The Novel N-Methyl-D-aspartate Receptor Modulator NYX-783 Exhibits Therapeutic Effects in Rodent Models Useful for the Study of Post-Traumatic Stress Disorder and Comorbid Alcohol Use Disorder

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Introduction

NYX-783 is a novel N-methyl-D-aspartate receptor (NMDAR) modulator currently in Phase 2 clinical development as a treatment for post-traumatic stress disorder (PTSD).

Both PTSD and alcohol use disorder (AUD) are prevalent, chronic and frequently comorbid diseases. In fact, individuals with PTSD are at a three-fold greater risk of developing AUD than the general population (Hasin, Stinson *et al*. 2007, Milliken, Auchterlonie *et al*. 2007). In addition, AUD comorbid with PTSD increases the number and severity of symptoms and worsens treatment outcomes than when these conditions occur independent of one another (Ralevski *et al*. 2014).

AUD and PTSD share several neurobiological correlates. Both AUD and PTSD are largely intractable conditions in part because continued alcohol use and relapse/PTSD flashbacks can be triggered by normally neutral environmental stimuli that have been associated with alcohol and/or trauma. Alcohol is also often used to self-medicate the anxiety that is a hallmark of both AUD and PTSD Leeies *et al.* 2010; Ebbert *et al.* 2018). Available pharmacotherapies for the treatment of AUD, PTSD, or AUD comorbid with PTSD are lacking and new approaches are needed (Taylor *et al*. 2017).

PTSD and AUD can be treated using exposure therapy and cognitive behavioral therapy aimed at reducing the ability of interoceptive and environmental stimuli to precipitate relapse via formation of new associations between alcohol/trauma-related stimuli. This process requires active, new learning, which is known as extinction, and relies upon the glutamatergic systems that mediate plasticity and cognitive functioning (Cleva *et al*. 2010). Thus, increasing attention is being given to the therapeutic potential of glutamatergic modulators in potentiating extinction learning for the treatment of AUD and PTSD.

The glutamatergic NMDAR is enriched in brain regions that mediate both AUD and PTSD symptoms, and NMDAR modulators such as D-cycloserine (DCS) have shown promise in treating both AUD and PTSD (Mataix-Cols *et al*. 2017, Kiefer *et al.* 2015). In the present studies, we used rat models useful for the evaluation of AUD with or without comorbid PTSD to determine if the NDMAR modulator NYX-783 prevents stress-induced deficits in extinction learning and relapse-like behavior.

Methods

Study 1: Post-traumatic Stress Disorder.

- *Conditioned fear. Continuous reinforcement and spontaneous recovery:* On Day 1, male Sprague Dawley rats (PD50) were placed into a foot-shock chamber for 7.5-min. Shocks (0.5 mA, constant current) were delivered for 1-sec at 90, 210, and 330-sec. Animals had 6 daily extinction trials, where they were placed in the foot-shock chamber for 5-min with no foot-shock. Animals were dosed with vehicle, NYX-783, or DCS a single time, 1-hr prior to extinction session 1 only. Following extinction, rats were left undisturbed in their home cage for 1-wk. Animals were returned to the shock chamber on day 14 to measure spontaneous recovery of conditioned fear
- *Partial reinforcement*. Male, C57BL/6 mice (PD60) were placed into a foot-shock chamber for 6 daily, 3-min sessions. Mice experienced foot-shock (2-sec, 0.7 mA, constant current) on days 1, 4, and 6. Extinction began the following day and continued for the next 6 days. Extinction sessions were identical to conditioning sessions except that no foot-shock was delivered. Animals were dosed with vehicle, NYX-783, or DCS 1-hr prior to each extinction session.

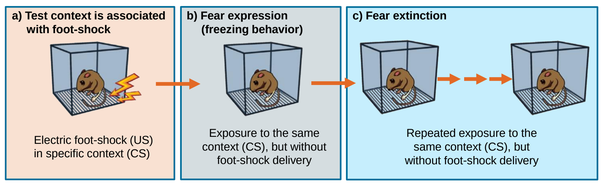
Study 2: Comorbid Alcohol Use Disorder.

