NYX-783, a novel NMDAR modulator, rescues the detrimental effects of encephalitis-causing anti-NMDAR antibodies on GluN2B-NMDAR expression *in vitro*

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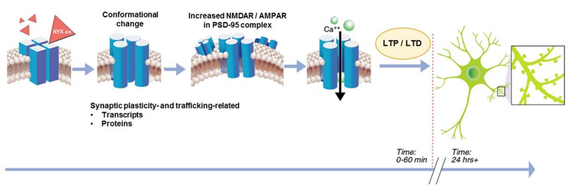
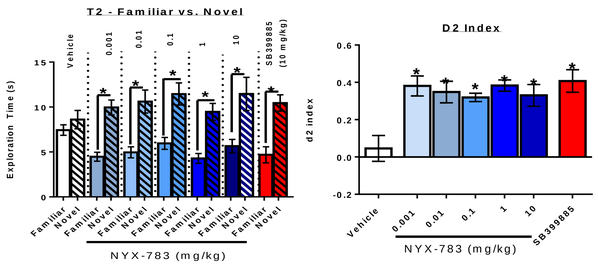
INTRODUCTION

N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors found in the central nervous system (CNS) and are critical for synaptic plasticity processes underlying numerous cognitive functions, including learning and memory. Loss of NMDAR function is implicated in many psychiatric and CNS disorders, including anti-NMDAR encephalitis (ANRE). Symptoms of ANRE begin with severe psychiatric problems and progress to seizures, movement impairments, and autonomic dysfunction. ANRE is an autoimmune disorder caused by the production of antibodies against the obligatory GluN1 subunit of the NMDAR. Binding of anti-GluN1 antibodies to the NMDAR leads to receptor internalization and thereby a reduction in functional NMDARs. Although ANRE can be treated with various immunotherapies, many patients have persistent cognitive deficits.

Here we show that NYX-783, a small molecule NMDAR modulator, improved rats' performance in the novel object recognition (NOR) paradigm, as well as enhanced long-term potentiation (LTP) in rat hippocampal and medial prefrontal cortex (mPFC) slices. In HEK cells expressing recombinant NMDARs, NYX-783 increased GluN2B-NMDAR surface expression. Given the effects of NYX-783 on cognitive function, NMDAR-dependent synaptic plasticity, and NMDAR surface/synaptic expression, we sought to determine whether it could rescue NMDAR internalization in response to anti-GluN1 antibody exposure.

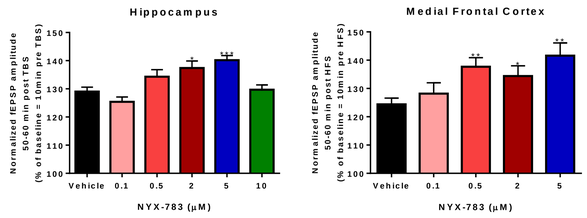


1 NYX-783 Enhances Recognition Memory in the NOR Assay



NYX-783, across all doses tested, significantly increases D2 index compared to vehicle. Animals are presented with 2 identical objects (T1), and 24hr later (T2), animals are presented with one novel and one familiar object. Rats are assessed in T2 for time spent exploring the novel vs. familiar object. The D2 index, a normalized difference score, is calculated as follows: (novel-familiar)/(novel+familiar). SB-399885, a 5-HT_6 receptor antagonist, was used as a positive control. NYX-783 was administered 1 h prior to T1; SB-399885 was administered 4 h prior to both T1 and T2. Significance by ANOVA *$p \leq 0.05$.

2 NYX-783 enhances long-term potentiation in the hippocampus and medial prefrontal cortex



NYX-783 enhances LTP in both the hippocampus and mPFC. For LTP recordings in hippocampal slices, field potentials (fEPSPs) were recorded from stratum radiatum of CA1 by stimulating the Schaffer collateral. LTP was induced by theta burst stimulation (5 pulses at 100Hz, 10 trains with 200ms intervals, 2 sets with 3 min intervals). For LTP recordings in mPFC slices, fEPSPs were recorded from layer 5 of rat mPFC slices by stimulating layer 2. LTP was induced by high frequency stimulation (100 pulses at 100Hz: 3 trains with 10 min intervals). LTP fold changes (50-60 min following the third tetanus) were analyzed using unpaired Student's t test; *p<0.05, **p < 0.01, ***p<0.001.

