

In the first clinical study on alcohol-dependent patients, intranasal OX was found to lessen alcohol withdrawal symptoms [17]. A separate clinical study further observed that the cue-induced alcohol craving was decreased by intranasal OX in the patients with high anxiety [18]. In a functional magnetic resonance imaging study with human heavy social drinkers, it was found that intranasal OX decreased neural reactivity to alcohol-related cues [4]. However, other clinical data have found that OX either has negative outcome or modest therapeutic value [19-22].

In human alcoholics, nalmefene, a mixed mu-opioid receptor (MOR) antagonist and partial kappa-opioid receptor (KOR) agonist, decreases excessive alcohol consumption, craving and relapse episodes [23]. Nalmefene was later approved for reducing alcohol consumption in alcohol-dependent patients in Europe [24]. In alcohol-dependent rats and mice, nalmefene decreases alcohol intake [25, 26]. In line with these observations, we recently found that nalmefene reduced excessive consumption (intake and preference) in an intermittent-access drinking model, prevented relapse-like drinking in an alcohol deprivation effect (ADE) model, and 'binge' drinking in a drinking-in-dark model in both male and female mice [27]. In addition, the nalmefene effect at the dose range <0.5 mg/kg was specific to alcohol, as shown by the lack of the effect on sucrose or saccharin drinking.

As OX has been recently applied in the clinical studies and trials of treating alcoholism or other diseases [28, 29], OX and nalmefene are two good candidates for exploring the potential advantage of the OX and nalmefene combination. By targeting three neurotransmitter systems (OXTR agonism for OX, MOR antagonism for nalmefene and partial agonism for nalmefene) involved in different components of alcohol addiction, the combination may have an increased efficacy over the single-target approach (like OX alone), with possible less adverse effects [30- 33]. Hence, we hypothesized that OX and nalmefene combination reduced excessive alcohol drinking in a synergistical manner, and our results may suggest new information about the medical use of OX for alcoholism treatment.

Therefore, the main subject of the present study is to test whether excessive or relapse-like alcohol drinking could be effectively reduced by the proper OX and nalmefene combination. For this purpose, the first objective was to evaluate the pharmacological effect of OX alone in both male and female mice after 3-week intermittent-access alcohol (IA) drinking and after 1-week abstinence (the ADE), which mimic excessive, escalation and 'relapse' alcohol intake [5, 32]. After the sub-effective doses of OX were determined, we specifically tested several combinations of OX and nalmefene using the sub-effective doses of each drug. The sub-effective doses of nalmefene have been determined in our early studies using the same IA and ADE paradigms in mice [27, 32].

## 2. MATERIALS AND METHODS

### 2.1. Animals

Male and female adult C57BL/6J mice (7-8 weeks of age) from The Jackson Laboratory (Bar Harbor, ME, USA) were kept on a 12-hour reverse light-dark cycle (lights off at 7:00 am) upon arrival and acclimated for about 2 weeks before experiments. Mice were then individually housed in ventilated cages fitted with steel lids and filter tops and given *ad libitum* access to water and food in temperature-controlled rooms (21 ºC). Animal care and experimental procedures were conducted according to *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996) and were approved by the Institutional Animal Care and Use Committee of Rockefeller University.

### 2.2. Materials

Human synthetic oxytocin (CellSciences, Canton, MA) and nalmefene hydrochloride (Baker Norton Pharmaceuticals) were dissolved in physiological saline. Ethanol solutions were prepared from 190 proof absolute ethyl alcohol (Pharmco-AAPER, Brookfield, CT, USA) and dissolved in tap water.

### 2.3. Procedures

#### 2.3.1. Intermittent-access alcohol (IA) drinking

This model has been widely used in C57BL/6J mice by many laboratories and described in detail

in earlier reports [32]. During 3 weeks of alcohol drinking in their home cages, mice had free access to food and water. Briefly, beginning at 10:00 am (3 hours after lights off), both the alcohol and water sipper tubes were presented on their home cages, and their place on left or right side of the cage was randomly arranged to avoid any development of side preference. The alcohol tubes filled with fresh 15% alcohol solution were maintained for 24 hours before being replaced by the water tubes. Both alcohol and water values were recorded at 0 hour and after 4, 8 and 24 hours of alcohol access in the drinking days and calculated as consumed alcohol intake (g/kg) and relative preference ratio for alcohol vs water (alcohol intake/total fluid intake).

#### 2.3.2. OX, OX with nalmefene administration(s) after IA

On day 22, mice were randomly assigned as the drug- and vehicle-treated groups with similar alcohol intakes in the baseline sessions. An experimenter, who blinded to the experimental groups, administered the drug and vehicle. The mice in control groups received one vehicle injection or two; and the mice in drug groups received one drug (OX) or two drugs (OX followed by nalmefene). The OX doses were based on an earlier publication [13]: the mice in drug groups received one OX injection (0.01, 0.03 or 0.1 mg/kg, i.p.), and the mice in control groups received one vehicle injection (saline). The OX with nalmefene dose was based on the above experiments with OX alone and our recent nalmefene study [27, 32]: the mice in drugs' groups received the first i.p. injection of OX (0.01 or 0.03 mg/kg) followed by the second i.p. injection of nalmefene (0.125 mg/kg) 20 min later; and the mice in control groups received one saline followed by another saline. Finally, the alcohol tube was provided 10 min after the last drug or vehicle injection, and alcohol and water intakes were recorded after 4, 8 and 24 hours of alcohol access (Table S1A).

