Positive allosteric modulator of the NMDA receptor, NYX-783, reverses repeated stress-induced reduction of exploration and reduces fear to unpredictable threats in rats

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Introduction

Post-traumatic stress disorder, or PTSD, is a traumatic events-induced (either acute or developmental, chronic) neuropsychiatric disorder characterized by hyperarousal and hypervigilance (e.g. increased startle response), avoidance of trauma-triggering stimuli, and re-experiencing traumatic events typically manifesting as flashbacks, all of which lead to significant social, occupational, and interpersonal dysfunction. On a behavioral level, PTSD is characterized by increased fear reactivity in response to unpredictable threats, deficits in fear extinction and impaired fear discrimination – inability to discriminate between fear and safety signals. These behavioral manifestations of PTSD can be studied in a laboratory setting using rodent models. Current approved pharmacotherapy for PTSD remains limited to selective serotonin reuptake inhibitors (SSRI's) in conjunction with prolonged exposure therapy (PE). Although current pharmacotherapy for PTSD is limited, modulators of glutamatergic NMDA receptors (NMDAR) have received substantial attention. A recent phase II clinical trial (ClinicalTrials.gov: NCT04044664) demonstrated the efficacy of NYX-783 (Aptinyx, Inc., Evanston, IL), a positive allosteric modulator of NMDAR, in subjects with PTSD. In the current study, we investigated the effects of NYX-783 in rat models of the sensitization of acoustic startle reflex (SS), fear reactivity to unpredictable threats measured in anxiety-potentiated startle (APS), and repeated stress-induced reduction in exploration in the elevated plus-maze (EPM).

Methods

Animals: Male and female Sprague-Dawley rats (60-70 days old) were purchased from Envigo (Chicago, IL) and were acclimated to housing in the vivarium for at least 7 days before starting experiments.

Startle Sensitization (SS): SS protocol was modified based on the original study (Davis 1989). Experiments were conducted in SR-LAB startle chambers with cylindrical animal enclosures (San Diego Instruments). All rats are first subjected to acoustic startle response (ASR) testing (pre-test), during which ASR amplitude is measured in response to 30 startle-eliciting white noise bursts (WNB, 95 dB). Then, NYX-783 injections (1 or 10 mg/kg or saline ip) were given to male rats (n=12 per group) followed by an hour in their home cage before returning to chambers to receive 10-footshocks (0.5mA, one every second) immediately followed by 30 WNB (post-test). Startle responses from pre- and post-test were compared within subjects.

Anxiety-potentiated startle (APS): Sprague-Dawley female rats (n=10 per group) were tested for baseline ASR as above for two consecutive days. On day 3 (fear conditioning), rats were exposed to eight un-paired 0.5 s foot shock and eight pseudo-randomly presented 3.7 s cue lights. To prevent the cue from becoming a safety signal, two trials of footshocks were co-terminated with cue light. On day 4, rats were tested for cued and non-cued fear, and on day 5, rats were tested for contextual fear. NYX-783 (1, 10, or 30 mg/kg ip) or vehicle (saline) were injected one hour before recall tests. Cued and non-cued fear were calculated as a percentage change according to the diagram:

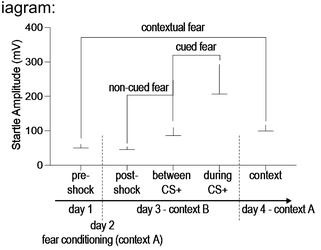
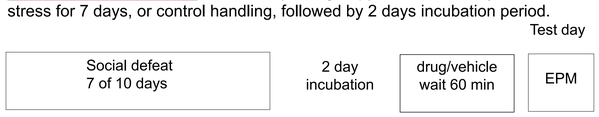


Fig. 1. APS components. Cued fear is calculated as a percent change score of ASR amplitude from 'between CS+' to 'during CS+'. Non-cued fear is calculated as a percent change score from post-shock to 'between CS+'. Contextual fear recall in context A with no CS presentations, calculated as percent change score from pre-shock on day 1 to ASR in context A on day 4.

