A role for the NMDA positive modulator NYX-783 in preclinical opioid use disorder research

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Abstract

Rates of opioid use disorder (OUD) and overdose deaths have increased dramatically in recent years. Currently approved medications for OUD include the opioid agonists methadone and buprenorphine, and the opioid antagonist naltrexone. However, relapse rates are high due to an impaired ability for addicts to control their urge to consume due to strong cravings, and the extreme severity of withdrawal symptoms. Additionally, the nature of the agonists can lead to abuse of those compounds as well. Non-opioid targets, such as glutamate receptors, are potentially ideal for developing intervention strategies with the goal of reducing OUD, relapse, and overdose death. NYX-783 is a small molecule positive modulator for the glutamate receptor NMDA. It has been shown to modulate learning and memory, both of which are impaired in drug addicts, and known play a role in relapse. Using mice, we have conducted preclinical studies to evaluate the potential for NYX-783 as a therapeutic for OUD with assessment of several outcomes: 1) respiratory depression, 2) consumption, 3) motivation for consumption, and 4) the development of aversion to withdrawal symptoms. For respiratory depression studies, mice were pretreated 1h prior with NYX-783 and respiratory rates were monitored for 15min after each escalating dose of oxycodone. No effects were seen across all doses of NYX-783 tested. To test for effects on drug consumption, mice were trained to orally self-administer oxycodone and then treated every two days with different doses of NYX-783. No effect on intake at clinically-relevant doses was observed. Mice that were self-administering oral oxycodone were then evaluated on a progressive ratio paradigm and no effect of NYX-783 was observed on rewards earned. We next evaluated withdrawal using two separate paradigms to test for effects of NTX-783 on, 1) for somatic withdrawal symptoms, 2) aversion to the state of withdrawal. For somatic withdrawal, using higher doses of naloxone (1 mg/kg), NYX-783 did not attenuate jumping behavior. For aversion to withdrawal, three aversion pairings were completed that consisted of oxycodone treatment, followed by NYX-783 preceding a low dose of naloxone (0.1mg/kg ip) immediately before pairing in a specific context. These alternated with neutral (saline) pairings daily. We observed a significant improvement in aversion scores in the NYX-783 treated mice. This suggests a potential therapeutic use for NYX-783 in reducing the negative state of withdrawal that can drive relapse in OUD

General Background

Opioid Use Disorder

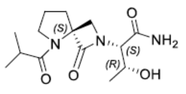
- The dramatic rise in opioid overdose deaths has designated OUD to be a major public health concern - Singh and Cleveland 2020
- Prescription opioids have played a major role in the development of the opioid epidemic; 25% of patients misusing them, and 5-10% developing OUD – Kallupi et al., 2020
- Treatment dropout rates and relapse are high among OUD patients – Coffa and Snyder 2019
- Lowering relapse rates continues to be a primary focus of OUD research – Volkow 2019

OUD, Altered Circuitry, Relapse, and NYX-783

- Chronic drug users perceive higher stress and cue-provoked cravings – Sinha 2009
- Disrupted neural circuits predict future drug relapse – Sinha 2006
- NMDA pathways are being targeted to explore neural circuit plasticity modulation to reverse or attenuate aspects of OUD
- NYX-783 is in a class of glutamate co-ligands or positive allosteric modulators for the NMDA receptor
- NYX-783 positive modulation of NMDAr reduces spontaneous recovery of fear in PTSD rodent models – Lee 2022
- **Goals: explore NYX-783 as a potential therapeutic for OUD.**

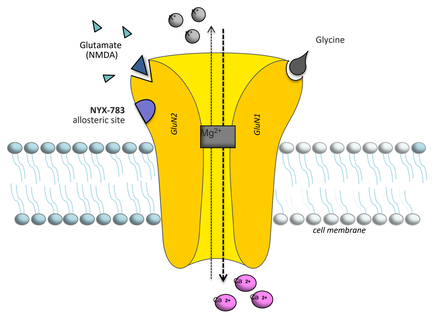
Presented Milestones of NYX-783 in OUD Mouse Models

1. Opioid-induced respiratory depression
2. Oxycodone consumption and motivation
3. Somatic withdrawal
4. Development of aversion during withdrawal