Of note, in both male and female mice, we observed comparable effects of OX, suggesting that the estrous cycle and associated hormones might be unimportant in the response to OX in females.

#### 2.3.3. The alcohol deprivation effect (ADE) after 1-week abstinence from IA

As described above, mice got access to IA alcohol drinking for 24 hours on alternating days for 3 weeks. In the baseline session, 15% alcohol and water intakes were recorded at 4, 8 and 24 hours. The alcohol tubes were taken away with only access to food and water. After 7 days of abstinence (day 30), new alcohol (30%) tubes were given back to the mice at 10:00 am (3 hours after lights off) and the alcohol and water intakes were recorded at 4, 8 and 24 hours in the ADE session. In the following experiments with both the male and female mice, we tested the effect of 0.03 mg/kg OX with 1 mg/kg nalmefene in the ADE model. The mice assigned to the drug- and vehicle-treated groups had similar alcohol intakes in the baseline session. Control groups: mice received vehicle injections before the ADE test; and Drug groups: mice received OX with nalmefene before the ADE test (Table S1B).

#### 2.3.4. Sucrose and saccharin drinking

The specific effect of 0.03 mg/kg OX with 0.125 mg/kg nalmefene was examined in sucrose (caloric reinforcer) or saccharin (non-caloric reinforcer) drinking. In these experiments, the IA alcohol drinking procedures were identical to those in the above experiment. After 3 weeks of IA, the alcohol tube was exchanged to sucrose for 3 sessions with stable intakes. The mice assigned to the combination- and vehicle-treated groups had similar sucrose intakes 24 hours before the test day. On the test day, sucrose (4%) and water intake values were recorded after 4, 8 and 24 hours of sucrose access. In separate experiments, saccharin drinking (0.1%) was tested similarly after 3 weeks of IA with an identical procedure.

### 2.4. Data analysis

We predicted that these studies require 6-8 males per group, based on the levels of differences seen previously [31, 32]. In the IA experiments, alcohol intake differences across the different groups were analyzed using two-way analysis of variance (ANOVA). for treatment (vehicle vs drug doses) and for times (4, 8 vs 24 h) with repeated measures, with testing our *a priori* hypothesis that there were effects of OX, OX with nalmefene based on the published findings [32]

and our new hypothesis. In the ADE experiments, alcohol intake differences across the different groups were analyzed using two-way ANOVA for treatment (vehicle vs drug) and for session (baseline vs ADE), with testing our *a priori* hypothesis that there was an ADE based on the published findings [32]. All the ANOVAs were followed by Newman-Keuls *post-hoc* tests. The accepted level of significance for all tests was $p<0.05$. All statistical analyses were performed using *Statistica* (version 5.5, StatSoft Inc, Tulsa, OK).

## 3. RESULTS

### 3.1. Effect of OX on alcohol intake and preference after IA in both male and female mice

In males, OX at 0.1 mg/kg significantly decreased alcohol intake at 4 hours (Table 1A). At this dose, there was a reduced preference ratio at 4 hours (Table 1A). However, there was a compensatory increase in water intake, resulting in nearly unchanged total fluid intake (Data not shown).

**Table 1.** Effects of oxytocin (OX) (0.1 mg/kg) on alcohol intake and preference ratio in male (A, n=6-8) and female (B, n=8) mice after 3-week intermittent-access alcohol drinking. Alcohol and water intake values as mean ± SEM were recorded after 4, 8 and 24 hours of alcohol access.

**A.** Male. A decreased alcohol intake (two-way ANOVA with repeat measure $F(1,13)=5.1$, $p<0.05$) at 4 hours (Newman-Keuls *post-hoc* test $p<0.05$); A reduced preference ratio (two-way ANOVA with repeat measure $F(1,13)=6.1$, $p<0.05$) at 4 hours (Newman-Keuls *post-hoc* test $p<0.05$). *$p<0.05$ vs. vehicle control at the same time point.

| | **Time** | **Vehicle** | **0.1 mg/kg OX** |
|---|---|---|---|
| Alcohol intake (g/kg) | 0-4 h | 5.1 ± 0.2 | 3.4 ± 0.4* |
| | 4-8 h | 3.1 ± 0.4 | 2.7 ± 0.4 |
| | 8-24 h | 7.9 ± 0.3 | 8.3 ± 0.7 |
| Preference ratio | 0-4 h | 0.77 ± 0.03 | 0.52 ± 0.04* |
| | 4-8 h | 0.45 ± 0.05 | 0.43 ± 0.06 |
| | 8-24 h | 0.51 ± 0.04 | 0.53 ± 0.08 |

