The Novel NMDAR Modulator NYX-783 Facilitates Extinction of Ethanol-seeking Behavior and Blocks Relapse-like Behavior Primed by Ethanol-associated Cues or Prior Stress in Rats

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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). NYX-783 appears to enhance synaptic plasticity processes in the brain and improve learning. In previous rodent fear conditioning studies, NYX-783 was shown to enhance extinction learning and prevent spontaneous recovery of the fear response.

Alcohol use disorder (AUD) is highly comorbid with PTSD, with rates as high as 59% (Jacobsen et al. 2001). The use of alcohol to dampen stress/trauma-related anxiety is hypothesized to initiate a potent feed-forward cycle where stimulus-response outcomes become overlearned to a point where it is difficult to create new associations (Gass and Chandler 2013), and treatment outcomes are worsened (Ralevski et al. 2014). With a paucity of options to treat AUD comorbid with PTSD, development of new pharmacotherapies is paramount.

Perturbations in glutamatergic circuitry are well known to play critical roles in the development and maintenance of both AUD and PTSD. Preclinical data show increased prefrontal dysfunction in response to stress and trauma that affects glutamatergic components including NMDARs and their ability to induce synaptic plasticity (Averill et al. 2017). This is also true for neuronal circuits involved in addiction, since alcohol alters NMDAR function across a number of brain regions including the prefrontal cortex (Hopf 2017).

NMDARs are also critically involved in extinction learning, a process of active learning where new associations are formed between cues and their previously associated outcomes. The neuronal systems required for extinction learning are negatively impacted by alcohol and stress/trauma, rendering AUD co-morbid with PTSD resistant to treatment. The NMDAR modulator NYX-783 may be an effective therapeutic in this condition due to its potential to normalize alcohol- and stress-induced deficits, in part, by enhancing plasticity and learning processes in rats. In the present study, we sought to determine if NYX-783 had an effect on extinction learning and relapse-like behavior in animal models of AUD and comorbid PTSD.

Methods

- *Acquisition*. Wistar rats (male, PD50) were trained in operant ethanol self-administration (20% 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 (~ 9 sessions), animals were exposed to chronic intermittent ethanol (CIE) or air control.
- *Chronic Intermittent Ethanol (CIE)*. Cage mate pairs were placed into the same vapor chamber (24 x 24 x14"; Plas Labs, Lansing, MI) at 1800 hours and removed at 0800 hours. CIE (14 hrs on/10 hrs off) was followed by a two-day break (Gass et. al. 2017). This cycle was repeated for a total of 8 ethanol exposures that resulted in ~ 250 mg/dl blood ethanol concentration. Control animals were exposed to air. Food and water were available *ad libitum*.
- *Maintenance*. Following CIE/Air, animals returned to self-administration sessions for 10% ethanol (v/v), and response criteria increased to an average of 60 active lever presses over the final two sessions.
- *Extinction and drug administration*. NYX-783 (0.1 or 6 mg/kg, ip) or vehicle (0.5% carboxymethylcelullose, 1 ml/kg, ip) was given only once (1 hr prior to the first extinction session) and responding extinguished during daily 30 min sessions; active lever presses activated cues, but not ethanol reinforcement. Sessions continued until criteria were met (lever pressing for each rat fell below 20% of that recorded during the last 2 days of self-administration training).
- *Spontaneous recovery.* After extinction criteria were met, rats were returned to the homecage for 3 weeks, and no ethanol exposure occurred. Next, the effect of prior NYX-783 treatment on spontaneous recovery of ethanol-seeking behavior was measured (for CIE/Air exposed rats that had voluntarily self-administered ethanol). During this 30 min session, responding on the previously ethanol-paired lever activated ethanol-associated cues, but lever responding was not reinforced with ethanol.
- *Restraint stress*. Starting on PD47, rats 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. Control animals were placed into a new cage in the presence of sandalwood odor. Sandalwood is considered a neutral odor. Restraint occurred between 1100 to 1300 hours on the Friday before alcohol self-administration. Food and water access was removed during this two-hour stress/sham stress session.
- *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 (0.1 or 6 mg/kg, 1 ml/kg, in 0.5% CMC, ip) was administered 1 hr prior to the test session.