- *Restraint stress*. Male Wistar rats (PD47) underwent acute restraint stress (3.25" x 8" tube, BrainTree Scientific, Inc.) during a single, 2-hr session in the presence of 3 mL sandalwood essential oil (neutral odor). Control animals were placed into a new cage in the presence of sandalwood odor. Food and water access was removed during this two-hour stress/sham-stress session, which occurred 3 days prior to the first alcohol self-administration session.
- *Acquisition*. Rats were trained in operant ethanol self-administration (10% v/v, FR1TO4) during 1-hr sessions (M, W, F; Gass et al. 2014). Each active lever-press triggered ethanol delivery (~ 45 µL), illumination of a stimulus light above the lever, tone activation (2900 Hz, 65 dB), and a 4-sec time-out where responses were recorded, but had no programmed consequence. Infrared sensors monitored ethanol port entry. After response criterion (30 active lever presses within one session) was met (~ 15 sessions), animals entered extinction training.
- *Ethanol extinction and drug administration*. NYX-783 or vehicle was given only once (1-hr prior to the first extinction session) and responding extinguished during daily 30-min sessions where responding on the previously ethanol-paired lever was reinforced by ethanol-associated cues, but not by ethanol itself. Sessions continued until extinction criteria were met (lever pressing for each rat fell below 20% of that recorded during the last 2 days of self-administration training).
- *Reinstatement*. Sandalwood odor was used to reinstate ethanol-seeking behavior in rats that previously received stress/sham stress. Reinstated ethanol-seeking behavior was measured under extinction conditions. NYX-783 or vehicle was administered only once, 1-hr prior to the test session.

Control measures:

- *Rotarod*. After being habituated to the rotarod for 3, 2-min sessions (4 rpm for the first min accelerating to 12 rpm over the next min) with a 30-min break between sessions, baseline performance was measured the following day (4 – 20 rpm over 2.5-min). Rats navigating the rod for 110-sec during baseline assessment were tested the following day (4 – 40 rpm over 5-min). Rats were dosed 1-hr prior to the test session, and terminal rpm was recorded at 15, 30, 60, and 120-min after drug administration.
- *Elevated plus maze*. Rats were dosed with NYX-783 or clobazam (benzodiazepine; 64 mg/kg), as positive control, and 12-hr later placed in the center of a 4-arm elevated maze with 2 walled arms and 2 open arms. Rats were assessed for entries into and time spent in the open arms.

Experimental approach: Study 1 (PTSD)

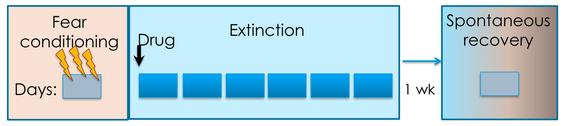


Adapted from Floes Â *et al*., (2015) *Trends Neurosci.*

Figure 1. Contextual fear conditioning, extinction, and spontaneous renewal overview. a) Rodents are placed into a neutral, novel context that the rodent associates with fear upon experiencing aversive foot-shock stimulus in the test chamber. **b)** Conditioned fear is quantified by the extent of freezing behavior expressed by the animal upon being returned to the test chamber where foot-shocks were previously received. **c)** Repeat exposure to the test chamber in the absence of foot-shock leads to extinction of freezing behavior that, after time (away from the test chamber), can be renewed by returning the subject to the test chamber (again without foot-shock delivery).

Study 1a: A single NYX-783 administration facilitates extinction and prevents spontaneous recovery of conditioned fear in a continuous reinforcement paradigm

Experimental approach: continuous reinforcement



Results:

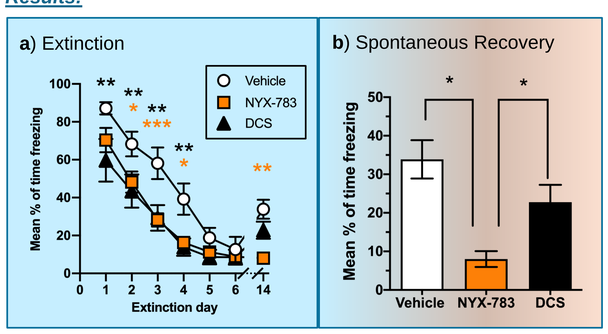
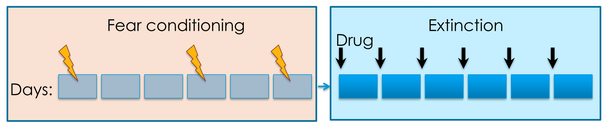


Figure 2. NYX-783 reduces fear conditioned via continuous reinforcement. a) Rats were treated with vehicle (1 ml/kg, PO or SC), D-cycloserine (DCS; 15 mg/kg, SC), or NYX-783 (1 mg/kg, PO) 1-hr prior to the first extinction session only. NYX-783 significantly facilitates fear extinction on days 2 - 4 whereas DCS significantly facilitates fear extinction on days 1 - 4. **b)** Unlike DCS, NYX-783 preventes spontaneous recovery of conditioned fear on day 14. Data represent mean ± SEM * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n = 11, repeated measures or one-way ANOVA followed by Dunnett's post-hoc.