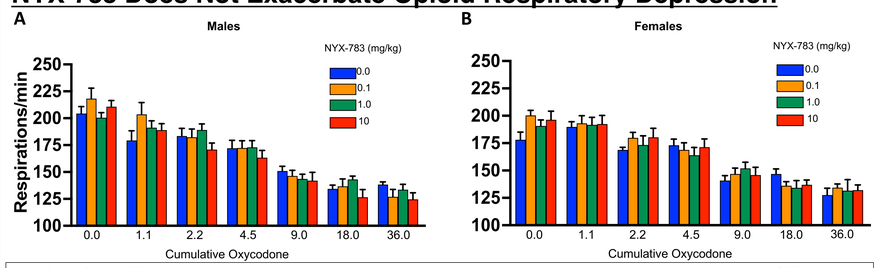
Molecular Formula: C14H23N3O4
Molecular Mass: 297.36 g/mole
Physical Description: White powder
Solubility: >50 mg/mL in water

(2S, 3R)-3-hydroxy-2-((S)-5-isobutyryl-1-oxo-2,5-diazaspiro[3.4]octan-2-yl)butanamide, monohydrate



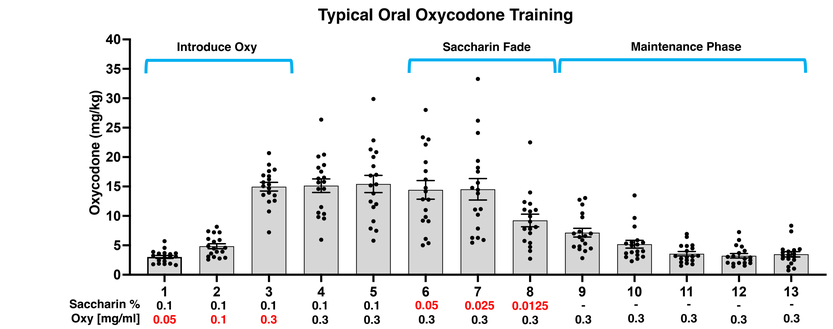
Results

NYX-783 Does Not Exacerbate Opioid Respiratory Depression

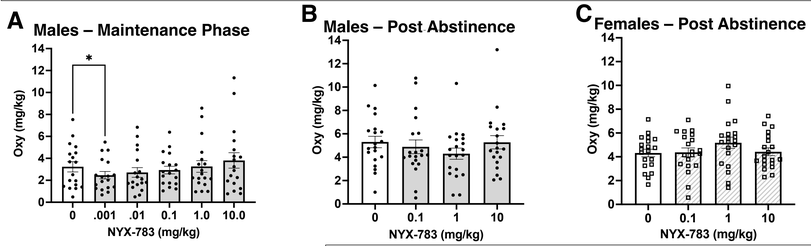


No effect of NYX-783 on oxycodone-induced respiratory depression in male or female mice. The day prior to testing, C57BL/6J mice underwent neck hair removal with Nair while anesthetized with isoflurane. On test day, mice were habituated to the **MouseOx Plus** collars (Starr Life Sciences) for 1hr. Mice were then pretreated with NYX-783 (0.1, 1.0, or 10.0 mg/kg or veh (saline, i.p.), and 1hr later underwent increasing the following doses of oxycodone (0, 1.1, 1.1, 2.2, 4.5, 9.0, and 18.0 mg/kg i.p. in saline) every 20 min. Respiration was monitored at 5 Hz over 15 min after each injection. Occasional loss of signal occurred due to opioid-induced locomotor activity occurred, resulting in a range of n's per dose. **(A)** Male mice (n= 6-11) showed no effect of NYX-783 on opioid-induced respiratory depression. **(B)** Female mice (n = 6-8) showed no effect of NYX-783 on opioid-induced respiratory depression.