**B.** Female. A decreased alcohol intake (two-way ANOVA with repeat measure $F(1,14)=5.5$, $p<0.05$) at 4 hours (Newman-Keuls *post-hoc* test $p<0.05$); A reduced preference ratio (two-way ANOVA with repeat measure $F(1,14)=5.7$, $p<0.05$) at 4 hours (Newman-Keuls *post-hoc* test $p<0.05$). *$p<0.05$ vs. vehicle control at the same time point.

| | **Time** | **Vehicle** | **0.1 mg/kg OX** |
|---|---|---|---|
| Alcohol intake (g/kg) | 0-4 h | 7.1 ± 0.6 | 4.5 ± 0.5* |
| | 4-8 h | 6.1 ± 0.5 | 5.7 ± 0.6 |
| | 8-24 h | 13 ± 0.8 | 14 ± 1.1 |
| Preference ratio | 0-4 h | 0.83 ± 0.05 | 0.55 ± 0.07* |
| | 4-8 h | 0.65 ± 0.04 | 0.63 ± 0.05 |
| | 8-24 h | 0.55 ± 0.06 | 0.56 ± 0.09 |

As there was no significant sex difference in the effect of OX on alcohol consumption, the female data at 0.1 mg/kg are presented separately in Table 1B. For both intake and preference ratio, there were significant effects of OX, with less intake and preference at 4 hours. Like males, there was no change of total fluid intake in females (Data not shown).

Figure 1 represents the full-dose response of OX (0, 0.01, 0.03 and 0.1 mg/kg) in alcohol intake and preference ratio at the 4-hour time point in both male and female mice. Compared with the control, the mean alcohol intake in OX-treated mice at 0.03 mg/kg was slightly reduced in both males and females, without reaching significant effects. At 0.1 mg/kg, however, there were significant decreases on alcohol intake (Figure 1A) and on preference ratio (Figure 1B) in both sexes.

### 3.2. Effect of OX and nalmefene combination on alcohol intake and preference ratio after IA

Figure 2A represents alcohol intake and preference ratio in males recorded at 4, 8 and 24 hours, following a combination dose (0.03 mg/kg OX with 0.125 mg/kg nalmefene): there were significant reductions of alcohol intake and preference ratio after 4 hours. Similarly, in females (Figure 2B), significant reductions of alcohol intake and preference ratio were observed after 4 hours. There was no effect on total fluid intake in either sex (Table S2).

The effects of OX (0.03 mg/kg) combined with nalmefene (0.125 mg/kg) on alcohol intake and preference at the 4-hour time point was compared with the ones of OX (0.03 mg/kg) alone, as shown in Figure 1. The OX with nalmefene showed more reductions than 0.03 mg/kg OX alone in both the