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NASDQ: APTX

Experimental approach: Study 1a

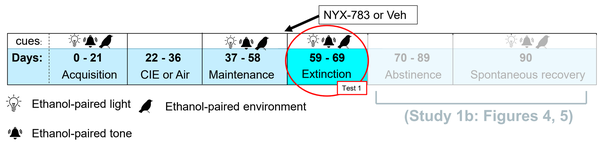
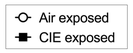
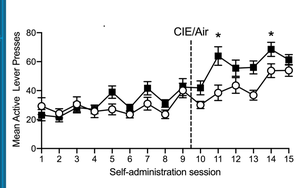


Figure 1. Approach to study the effect of NYX-783 on extinction of ethanol self-administration. After rats acquired ethanol self-administration, half of these rats were made ethanol-dependent by chronic intermittent ethanol vapor (CIE; blood ethanol concentration ~ 250 mg/dl). The other half were exposed to air during this period. After 1-week abstinence from CIE/Air procedures, voluntary operant ethanol self-administration resumed for 21 days. Ethanol was then removed, and the effect of NYX-783 on extinction of ethanol self-administration measured. Vehicle or NYX-783 was given once (0.1 or 6 mg/kg, ip, 1 hour prior to first extinction session).

Study 1a: A single NYX-783 administration reduces ethanol-seeking behavior regardless of prior ethanol dependence

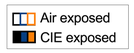
a) CIE increases ethanol self-administration

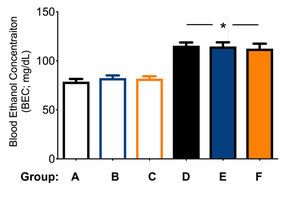




Comparing AIR vs CIE: F(1,975) = 224.28, n = 10 – 12 per group, *p < 0.0001

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

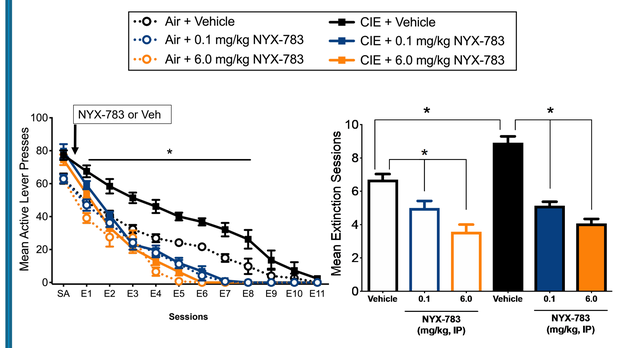




Comparing AIR vs CIE: F(5,56) = 22.72, n = 10 – 12 per group, *p < 0.0001

c) Extinction responding over days is reduced by NYX-783

d) Mean extinction sessions to reach criteria are reduced by NYX-783



Treatment (compared to CIE + Veh): F(2,385) = 224.7, n = 12 per group, *p < 0.0001
Also significantly different from Air + Veh
SA: last self-administration session; E#: extinction session

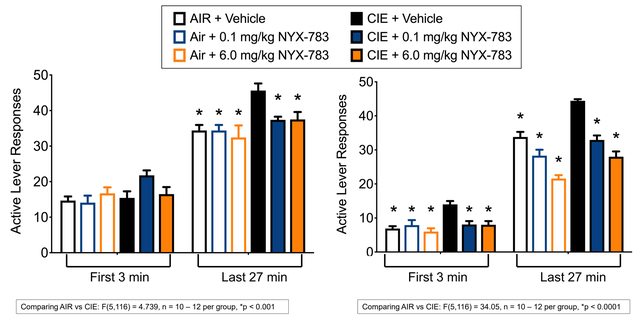
Compared to Air + Veh: F(2,24) = 14.98, n = 7 - 10 per group, *p < 0.0001
Also significantly different from CIE + Veh