NYX-783 Does Not Alter Oral Oxycodone Self-Administration or Motivation to Earn Oxycodone



Typical progression of oral oxycodone self-administration training for C57BL/6J mice. Shown here, male mice (n=18) were maintained on food restriction (85-90%) of their starting bodyweight throughout operant behavior. Mice were first trained to self-administer a saccharin solution in **Med Associates** operant boxes, with multiple nose poke ports on one wall, and a magazine for reward retrieval on the opposite wall. A cue light for a nose poke port would be turned on indicating an active port, and then turned off when a correct nose entry was detected, resulting in a reward delivery accompanied with an indicating magazine cue light. Upon a detected nose entry in the magazine, the magazine cue light would turn off, and after a 3 sec delay, the nose poke port would become active again. Each correct nose poke resulted in a 20μl saccharin reward deposited in the magazine (data not shown). After learning the task on an FR1 schedule (fixed ratio of 1 nose poke response/reward), mice progressed to an FR3 (3 nose pokes/reward) schedule. Oxycodone was then gradually introduced over three days. On Day 6, a saccharin fade was initiated and completed on Day 9. Mice maintained their operant self-administration for a solution of oxycodone in water, suggesting a level of dependence. Magazines were checked after each session to confirm that the mice consumed their earned rewards. All error bars are S.E.M.



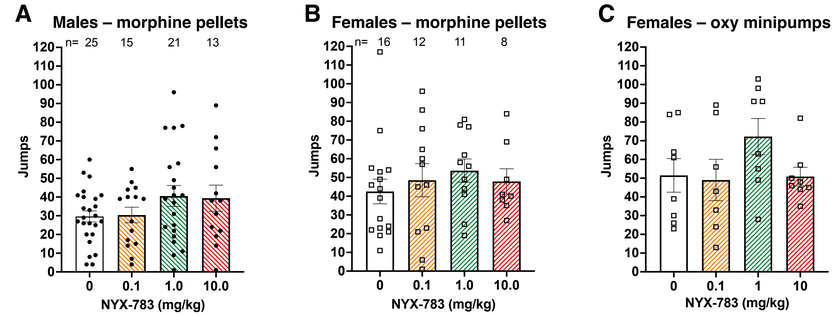
No effect of NYX-783 on oral oxycodone self-administration at clinically relevant doses during maintenance phase, post-abstinence, or progressive ratio (PR). We used a Latin Square dosing design for testing at **Maintenance** and **Post Abstinence** phases during self-administration sessions. All mice were split into four groups and injected with a dose of NYX-783 1hr prior to a 3hr self-administration test session. Test sessions alternated with a normal 3hr self-administration session the following day. No effects of the previous dosing from a Test day were observed. Each group of mice went through all doses shown in the graphs. **(A)** Male mice (n=18) were tested under Latin Square design beginning on Day14, and a significant reduction in consumption was observed on the 0.001 mg/kg (paired t-test *p=0.04087). **(B)** Additional male mice (n=20) were trained in identical fashion and then subjected to an abstinence period of 7 days after their final maintenance Day 13 session. This involved returning them to *ad libitum* chow and depriving them of the operant boxes. The night before initiating Latin Square testing, they were food restricted and resumed self-administration for testing. No effect of NYX-783 was observed. **(C)** Female mice (n=20) were trained and subjected to abstinence in an identical fashion. Latin Square testing showed no effects of NYX-783 at any dose.

(D, E) A separate cohort of females was trained and tested under a progressive ratio (PR) paradigm, which introduces an increasing number of nose poke requirements for each subsequent reward. PR is commonly used to assess motivation. The exponential nose poke criteria for the first ten rewards was as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, and 32. A breakpoint of 25 min was used to determine the threshold for a lack of seeking, and sessions were terminated upon a lack of active nose pokes for 25 min. Breakpoints are graphed by the number of nose pokes required for the previous reward achieved. On test day, mice were balanced (based on their averaged three days prior performance) into Veh (n=9 saline i.p.) or NYX-783 (n=10, 1 mg/kg i.p.) groups and treated 1hr prior to their 3hr PR session. No effect on rewards earned or breakpoint was observed. All error bars are S.E.M.

