NMDA Receptor Modulation with NYX-458 Rescues Cognitive Impairment and Peripheral Growth Hormone Levels in a Clinically Relevant Model of Repeat Concussion

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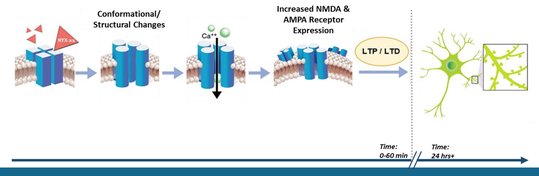
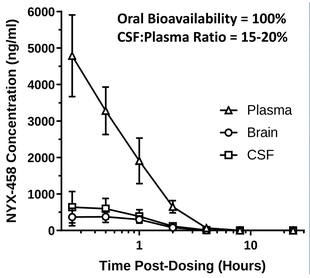
INTRODUCTION

N-Methyl-D-Aspartate receptors (NMDARs) are a family of ligand-gated ionotropic glutamate receptors that are found predominantly in the central nervous system (CNS) and play a pivotal role in mediating normal neuronal functions. NMDAR dysfunction has been implicated in a variety of CNS disorders, including post-traumatic stress disorder (PTSD), traumatic brain injury (TBI), cognitive impairment, mood disorders, and neuropathic pain. Aptinyx has developed a family of novel, small molecule, orally bioavailable synthetic NMDAR modulators. Mechanistically, these molecules bind directly to NMDARs, modulating (rather than inhibiting or over-activating) them and triggering a neurobiological cascade leading to enhancement of synaptic plasticity. Here, a member of this family, NYX-458, was evaluated for its ability to ameliorate affective and cognitive deficits, as well as peripheral hormone levels in a preclinical model of repeat concussion.

In a Phase 1 clinical study in healthy human volunteers, NYX-458 demonstrated a favorable safety and tolerability profile with dose-dependent and predictable pharmacokinetics. NYX-458 is currently in clinical development for the treatment of cognitive impairment associated with Parkinson's disease.



1. NYX-458 is orally bioavailable, a CNS penetrant, and demonstrates a favorable toxicology profile in rats and dogs

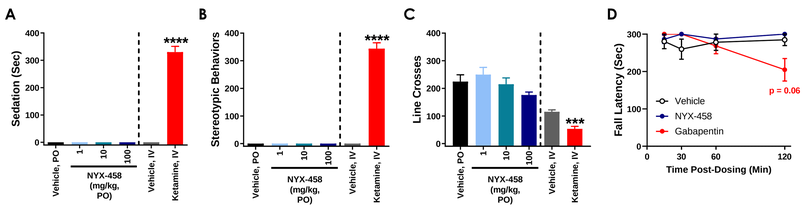


Parameter	Maximum Tolerated Dose Study (Rat)
Doses (mg/kg)	750, 1000
N / Group	3 male, 3 female
Maximally Tolerated Dose	1000 mg / kg
Noteworthy Findings	No treatment-related findings at max dose

Parameter	28-Day Repeat Dose Toxicology Studies	
Species	Rat	Dog
Doses (mg/kg)	50 - 250 mg/kg	5 - 75 mg/kg
N / Group	13 male, 13 female	5 male, 5 female
NOEL	250 mg / kg / day	75 mg / kg / day
Noteworthy Findings	No treatment-related findings in either species	