Study 1b: Repeat NYX-783 administrations facilitate extinction of conditioned fear normally resistant to extinction in a partial reinforcement paradigm

Experimental approach: partial reinforcement



Results:

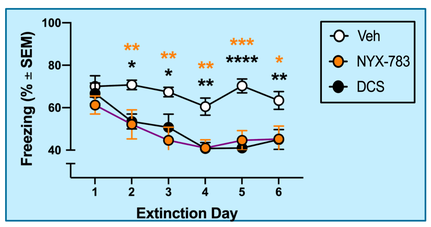
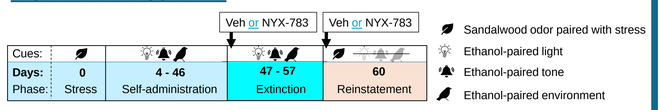


Figure 3. NYX-783 reduces fear conditioned by partial reinforcement. NYX-783 (10 mg/kg, ip), D-cycloserine (DCS; 15 mg/kg, ip), or vehicle (1 ml/kg) were given 1-hr prior to each extinction session. Both NYX-783 and DCS significantly reduces fear conditioned via partial reinforcement. Data represent mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n = 8. one-way ANOVA followed by Tukey's post-hoc test.

Experimental approach: Study 2 (AUD comorbid with PTSD)

Experimental approach:



Results:

Study 2a: Prior restraint stress increases ethanol self-administration

a) Restraint stress increases ethanol self-administration

b) Groups to be NYX-783 treated (Study 2b) were balanced by BEC

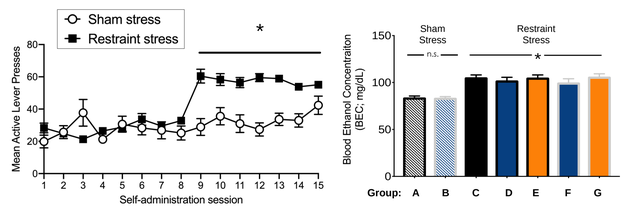
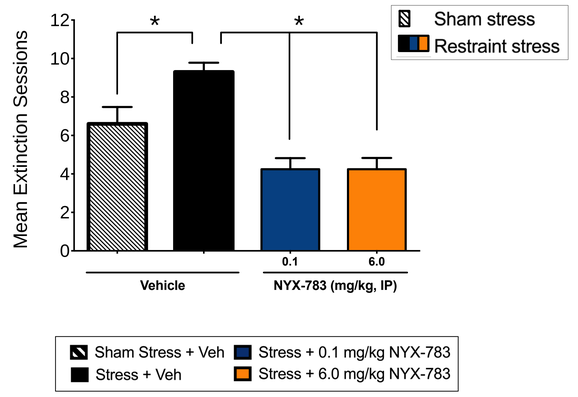


Figure 4. Ethanol consumption increases following restraint stress. A single, 2-hr restraint-stress session increases both **a**) ethanol self-administration and **b**) blood ethanol concentration (BEC). Subsequent treatment groups were balanced by BEC. Data represent mean ± SEM, one-way or repeated measures two-way ANOVA followed by a Holm-Sidak post-hoc; * $p < 0.05$, n = 7 – 9 per group. n.s.: not significant.

Study 2b: NYX-783 facilitates extinction of ethanol-seeking behavior in a model of AUD comorbid with PTSD

a) A single NYX-783 administration facilitates ethanol extinction



b) Extinction Session 1: ethanol seeking

c) Extinction Session 2: ethanol seeking

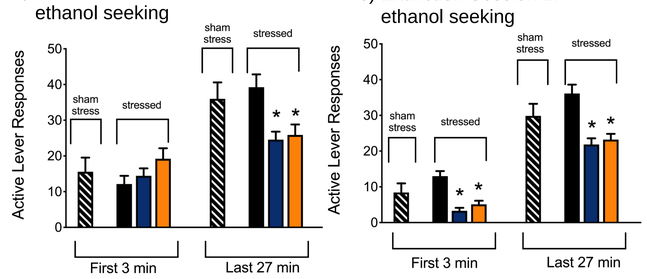


Figure 5. NYX-783 reduces ethanol-seeking behavior in a rat model of AUD with comorbid PTSD. The effect of NYX-783 on extinction of ethanol self-administration was evaluated in some rats from Figure 4 (restraint stress exacerbates ethanol self-administration). *Rats were given NYX-783 one time; 1-hr prior to the first extinction session.* **a**) Rats with prior stress were resistant to extinction. A single administration of NYX-783 (1-hr prior to the first extinction session) facilitates extinction of ethanol-seeking behavior despite prior restraint stress. **b**) Analysis of within-session responding reveals that NYX-783 enhances extinction learning (during the last 27-min of the first extinction session) without motoric effect (first 3-min, p = 0.35). **c**) The ability of NYX-783 to enhance extinction learning (acquired during Session one) was apparent throughout Extinction Session two. NYX-783 was given only once; 1-hr prior to the first extinction session. Data represent mean ± SEM; analyzed by a one-way or repeated measures two-way ANOVA followed by a Holm-Sidak post-hoc, * $p < 0.05$, n = 7 – 9 per group.