NYX-783 Does Not Increase Somatic Withdrawals (Jumps)

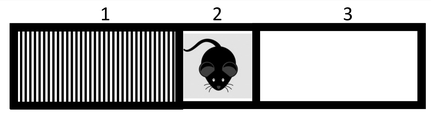
Two approaches were used to assess somatic withdrawals

- Subcutaneous morphine pellets for 5 days
- 3-day oxycodone minipumps delivering 130 mg/kg-BW/Day



No effect of NYX-783 on somatic withdrawals. Male and female C57BL/6J mice were exposed to chronic opioids to assess the effects of different doses of NYX-783 during naloxone-induced withdrawals. Subcutaneous morphine pellets (25mg) for 5 days, or 3-day oxycodone minipumps (130 mg/kg-BW/Day) were surgically implanted. On test day, mice were treated with NYX-783 1hr prior to naloxone (1.0 mg/kg ip). Immediately after naloxone, mice were placed in an empty mouse bucket with a high profile lid, with raised **Med Associates** locomotor beams set high enough to register a beam break only when a mouse executed a full jump (all four paws leaving the cage floor). A 0.5 sec timeout after a beam break was incorporated to the program to make sure each jump was only counted once. **(A, B)** Male and female mice with morphine pellets were tested 5 days after surgery. No effect of any dose of NYX-783 was observed. See panels for n's. **(C)** Female mice with oxycodone minipumps were tested 3 days after surgery. No effect of any dose of NYX-783 (n=8, 7, 8, 8) was observed. All error bars are S.E.M.

NYX-783 Improves Aversion Scores To The State Of Withdrawal



Aversion pairing days

oxycodone 5mg/kg, 2hr prior to chamber pairing
NYX-783 10mg/kg or Saline, 1hr prior to chamber pairing
naloxone 0.1mg/kg, just prior to chamber pairing

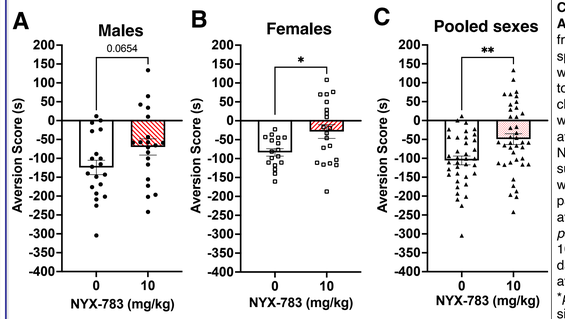
Neutral pairing days

saline 2hr prior to chamber pairing
saline 1hr prior to chamber pairing
saline, just prior to chamber pairing

Conditioned Place Aversion Paradigm Sequence

- **Day 1: Habituation** - 15min (Free access to all 3 chambers)
- **Day 2, 4, 6: Aversion pairing** - 30min (confined to chamber 1 or 3)
- **Day 3, 5, 7: Neutral pairing** - 30min (confined to the opposite chamber)
- **Day 8: Test** - 15min (Free access to all 3 chambers)

Aversion Score = (Time spent in the aversion chamber on Test day) - (Time spent in the aversion chamber on Habituation day)



NYX-783 improves aversion scores of C57BL/6J mice. A typical 3 chamber **Med Associates** CPA/CPP box was used. Entry/exit from one chamber to another, as well as time spent in each of the 3 chambers, was monitored with infrared beams. Habituation data was used to determine the context pairing for the chambers. A low 0.1 mg/kg dose of naloxone was chosen to induce a moderate state of aversion. The majority of control mice (0 mg/kg NYX-783) had moderate aversion scores, supporting this approach. **(A)** Male mice treated with 10 mg/kg NYX-7834 on their aversion pairing days showed a trending improvement in aversion score compared to Veh mice (n=20, 20; p=0.0654 t-test). **(B)** Female mice treated with 10 mg/kg NYX-7834 on their aversion pairing days and showed a significant improvement in aversion score compared to Veh mice (n=17, 20; *p=0.0158 t-test). **(C)** Pooling sexes revealed a significant improvement effect of NYX-783 (n=37, 40; **p=0.0032 t-test).

Conclusions

- **NYX-783 safety milestone criteria of preclinical models of OUD were met**
  - Respiratory depression did not worsen
  - Oxycodone self-administration or motivation for oxycodone did not increase
  - Somatic withdrawals did not increase
- **NYX-783 reduced the aversion caused by opioid withdrawal, supporting a clinical benefit in reducing negative emotional states and prevent relapse.**

Acknowledgements

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