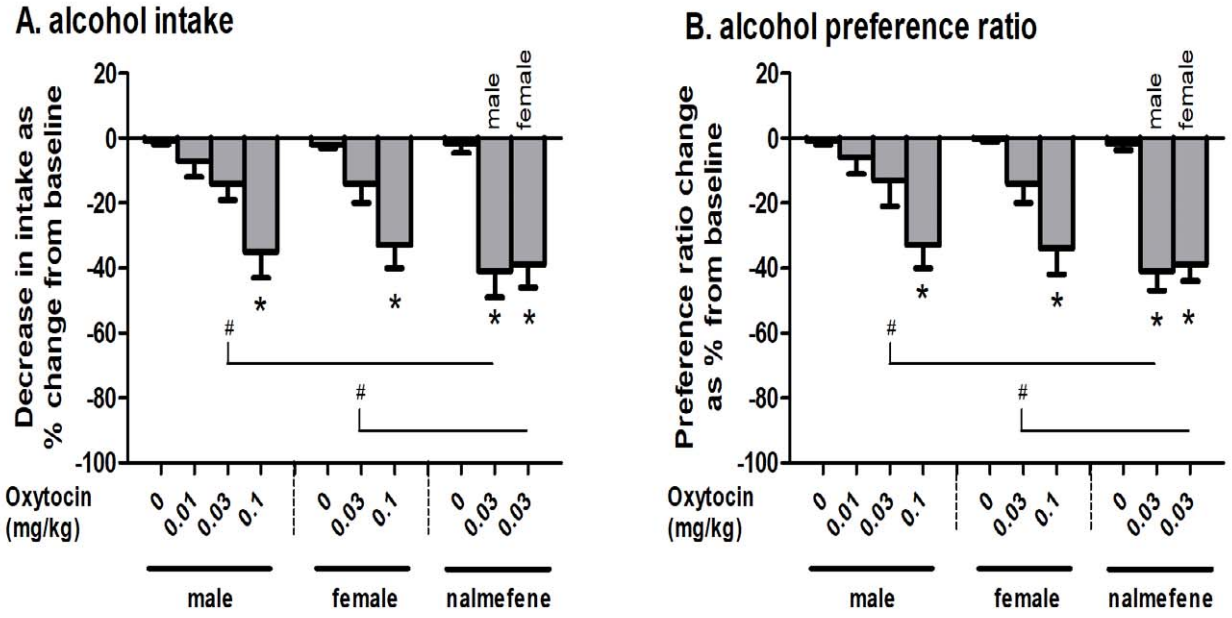


**Figure 1.** Dose responses of administration of oxytocin (OX) alone (0, 0.01, 0.03 or 0.1 mg/kg) and in combination with nalmefene (0 or 0.125 mg/kg) on reducing 15% alcohol intake (A) and preference ratio (B) in mice (n=6-8) after 3-week intermittent-access alcohol drinking. Data were collected at the 4-hour time point on the testing day and are expressed as a percentage of alcohol intake or preference ratio in vehicle-treated mice. Data are presented as mean + SEM. Males at 0.1 mg/kg OX, decreases on alcohol intake (one-way ANOVA $F(9,62)=12$, $p<0.005$; Newman-Keuls *post-hoc* test $p<0.05$) (A) and on preference ratio (one-way ANOVA $F(9,62)=10$, $p<0.005$; $p<0.05$) (B); Females at 0.1 mg/kg OX, decreases on alcohol intake (Newman-Keuls *post-hoc* test $p<0.05$) (A) and on preference ratio ($p<0.05$) (B). *$p<0.05$ vs. control (OX at 0 mg/kg); and in both males and females, the OX with nalmefene showed more reductions than 0.03 mg/kg OX alone in alcohol intake (A) and preference ratio (B) in both sexes ($p<0.05$). # $p<0.05$ vs. 0.03 mg/kg OX alone.

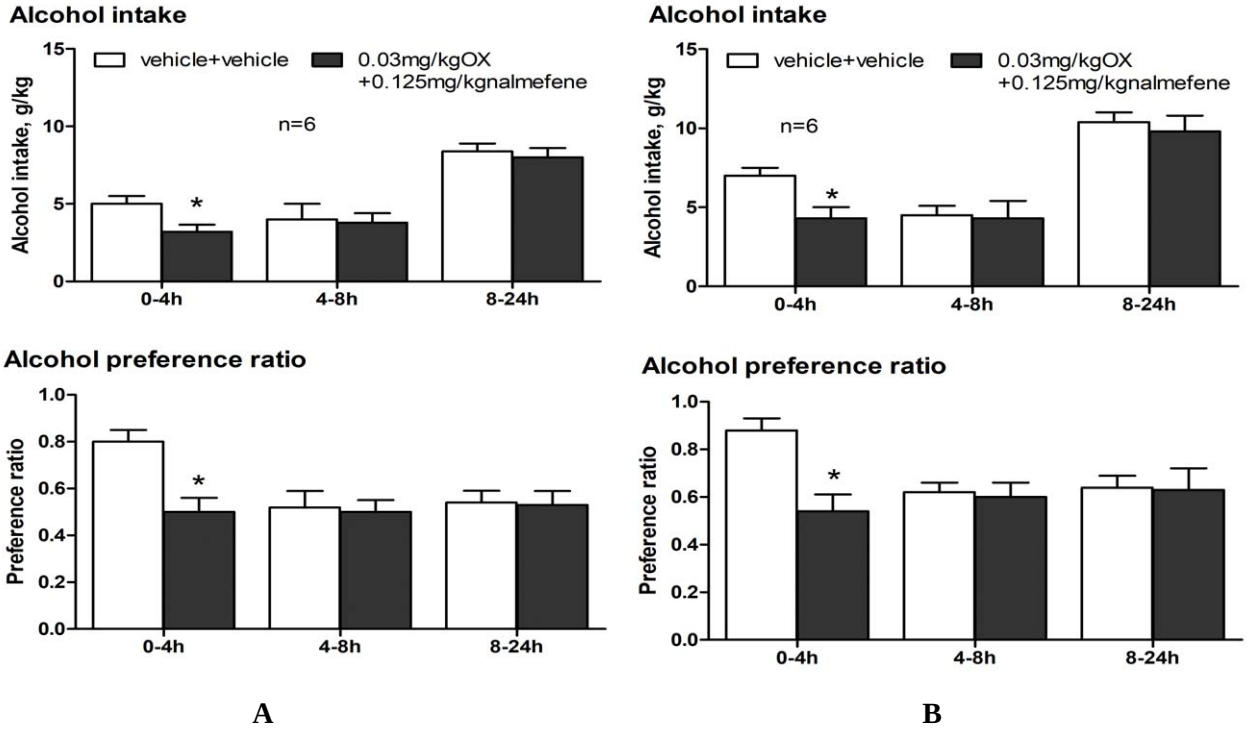


**Figure 2.** Effect of 0.03 mg/kg oxytocin (OX) and 0.125 mg/kg nalmefene combination on alcohol intake and preference ratio after 3-week intermittent-access alcohol drinking in male mice (A, n=6) and female mice (B, n=6). Data as mean + SEM are presented after 4, 8 and 24 hours of alcohol access.