Figure 2. NYX-783 facilitates extinction of ethanol-seeking behavior in rats with or without prior ethanol dependence. All rats underwent voluntary ethanol self-administration; some rats were made ethanol-dependent via chronic intermittent ethanol vapor exposure (CIE). Air exposure served as a control. **a**) CIE increased ethanol self-administration and **b**) blood ethanol concentration (BEC). **c**) Rats were dosed with NYX-783 or vehicle only once; 1 hr prior to the first extinction session (please see timeline in Figure 1). Rats with prior ethanol dependence (CIE; closed, black squares) were resistant to extinction when compared to rats that self-administered ethanol, but that had no history of ethanol-dependence (Air; open, black circles). NYX-783 reduced rat ethanol-seeking behavior regardless of prior ethanol dependence. Significant treatment effects were seen from extinction session 1 through 8. **d**) Similar to responding on the ethanol-paired lever, NYX-783 reduced the number of extinction session required to reach criteria (< 20% of responding during the last 2 self-administration sessions). Data represent mean ± SEM, and were analyzed by a one- or two-way ANOVA followed by a Dunnett's post-hoc; * p < 0.0001.

Study 1a: NYX-783 facilitates extinction learning

a) Extinction Session 1: ethanol seeking

b) Extinction Session 2: ethanol seeking



Comparing AIR vs CIE: F(5,116) = 4.739, n = 10 – 12 per group, *p < 0.001

Comparing AIR vs CIE: F(5,116) = 34.05, n = 10 – 12 per group, *p < 0.0001

Figure 3. Early (first 3 min) and late (last 27 min) session lever responding during the first and second extinction sessions. a) During the first extinction session, NYX-783 enhanced extinction learning (last 27 min) without motoric effect (First 3 min). **b**) The ability of NYX-783 to enhance extinction learning (acquired during Session 1) was apparent throughout Session 2. NYX-783 was given only once; 1 hr prior to the first extinction session. Data represent mean ± SEM, and were analyzed by a repeated measures two-way ANOVA followed by a Holm-Sidak post-hoc; * p < 0.001.

Experimental approach: Study 1b

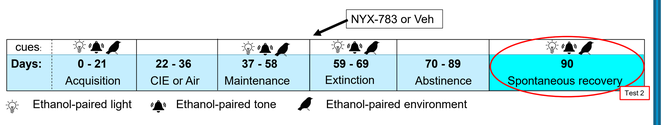
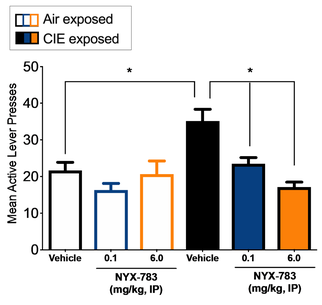


Figure 4. Approach to study effect of NYX-783 on relapse-like behavior. After meeting extinction criteria, rats were returned to the home cage for 3 weeks prior to being returned to the operant chamber where the effect of prior NYX-783 treatment on spontaneous recovery of ethanol-seeking behavior was measured under extinction conditions.

Study 1b: NYX-783 reduces relapse-like behavior in rats with prior ethanol dependence



F(2,26) = 12.56, n = 7 - 10 per group, * p < 0.001

Figure 5. NYX-783 decreases spontaneous recovery of ethanol-seeking behavior. After meeting extinction criteria, rats from Study 1a remained in the home cage for 3 wks. Spontaneous recovery of operant responding was then measured under extinction conditions. Ethanol-seeking behavior was increased by prior exposure to chronic intermittent ethanol (CIE), and this effect was reversed by NYX-783. Rats received NYX-783 only once (1 hr prior to the first extinction session). No NYX-783 effect on spontaneous recovery of ethanol-seeking behavior was seen in rats without a history of ethanol dependence (Air controls). Data represent mean ± SEM, and were analyzed by a one-way ANOVA followed by a Dunnett's post-hoc; * p < 0.001.

Experimental approach: Study 2

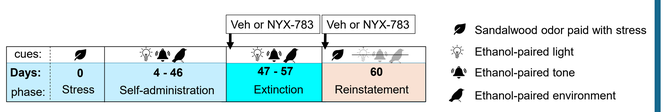
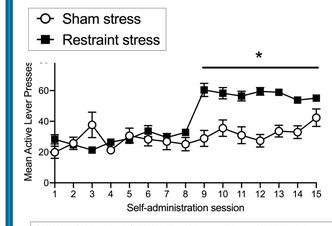


Figure 6. Approach to study effect of NYX-783 on ethanol-seeking behavior in a model of comorbid PTSD. A separate rat cohort underwent restraint stress (sham stress served as a control) in the presence of sandalwood odor 4 days prior to voluntary ethanol self-administration (10% v/v, ~40 days, ≥ 80 mg% BEC) that was followed by extinction and reinstatement precipitated by sandalwood odor. Vehicle or NYX-783 was given once (0.1 or 6 mg/kg, ip, 1 hour prior to the first extinction or reinstatement session).