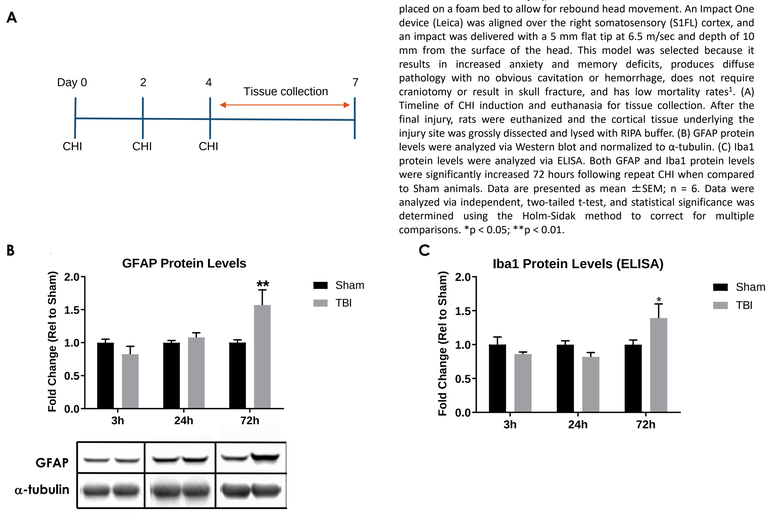
Pharmacokinetics and toxicology data in a maximum tolerated dose study and 28-day repeat dose study with NYX-458 in rat and dog. *(Left panel)* Three male Sprague Dawley rats each received 10 mg/kg NYX-458 (PO). The Tmax was 0.25 hours with a Cmax of 4784, 638, and 374 ng/ml in plasma, CSF, and brain, respectively. *(Right panel)* For toxicology studies, animals were observed for behavioral changes, mortality, body weight, and food consumption. Clinical chemistries were evaluated throughout the study. At necropsy, macroscopic evaluations were conducted followed by histopathological evaluation on select tissues.

2. NYX-458 has no effect on locomotor activity and does not cause sedation or stereotypies in rats



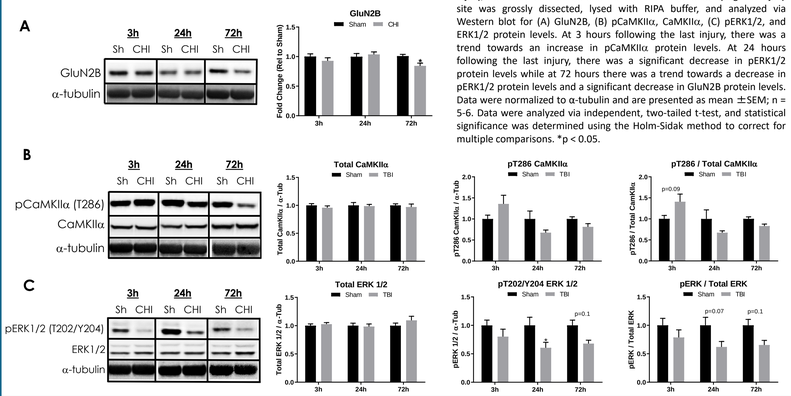
Open field behavior and RotaRod performance in rat. Male Sprague Dawley rats were dosed with vehicle (PO or IV), NYX-458 (1, 10 or 100 mg/kg, PO), or ketamine (10 mg/kg IV) and placed in an open field for 20 minutes immediately after dosing (ketamine group) or 1h after dosing with NYX-458. Incidents of sedation behaviors (A), stereotypic behaviors such as repetitive circling or head bobbing (B), and line crosses (C) were scored by a blind observer. No significant changes in sedation, stereotypic behaviors, or line crosses were observed for any dose of NYX-458 when compared to vehicle. (D) In a separate study, rats were administered vehicle (PO), NYX-458 (100 mg/kg, PO) or gabapentin (300 mg/kg, PO) and placed on a RotaRod (16 RPM for 300 sec). All animals were tested 15, 30, 60 and 120 min. after dosing and latency to fall was recorded. NYX-458 (100mg/kg, PO) did not significantly alter the fall latency when compared to vehicle. For panels A, B, and C, data from PO-dosed rats were analyzed via one-way ANOVA (all non-significant), and data from IV-dosed rats were analyzed via independent, two-tailed t-test (sedation t_{15} = 14.84; stereotypies t_{16} = 14.52; line crosses t_{15} = 5.22). For panel D, data were analyzed via two-way ANOVA ($F_{6,93}$ = 5.96; p < 0.001 for time by condition interaction), followed by Dunnett's post-hoc test (for each time point, all conditions compared to Vehicle). n = 2-12. ***p < 0.001; ****p < 0.0001.