**A.** Reductions of alcohol intake (upper) (two-way ANOVA with repeat measure $F(1,10)=5.9$, $p<0.05$) at 4 hours (Newman-Keuls *post-hoc* test $p<0.05$) and preference ratio (lower) (two-way ANOVA with repeat measure $F(1,10)=6.0$, $p<0.05$) after 4 hours (Newman-Keuls *post-hoc* test $p<0.05$). *$p<0.05$ vs. vehicle control.

**B.** Reductions of alcohol intake (upper) (two-way ANOVA with repeat measure $F(1,10)=6.2$, $p<0.05$) at 4 hours (Newman-Keuls *post-hoc* test $p<0.05$) and preference ratio (lower) (two-way ANOVA with repeat measure $F(1,10)=6.4$, $p<0.05$) after 4 hours (Newman-Keuls *post-hoc* test $p<0.05$). *$p<0.05$ vs. vehicle control.

intake (Figure 1A) and preference ratio (Figure 1B) in both sexes. At a lower dose 0.01 mg/kg, OX combined with 0.125 mg/kg nalmefene had no significant effect on alcohol intake, water intake or alcohol preference ratio in either male or female mice (data not shown).

### 3.3. Effect of OX and nalmefene combination on ADE

In a pilot with a small number of mice, OX at 0.01 mg/kg combined with nalmefene (0.125 mg/kg) was tested and no effect was observed in either sex (data not shown).

In males at 0.03 mg/kg OX with nalmefene (0.125 mg/kg) (Figure 3A), the combination reduced alcohol intake in the ADE session. There were no significant effects of ADE or the combination after 8 or 24 hours (data not shown). Like the males, there was a significant ADE effect in females and the combination blunted the ADE after 4 hours (Figure 3B), but not 8 or 24 hours (data not shown).

### 3.4. Effect of OX and nalmefene combination on sucrose or saccharin drinking

The specificity of the effect of the combination on alcohol (a caloric reinforcer) was determined by testing its effect on sucrose (caloric reinforcer) and saccharin (non-caloric reinforcer) drinking after IA. However, no effect of OX and nalmefene (0.03 mg/kg and 0.125 mg/kg, the most effective combination for reducing alcohol) was found on either 4% sucrose drinking (Table S3 A, B) or 0.1% saccharin drinking (Table S3 C, D) in either sex.

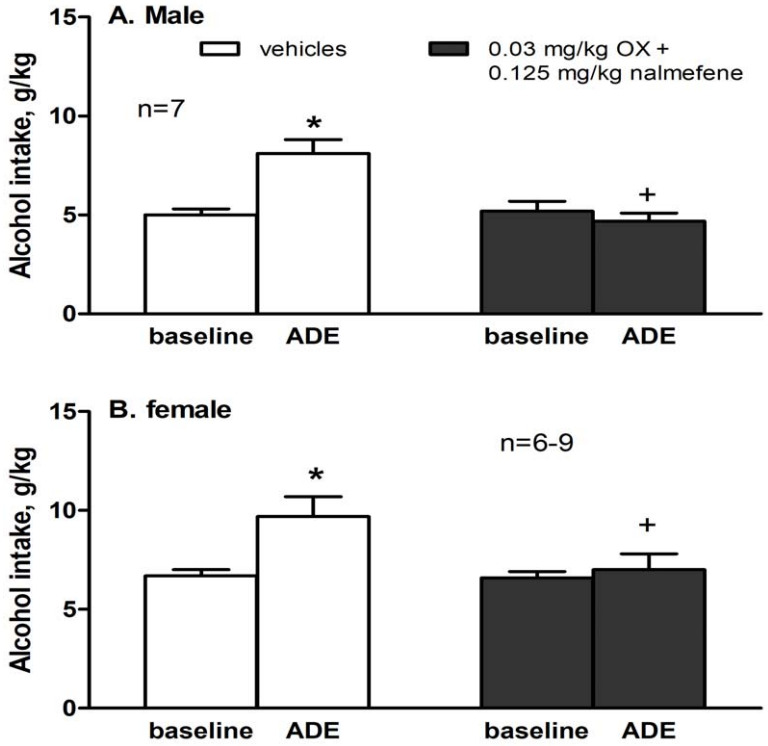


**Figure 3.** Effect of 0.03 mg/kg oxytocin (OX) and 0.125 mg/kg nalmefene combination on alcohol intake (g/kg) after 1-week abstinence (alcohol deprivation effect, ADE) from 3 weeks of intermittent-access alcohol drinking in male (A, n=7) and female (B, n=6-9) mice. Data as mean + SEM are presented after 4 hours of alcohol access. (A) Males. Two-way ANOVA revealed significant effects of the combination ($F(1,24)=4.7$, $p<0.05$) and session x combination interaction ($F(1,24)=4.7$, $p<0.05$): the combination-treated ones had less intake than the vehicle-treated ones in the ADE session ($p<0.05$). To test our *a priori* hypothesis that an ADE occurred, the *post-hoc* test showed that in the vehicle-treated mice, the ADE was significant ($p<0.05$). *$p<0.05$ vs. vehicle control at baseline; and + $p<0.05$ vs. ADE. (B) Females. Two-way ANOVA revealed a significant effect of session ($F(1,26)=5.3$, $p<0.05$) and session x combination interaction ($F(1,26)=4.9$, $p<0.05$), and Newman-Keuls *post hoc* analysis showed that the control mice had more intake in the ADE session than the baseline ($p<0.05$), and the combination-treated group had less intake than the vehicle control group in the ADE session ($p<0.05$). *$p<0.05$ vs. vehicle control at baseline; and + $p<0.05$ vs. ADE.