Repeated Social Defeat: Male rats (n=12/group) underwent daily resident-intruder stress for 7 days, or control handling, followed by 2 days incubation period.



Elevated Plus Maze (EPM): On the testing day (day 10) , control or stressed rats received saline or NYX-783 (1, 10, or 30 mg/kg ip) one hour prior placement in the center of the maze for a 5-minute test period.

Statistical Analysis: Data was analyzed using GraphPad Prism 9.0 with a p-value <0.05 considered statistically significant. Data was analyzed with repeated measures (RM) two-way ANOVA, one-way ANOVA or un-paired t-test. Where the F-ratio is significant, all-pairwise *post-hoc* comparisons were made using Sidak's tests.

Results

Shock-Induced Startle Sensitization (SS)

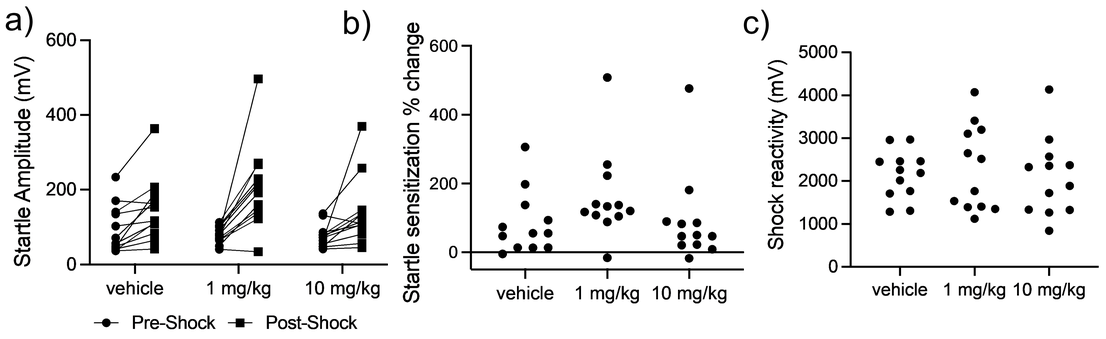
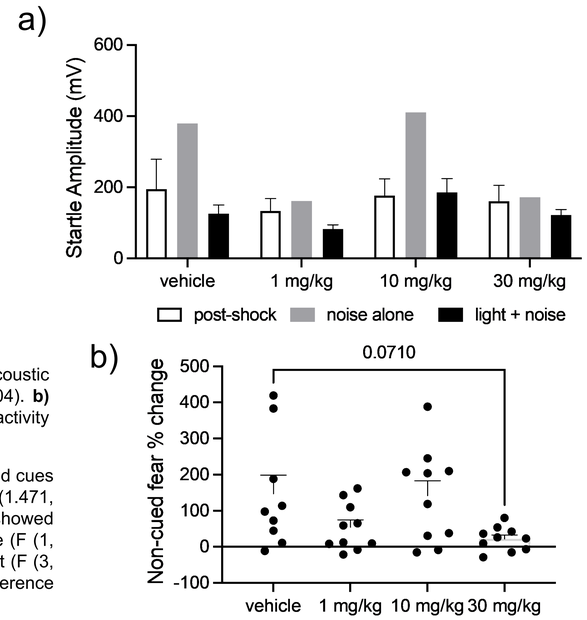


Figure 2. NYX-783 does not affect foot-shock induced startle sensitization in male rats. a) There was a significant foot-shocks effect on acoustic startle response (F (1, 33) = 35.42, P<0.0001, pre vs. post-shock startle) but no treatment effect of NYX-783 (F (2, 33) = 0.9954, P=0.3804). **b)** Ordinary one way ANOVA of percentage change of startle sensitization showed no treatment effect (F (2, 33) = 1.514, P=0.2350). **c)** Shock reactivity was not affected by treatment with NYX-783 (F (2, 33) = 0.1824, P=0.8341), n=12 per group.