4. Repeat closed head injury (CHI) results in glial activation in the cortex



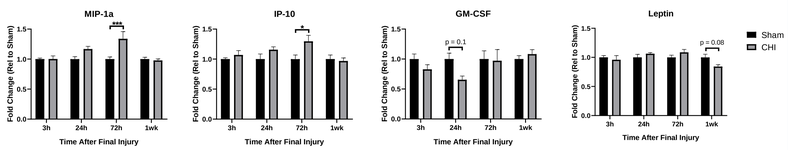
Induction of repeat CHI and resultant protein level changes in underlying cortical tissue. For each injury, rats were anesthetized with isoflurane and placed on a foam bed to allow for rebound head movement. An Impact One device (Leica) was aligned over the right somatosensory (S1FL) cortex, and an impact was delivered with a 5 mm flat tip at 6.5 m/sec and depth of 10 mm from the surface of the head. This model was selected because it results in increased anxiety and memory deficits, produces diffuse pathology with no obvious cavitation or hemorrhage, does not require craniotomy or result in skull fracture, and has low mortality rates[1]. (A) Timeline of CHI induction and euthanasia for tissue collection. After the final injury, rats were euthanized and the cortical tissue underlying the injury site was grossly dissected and lysed with RIPA buffer. (B) GFAP protein levels were analyzed via Western blot and normalized to α-tubulin. (C) Iba1 protein levels were analyzed via ELISA. Both GFAP and Iba1 protein levels were significantly increased 72 hours following repeat CHI when compared to Sham animals. Data are presented as mean ±SEM; n = 6. Data were analyzed via independent, two-tailed t-test, and statistical significance was determined using the Holm-Sidak method to correct for multiple comparisons. *p < 0.05; **p < 0.01.

5. Repeat CHI alters NMDAR-associated protein levels in the cortex



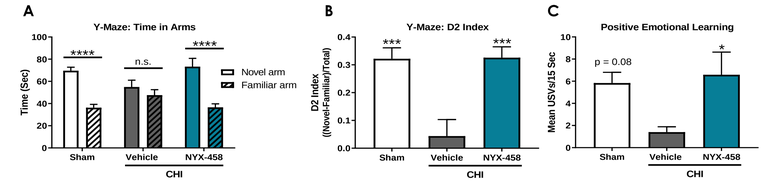
Repeat CHI alters NMDAR signaling. GluN2B, CaMKIIα, and ERK play important roles in synaptic plasticity processes and their expression has been shown to be altered in various animal models of TBI. After the final injury, rats were euthanized and the cortical tissue underlying the injury site was grossly dissected, lysed with RIPA buffer, and analyzed via Western blot for (A) GluN2B, (B) pCaMKIIα, CaMKIIα, (C) pERK1/2, and ERK1/2 protein levels. At 3 hours following the last injury, there was a trend towards an increase in pCaMKIIα protein levels. At 24 hours following the last injury, there was a significant decrease in pERK1/2 protein levels while at 72 hours there was a trend towards a decrease in pERK1/2 protein levels and a significant decrease in GluN2B protein levels. Data were normalized to α-tubulin and are presented as mean ±SEM; n = 5-6. Data were analyzed via independent, two-tailed t-test, and statistical significance was determined using the Holm-Sidak method to correct for multiple comparisons. *p < 0.05.

6. Repeat CHI alters cortical cytokine expression profile



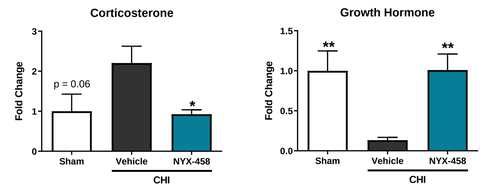
Altered cytokine expression profile following CHI in underlying cortical tissue. After the final injury, rats were euthanized and the cortical tissue underlying the injury site was grossly dissected and lysed with RIPA buffer. Samples were shipped to Eve Technologies and run on the cytokine/chemokine 27-plex discovery assay that included: Eotaxin, epidermal growth factor (EGF), fractalkine, IL-1α, IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, IL-17A, IL-18, IP-10, GRO/KC, interferon-gamma (IFN-γ), tumor necrosis factor (TNF)-α, G-CSF, GM-CSF, MCP-1, leptin, LIX, MIP-1α, MIP-2, RANTES, vascular endothelial growth factor (VEGF). At 24 hours following the last injury there was a trend towards a decrease in GM-CSF protein levels while at 72 hours there was a significant increase in MIP-1a and IP-10 protein levels. At 1 week following the last injury there was a trend towards a decrease in Leptin protein levels. Data were normalized to the sham condition and are presented as mean ±SEM; n = 5-6. Data were analyzed via independent, two-tailed t-test, and statistical significance was determined using the Holm-Sidak method to correct for multiple comparisons. *p < 0.05; ***p < 0.001