Similarly, there was no effect of OX with nalmefene on sucrose or saccharin drinking in alcohol-naïve mice (data not shown).

## 4. DISCUSSION

In the 3-week IA model, there is an escalating and excessive alcohol consumption after a period of excessive drinking mimicking a transition from alcohol abuse to dependence [15, 32]. Using this model, we recently report that OX significantly reduced alcohol intake and preference in a dose-dependent manner in male mice [34]. The present study replicated the results in male mice and extended by examining the female mice and found that OX significantly decreased alcohol drinking without sex difference (Table 1; Figure 1). Our new results in both male and female mice may constitute an extension to the anti-alcoholism properties of OX observed in male rodent studies only (see updated reviews [28, 35]).

Of interest, our new objective in this study provided initial evidence that the OX and nalmefene combination is more effective than either drug alone: [1] the treatments with OX and nalmefene efficiently decreased excessive alcohol drinking in mice without sex difference (Figure 1; Figure 2); and [2] as compared with the effects of OX or nalmefene alone, the combinations were

more effective than either drug only (Figure 1). The effect of this combination was alcohol-specific, as demonstrated by the lack of any effect on sucrose or saccharin consumption (Table S3). In the ADE model, the combination of OX and nalmefene had a synergistic effect on preventing ADE (Figure 3), as each compound only at this low dose did not have any significant effect. Our studies have provided promising *in vivo* data demonstrating that the combination of clinically utilized nalmefene and OX is more efficacious than either drug only in both mouse IA and ADE models. In line with this new result, our previous studies observed similar synergistic effects of vasopressin receptor V1b antagonist combined with naltrexone [31, 32], as OX and vasopressin exhibit opposite effects on alcohol intakes [31, 35-37]. Indeed, neurobiological studies have demonstrated that the multiple actions of alcohol in the brain include endorphin/MOR, OX/OXTR and dynorphin/KOR that are profoundly disrupted after chronic alcohol exposure in rodents [3-5, 15, 35-39]. As the effectiveness of nalmefene and OX could be mediated *via* multiple neuronal pathways (at least MOR, KOR and OXTR), the combination showed synergistic effects in reducing alcohol intake and seems likely to have improved efficacy over each single compound (Figure 1).

For mechanistic point of view, alcohol activates both MOR and KOR and consequently produces drug rewarding effects, positive/negative reinforcing actions and motivational behaviors of alcohol drinking in rodents [38-40]. The OX neuronal activity and release is connected with activation of MOR and KOR [41-43], and the potential interaction of MOR, KOR and OXTR is further supported by the co-localization of the MOR, KOR and OXTR expression in specific brain regions across rodents and humans [33]. After chronic alcohol exposure, therefore, the MOR, KOR and OXTR neuronal activities, expressions and their interactions could be disrupted as found before in both rodents and humans. Therefore, OX combined with nalmefene may reverse those disruptions by normalizing the OX deficiency and reducing MOR overactivity, at least as supported by our current data.

## 5. CONCLUSION

Consistent with many studies on alcohol consumption in different rodent models, our current new finding provided further promising *in vivo* data demonstrating that the use of oxytocin, in combination with nalmefene, is a novel approach for the treatment of alcoholism, with an improved efficacy.

## SUPPLEMENTARY MATERIAL

**Table S1.** Experimental timelines. The 3-week intermittent-access alcohol drinking (15% alcohol vs. water) or after 1-week abstinence (alcohol deprivation effect, ADE) with oxytocin (OX), nalmefene or their combinations.

**A.**

| Weeks 1-3 | Day 22 |
|---|---|
| Intermittent-access in a 2-bottle choice (15% alcohol vs. water) with 24-h access every other day | OX or OX + nalmefene |

**B.**

| Weeks 1-3 | Week 4 | Day 30 |
|---|---|---|
| Intermittent-access in a 2-bottle choice (15% alcohol vs. water) with 24-h access every other day | 1-week abstinence | OX or OX + nalmefene |

**Table S2.** No effects of oxytocin (OX) (0.03 mg/kg) and 0.125 mg/kg nalmefene combination on total fluid intake in male (A) and female (B) mice after 3-week intermittent-access alcohol drinking in mice (n=6). Data (mean ± SEM) are presented after 4, 8 and 24 hours of alcohol access.