Figure 3. NYX-783 reduces recall of anxiety-potentiated startle (APS) in female rats. Female rats were subjected to unpaired foot-shocks and cues during fear conditioning. **a)** During the second recall test (APS2), there was a significant trial effect on acoustic startle response for all trials (F (1.471, 51.47) = 13.10, P=0.0001), as well as for noise alone vs. post-shock startle trials (F (1, 35) = 13.59, P=0.0008), suggesting that female rats showed robust non-cued fear expression during the second recall test. Furthermore, there was a significant interaction between treatment and trial type (F (1, 35) = 13.59, P=0.0008). **b)** One way ANOVA of percentage change of non-cued fear showed a significant treatment effect of NYX-783 treatment (F (3, 35) = 3.355, P=0.0297), with posthoc showing a trend between vehicle and NYX-783 (P=0.0710). Finally, unpaired t-test showed a significant difference between saline and 30 mg/kg of NYX=783 (t=2.469, df=17, p = 0.0244), n=10 per group. ⟶

Anxiety-potentiated Startle (APS)



Stress-induced deficits in exploration (EPM)

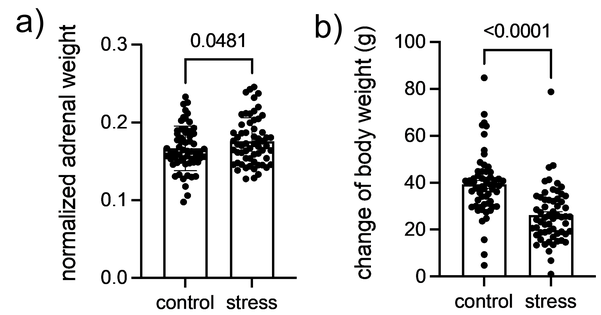
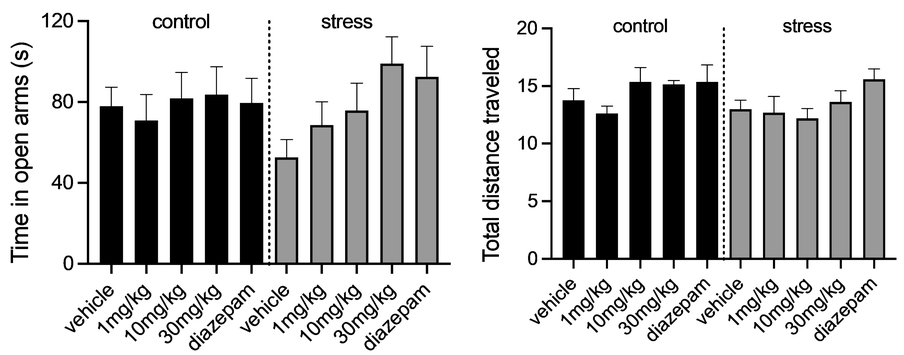


Figure 4. NYX-783 reduces repeated stress-induced reduction of exploration in the elevated plus-maze (EPM). a) Repeated social defeat stress increases adrenal weight (normalized per total body weight, t=1.677, df=118, P=0.0481), and **b)** reduces body weight gain in male rats (t=5.671, df=118, P<0.0001). **c)** There was no effect of NYX-783 on time spent in open arms in the control group (one way-ANOVA, F (3, 33) = 0.2047, P=0.8924). After social defeat stress, there was a significant NYX-783 treatment effect on time spent in the open arms (F (3, 41) = 2.852, P=0.0489), **d)** with no significant effect of treatment on total distance traveled in the EPM (F (9, 95) = 1.675, P=0.1056), n=12 per group.



Conclusions

- NYX-783 (at 1 or 10 mg/kg) does not affect foot-shocks induced startle sensitization in male rats
- NYX-783 (at 30 mg/kg) reduces recall of anxiety-potentiated startle in female rats, suggesting that NYX-783, acutely, reduces fear to unpredictable threats measured in the APS
- NYX-783 (at 30 mg/kg), acutely, reverses repeated stress-induced reduction of open arms exploration in the EPM in male rats, with no significant effect on total distance traveled, suggesting that NYX-783 has anxiolytic effect in the EPM
- Overall, our results suggest that NYX-783 (at 30 mg/kg) demonstrates pharmacotherapeutic potential for treatment of PTSD

References

Acknowledgements

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