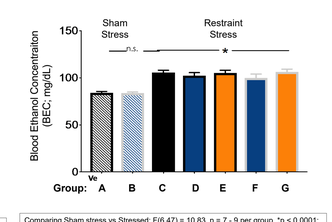
Study 2: NYX-783 reduces ethanol-seeking behavior in a model of AUD comorbid with PTSD

a) Restraint stress increases ethanol self-administration



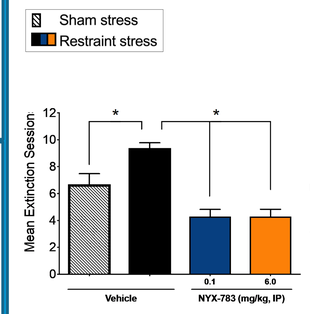
Comparing Sham stress vs Stressed: F(1,960) = 67.12, n = 7 - 9 per group, *p < 0.0001

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



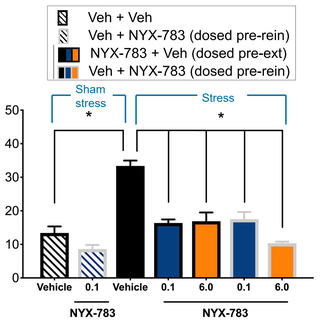
Comparing Sham stress vs Stressed: F(6,47) = 10.83, n = 7 - 9 per group, *p < 0.0001; n.s. = not significant

c) NYX-783 facilitated extinction (made resistant by prior stress)



Treatment (compared to Stressed + Veh): F(2,21) = 26.42, n = 8 - 9 per group, *p < 0.0001
Also significantly different from Sham Stress + Veh

d) NYX-783 reduced relapse-like behavior precipitated by memory of stress



Treatment (compared to Stressed + Veh): F(6,47) = 14.63, n = 7 - 8 per group, *p < 0.0001; all post-hoc comparisons were p <0.05

Figure 7. NYX-783 reduced ethanol-seeking behavior in a rat model of comorbid PTSD. Exposure to a single, 2-hr restraint-stress session increased both **a**) ethanol self-administration and **b**) blood ethanol concentration (BEC). Treatment groups were balanced by BEC. **c**) Rats with prior stress were resistant to extinction. A single administration of NYX-783 (1 hr prior to the first extinction session) facilitated extinction of ethanol-seeking behavior despite prior restraint stress. **d**) Sandalwood odor that was associated with prior restraint stress precipitated ethanol-seeking behavior, and this effect was blocked by a single NYX-783 administration. NYX-783 was equally effective regardless of being administered 1 hr prior to extension session 1 or 1 hr prior to the reinstatement test. Data represent mean ± SEM; * p < 0.05

NYX-783 does not impact motoric capacity

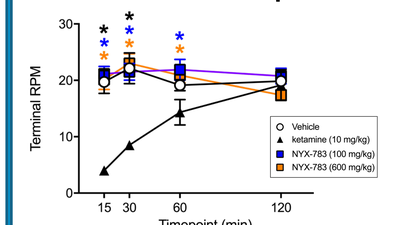


Figure 8. Accelerating rotarod performance was unaffected by NYX-783. Drug and ethanol-naive rats were placed on an accelerating rotarod 1 hr after administration of high NYX-783 doses. Data represent mean ± SEM; F(3,25) = 13.05, * p < 0.05

Conclusions

1. The novel NMDAR modulator NYX-783 significantly reduced ethanol-seeking behavior in rats with or without a history of ethanol dependence.
2. NYX-783 also significantly reduced relapse-like behavior precipitated by cues associated with ethanol (in rats with a history of ethanol dependence) or stress.
3. These preclinical data support the further investigation of NYX-783 for alcohol use disorder and in PTSD subjects who are alcohol seeking.