Study 2c: NYX-783 reduces relapse-like behavior

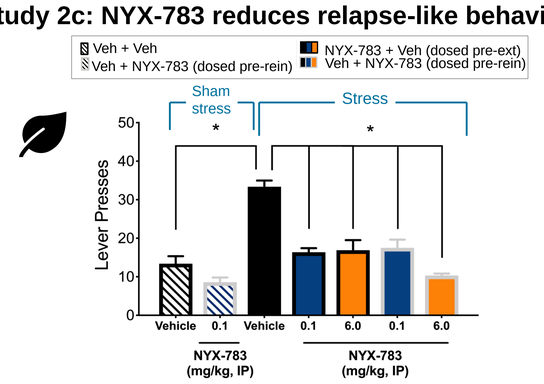


Figure 6. NYX-783 reduces relapse-like rat behavior precipitated by odor cue previously associated with restraint stress. Sandalwood odor (represented by the leaf symbol;) that is associated with prior restraint stress reinstates ethanol-seeking behavior, and this effect is blocked by a single NYX-783 administration. NYX-783 appears to be equally effective regardless of being administered 1-hr prior to extinction session one or 1-hr prior to the reinstatement test, as NYX-783 was given only a single time either prior to extinction or prior to reinstatement. Data represent mean ± SEM one-way ANOVA followed by a Holm-Sidak post-hoc; * $p < 0.05$, n = 7 – 9 per group.

NYX-783 does not impact motoric capacity

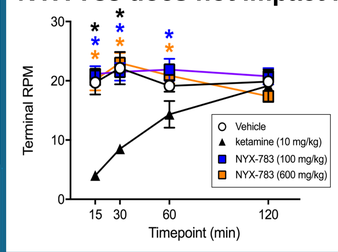


Figure 7. Accelerating rotarod performance is unaffected by NYX-783. Drug- and ethanol-naive rats are placed on an accelerating rotarod 15, 30, 60, and 120-min after administration of high NYX-783 doses (PO) or ketamine (IV). Ketamine initially produces ataxia and/or incoordination, but no adverse effects are observed with NYX-783. Data represent mean ± SEM one-way ANOVA followed by Tukey's post-hoc; * $p < 0.05$, n = 8.

NYX-783 does not impact anxiety-like behavior

a) Total arm entries **b**) Open arm entries

c) Time in open arms

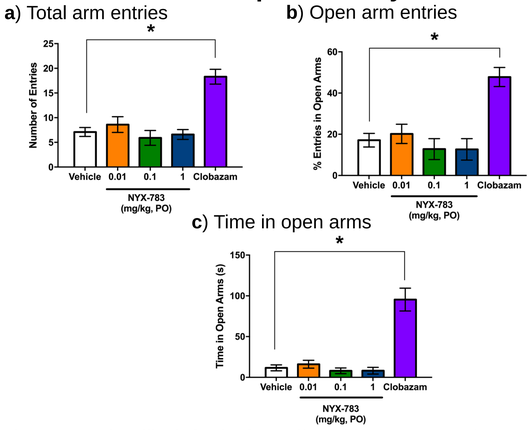


Figure 8. Anxiety-like behavior is unaffected by NYX-783. Using a separate cohort, anxiety-like behavior is assessed in the elevated plus maze. Rats are dosed with NYX-783 or the positive control clobazam, and 1-hr later placed in the center of a 4-arm elevated maze with 2 walled (closed) arms and 2 open arms. Rats are assessed for **c)** total number of arm entries, **d)** % entries into open arms **e)** total time spent in an open arm. Compared to vehicle, clobazam produces anxiolytic-like behavior, but no effect is observed with NYX-783. Data represent mean ± SEM, one-way ANOVA followed by Tukey's post-hoc, * $p < 0.05$, n = 10.

Conclusions

1. NYX-783 facilitates learning and memory without sedative properties.
2. The novel NMDAR modulator NYX-783 significantly reduces conditioned fear in both partial and continuous fear models across rodent species.
3. NYX-783 reduces ethanol-seeking behavior in rats normally resistant to extinction due to prior stress.
4. NYX-783 also significantly reduces ethanol relapse-like behavior precipitated by cues associated with stress.
5. These preclinical data support further investigation of NYX-783 for AUD and for PTSD with comorbid AUD.

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Nasdaq: APTX