7. Repeat CHI results in spatial and affective learning deficits, which are rescued by NYX-458



Performance in the forced alternation version of the y-maze and positive emotional learning tasks by sham-treated and CHI rats. Male Long Evans rats were given three closed-head injuries (CHI) and were dosed with vehicle or NYX-458 (10 mg/kg, IP) 1 hr after each injury. Sham rats received anesthesia only and were dosed with vehicle. Three days after the final injury, rats explored two open arms of a y-maze for five minutes. Two hours later, they were again placed in the y-maze and explored three open arms for 5 min. The start arm was held constant for all rats, and the novel and familiar arms were counterbalanced. Time spent actively exploring the novel and familiar arms (A) was scored from video by a blind observer, and the D2 index (B) was calculated. Y-maze results indicate that injured rats spent less time in the novel arm versus the familiar arms when compared to shams and this deficit was rescued in animals dosed with NYX-458. Data are presented as mean ±SEM; n = 12-14. Exploration time was analyzed via two-way ANOVA ($F_{2,36}$ = 8.06; p = 0.0013 for arm x condition interaction) followed by Sidak's post-hoc test (within each condition, novel arm compared to familiar arm). D2 index was analyzed via one-way ANOVA ($F_{2,36}$ = 6.27; p = 0.0046) followed by Dunnett's post-hoc test (all groups compared to CHI+Vehicle). (C) A separate cohort of rats received the same injury and drug treatment schedule. Two days after the final injury, rats were engaged in heterospecific rough-and-tumble play, and ultrasonic vocalizations (USVs) were recorded between play bouts as a measure of positive emotional learning. Repeat CHI resulted in a decrease in USVs which was rescued in animals dosed with NYX-458. Data are presented as the mean (±SEM) number of hedonic USVs across six 15-sec trials; n = 5-6. Data were analyzed via one-way ANOVA ($F_{2,14}$ = 3.68; p = 0.052) followed by Dunnett's post-hoc test (all groups compared to CHI+Vehicle). *p < 0.05; ***p < 0.001; ****p < 0.0001.

8. Repeat CHI alters levels of circulating corticosterone and growth hormone, which are normalized by NYX-458



Serum corticosterone and growth hormone in sham-treated and CHI rats. Male Long Evans rats were given three closed-head injuries (CHI) and were dosed with vehicle or NYX-458 (10 mg/kg, IP) one hour after each injury. Sham rats received anesthesia only and were dosed with vehicle. Serum was collected four days after the final injury and analyzed via ELISA for both corticosterone and growth hormone. At this time point, repeat CHI resulted in an increase in serum corticosterone and a decrease in growth hormone, both of which were normalized in rats dosed with NYX-458. Data were normalized to the sham condition and are presented as mean ±SEM; n = 4-6. Data were analyzed via one-way ANOVA followed by Dunnett's post-hoc test (all conditions compared to CHI+Vehicle). Corticosterone: $F_{2,11}$ = 4.59; p = 0.036. Growth hormone: $F_{2,13}$ = 6.49; p = 0.011. *p < 0.05; **p < 0.01.

CONCLUSIONS

- In preclinical studies, NYX-458 is orally bioavailable, penetrates the CNS, demonstrates an excellent safety profile, and does not cause sedation or stereotypies.
- Repeat closed-head injury (CHI) resulted in decreased NMDAR-associated protein levels, altered cytokine expression profile, affective and spatial learning/memory impairments, and neuroendocrine dysfunction.
- These findings are consistent with other studies suggesting NMDAR hypofunction follows early excitotoxic events and results in persistent cognitive deficits[2,3].
- NYX-458 rescued affective and spatial learning/memory impairments induced by repeat CHI.
- NYX-458 rescued neuroendocrine dysfunction, as measured by altered circulating hormone levels induced by repeat CHI.
- Positive NMDAR modulation, rather than NMDAR antagonism, may therefore constitute an effective therapeutic strategy in TBI.
- Altogether, the robust activity demonstrated in the preclinical data presented here is suggestive of the therapeutic potential of NYX-458 in TBI and support further evaluation in clinical studies.

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FINANCIAL DISCLOSURE

LPC, KL, RJW, MES, AML, JSB, CC & JRM received financial compensation and stock from Aptinyx, Inc.
NASDAQ : APTX