**A. Male**

| Total fluid intake, ml | Vehicle + Saline | 0.03 mg/kg OX + 0.125 mg/kg nalmefene |
|---|---|---|
| 0-4 h | 1.6 ± 0.19 | 1.5 ± 0.21 |
| 5-8 h | 1.1 ± 0.25 | 1.2 ± 0.22 |
| 9-24 h | 3.0 ± 0.38 | 3.1 ± 0.47 |

**B. Female**

| Total fluid intake, ml | Vehicle + Saline | 0.03 mg/kg OX + 0.125 mg/kg nalmefene |
|---|---|---|
| 0-4 h | 1.9 ± 0.11 | 1.8 ± 0.22 |
| 5-8 h | 1.6 ± 0.26 | 1.6 ± 0.17 |
| 9-24 h | 3.2 ± 0.33 | 3.3 ± 0.35 |

**Table S3.** No effect of oxytocin (0.03 mg/kg) and 0.125 mg/kg nalmefene combination on 4% sucrose (A for males, and B for females) or 0.1% saccharin (C for males and D for females) intake, water intake and their preference ratio in mice (n=6-7). Data (mean ± SEM) are presented after 4 hours of sucrose or saccharin access.

**A. Male**

| Treatment | Vehicle + Saline | 0.03 mg/kg OX + 0.125 mg/kg nalmefene |
|---|---|---|
| 4% sucrose intake (g/kg/4h) | 9.7 ± 0.92 | 9.6 ± 0.99 |
| Preference ratio | 0.94 ± 0.05 | 0.95 ± 0.06 |

**B. Female**

| Treatment | Vehicle + Saline | 0.03 mg/kg OX + 0.125 mg/kg nalmefene |
|---|---|---|
| 4% sucrose intake (g/kg/4h) | 9.8 ± 0.79 | 9.9 ± 0.89 |
| Preference ratio | 0.95 ± 0.07 | 0.97 ± 0.04 |

**C. Male**

| Treatment | Vehicle + Saline | 0.03 mg/kg OX + 0.125 mg/kg nalmefene |
|---|---|---|
| 0.1% saccharin intake (g/kg/4h) | 0.13 ± 0.04 | 0.12 ± 0.05 |
| Preference ratio | 0.98 ± 0.03 | 0.96 ± 0.03 |

**D. Female**

| Treatment | Vehicle + Saline | 0.03mg/kg OX + 0.125 mg/kg nalmefene |
|---|---|---|
| 0.1% saccharin intake (g/kg/4h) | 0.138 ± 0.05 | 0.17 ± 0.06 |
| Preference ratio | 0.97 ± 0.06 | 0.98 ± 0.07 |

## ACKNOWLEDGEMENT

This work was supported by Robertson Therapeutic Discover Fund at the Rockefeller University (YZ) and NIH AA021970 (YZ).

## CONTRIBUTORS

YZ, DCZ designed the study, managed literature searches, undertook behavioral study, conducted statistical analysis and wrote the manuscript. Specifically, we remember Dr. Mary Jeanne Kreek for her contributions in medical research on drug addiction diseases.

## CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

## REFERENCES

1. Neumann, I. D. 2002, Prog. Brain Res., 139, 147.
2. Lee, M. R., Rohn, M. C., Tanda, G. and Leggio, L. 2016, CNS Drugs, 30, 109.
3. Silva, S. M., Madeira, M. D., Ruela, C. and Paula-Barbosa, M. M. 2002, Brain Res., 925, 76.
4. Hansson, A. C., Koopmann, A., Uhrig, S., Bühler, S., Domi, E., Kiessling, E., Ciccocioppo, R., Froemke, R. C., Grinevich, V. and Kiefer, F. 2018, Neuropsychopharm., 43, 1235.
5. Zhou, Y., Liang, Y., Low, M. J. and Kreek, M. J. 2019, Genes Brain Beh., 18, e12600.
6. Sivukhina, E. V., Dolzhikov, A., Morozov, I. E., Jirikowski, G. F. and Grinevich, V. 2006, Horm. Metab. Res., 38, 382.
7. Marchesi, C., Chiodera, P., Brusamonti, E., Volpi, R. and Coiro, V. 1997, Progress Neuro-psychopharm. Biol. Psychia., 21, 797.
8. McGregor, I. S. and Bowen, M. T. 2012, Horm. Behav., 61, 331.
9. Rodriguez, K. M., Smith, B. L. and Caldwell, H. K. 2020, Brain Beh., 10, e01749.
10. Bahi, A. 2015, Physiol. Beh., 139, 321.
11. Peters, S., Slattery, D. A., Flor, P. J., Neumann, I. D. and Reber, S. O. 2013, Addict. Biol., 18, 66.
12. MacFadyen, K., Loveless, R., DeLucca, B., Wardley, K., Deogan, S., Thomas, C. and Peris, J. 2016, Pharmacol. Biochem. Beh., 140, 27.
13. King, C. E. and Becker, H. C. 2019, Psychopharmacol., 236, 2613.
14. Caruso, M. A., Robins, M. T., Fulenwider, H. D. and Ryabinin, A. E. 2021, Psychopharmacol., 238, 899.
15. Koob, G. F. 2021, Pharmacol. Rev., 73, 163.
16. Tunstall, B. J., Kirson, D., Zallar, L. J., McConnell, S. A., Vendruscolo, J. C., Ho, C. P., Oleata, C. S., Khom, S., Manning, M. and Lee, M. R. 2019, PLoS Biol., 17, e2006421.
17. Pedersen, C. A., Smedley, K. L., Leserman, J., Jarskog, L. F., Rau, S. W., Kampov-Polevoi, A., Casey, R. L., Fender, T. and Garbutt, J. C. 2013, Alcohol. Clin. Exp. Res., 37, 484.
18. Mitchell, J. M., Arcuni, P. A., Weinstein, D. and Woolley, J. D. 2016, J. Addict. Med., 10, 182.
19. Flanagan, J. C., Allan, N. P., Calhoun, C. D., Badour, C. L., Maria, M.-S., Brady, K. T. and Back, S. E. 2019, Exp. Clin. Psychopharmacol., 27, 45.
20. Melkonian, A. J., Flanagan, J. C., Calhoun, C. D., Hogan, J. N. and Back, S. E. 2021, J. Clin. Psychopharmacol., 41, 465.
21. Melby, K., Gråwe, R. W., Aamo, T. O., Skovlund, E. and Spigset, O. 2021, Alcohol Alcohol., 56, 565.
22. Stauffer, C. S., Meinzer, N. K., Morrison, T., Wen, J. H., Radanovich, L., Leung, D., Niles, A., O'Donovan, A., Batki, S. L. and Woolley, J. D. 2019, Alcohol. Clin. Exp. Res., 43, 2627.
23. Malley, S., Jaffe, A., Chang, G., Schottenfeld, R., Meyer, R. and Rounsaville, B. 1992, Arch. Gen. Psychiatry, 49, 881.
24. Mason, B. J., Ritvo, E. C., Morgan, R. O., Salvato, F. R., Goldberg, G., Welch, B. and Mantero-Atienza, E. 1994, Alcohol. Clin. Exp. Res., 18, 1162.
25. Walker, B. M. and Koob, G. F. 2008, Neuropsychopharmacol., 33, 643.
26. Rose, J. H., Karkhanis, A. N., Steiniger-Brach, B. and Jones, S. R. 2016, Int. J. Mol. Sci., 17, 1216.

27. Zhou, Y. and Kreek, M. J. 2019, Brain Res., 1724, 146410.
28. Perry, C. J. and Cornish, J. L. 2022, Addict. Neurosci., 100018.
29. Zimmermann, S., Thomas, B. C., Krisam, J., Limprecht, R., Klose, C., Stenger, M., Pourbaix, M., Ries, M., Vollstaedt-Klein, S. and Koopmann, A. 2022, BMJ Open, 12, e059672.
30. Heyser, C. J., Moc, K. and Koob, G. F. 2003, Neuropsychopharmacol., 28, 1463.
31. Zhou, Y., Colombo, G., Carai, M. A. M., Ho, A., Gessa, G. L. and Kreek, M. J. 2011, Alcohol. Clin. Exp. Res., 35, 1876.
32. Zhou, Y., Rubinstein, M., Low M. J. and Kreek, M. J. 2018, Alcohol. Clin. Exp. Res., 42, 195.
33. Dal Monte, O., Piva, M., Anderson, K. M., Tringides, M., Holmes, A. J. and Chang, S. W. 2017, Proc. Natl. Acad. Sci., 114, 5247.
34. Zhou, Y., A. Baehr., Zhou, D.C. and Kreek. M.J. 2022, J Sub. Abuse Alcohol., 9, 1096.
35. Ryabinin, A. E. and Fulenwider, H. D. 2021, Neurosci. Biobeh. Rev., 127, 852.
36. Zhou, Y. and Kreek, M. J. 2018, J. Pharmacol. Exp. Ther., 366, 9.
37. Dannenhoffer, C. A., Kim, E. U., Saalfield, J., Werner, D. F., Varlinskaya, E. I. and Spear, L. P. 2018, Psychopharmacol., 235, 3065.
38. Olive, M. F., Koenig, H. N., Nannini, M. A. and Hodge, C. W. 2001, J. Neurosci., 21, RC184.
39. Zhou, Y., Colombo, G., Niikura, K., Carai, M., Femenía, T., García-Gutiérrez, M., Manzanares, J., Ho, A., Gessa, G. and Kreek, M. J. 2013, Alcohol. Clin. Exp. Res., 37, E131.
40. Marinelli, P. W., Quirion, R. and Gianoulakis, C. 2003, Psychopharmacol., 169, 60.
41. Bicknell, R. and Leng, G. 1982, Nature, 298, 161.
42. Summy-Long, J. Y., Rosella-Dampman, L. M., McLemore, G. L. and Koehler, E. 1990, Neuroendocrinol., 51, 376.
43. Douglas, A., Dye, S., Leng, G., Russell, J. and Bicknell, R. 1993, J. Neuroendocrin., 5, 307.