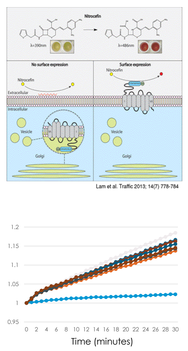
3 NYX-783 increases GluN2A- and GluN2B-NMDAR expression in the PSD of rat mPFC

Fold Change (NYX-783 compared to vehicle)

	15min	30min	60min	24hr
GluN2A	+2.228	ns	ns	ns
GluN2B	+2.308	ns	ns	ns

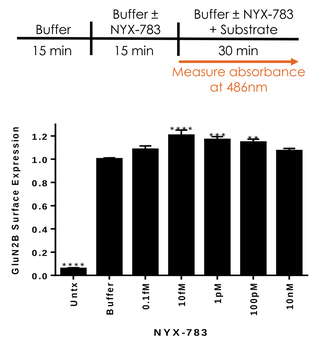
NYX-783 significantly increases PSD95-associated GluN2A- and GluN2B NMDA receptors in the rat mPFC 15 min post-dosing. Adult male Sprague Dawley rats were dosed with NYX-783 (1mg/kg) or vehicle and mPFC was isolated at the indicated times post-dosing. Total protein was co-immunoprecipitated with PSD95 and analyzed by nanLC-MS/MS. Data is presented as fold change compared to vehicle (n = 5 per group). Protein expression between drug-treated and vehicle groups were assessed by Student's t-Test $p \leq 0.05$, ns = not significant.

4 HEK cell β-lactamase assay developed to measure NMDAR surface expression *in vitro*.



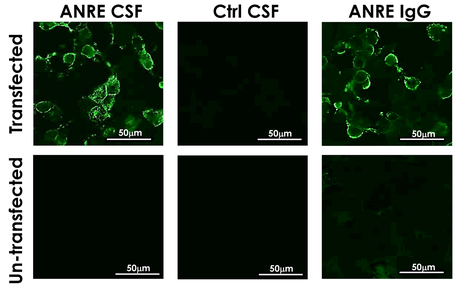
- hNR1/PSD95-expressing HEK cells transiently transfected with β-lactamase tagged GluN2B (N-terminal)
- β-lactamase cleaves its cell impermeable substrate, nitrocefin, resulting in a change in absorbance
- Buffer = HBSS + 10mM HEPES + 50μM Glutamate (pH7.4)
- 15min incubation ± NYX-783 at 37°C
- 30min assessment of ΔA_{486nm} with substrate ± NYX-783 at 37°C (slope is calculated for minutes 6-30 after substrate is added)
- Slope is directly proportional to GluN2B surface expression
- Changes in GluN2B surface expression are represented as fold change compared to buffer
- In ANRE experiments (Figures 6b & 7) cells are incubated at 37°C for 45 min with IgG or CSF then washed with buffer prior to β-lactamase assay

5 NYX-783 increases GluN2B–NMDAR surface expression in GluN1/GluN2B HEK cells.



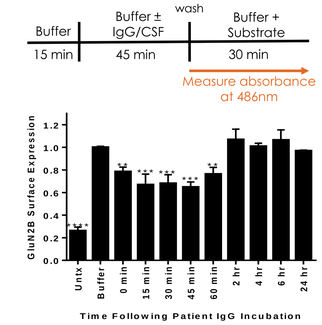
NYX-783 significantly increases GluN2B-NMDAR surface expression in a dose dependent manner. GluN2B-NMDAR surface expression measured by HEK cell β-lactamase assay following incubation with NYX-783, n=9-12. Untx=un-transfected HEK cells. Significance by ANOVA (Dunnett's) compared to buffer (vehicle), ** p≤0.01, *** p ≤ 0.001, **** p≤0.0001.

6a ANRE patient CSF and purified IgG contains NMDAR specific antibodies.



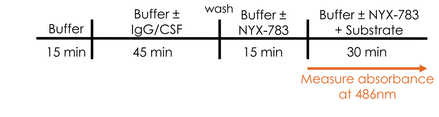
ANRE patient CSF and purified IgG show immunoreactivity to hNR1 transfected HEK cells while control CSF does not. Representative images for immunoreactivity of Left. ANRE patient CSF (1:10), Middle. Control non-ANRE patient CSF (1:10), or Right. purified ANRE patient IgG (1:250) to either hGluN1 transfected (top) or un-transfected (bottom) HEK cells.

6b ANRE patient antibody incubation decreases GluN2B-NMDAR surface expression in GluN1/GluN2B HEK cells.

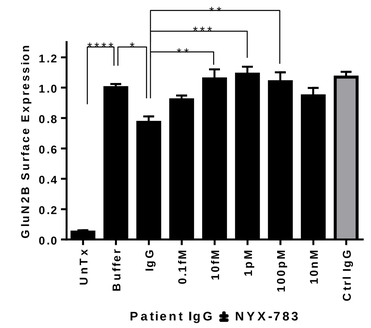


ANRE patient IgG incubation decreases GluN2B surface expression in a time dependent manner. GluN2B-NMDAR surface expression following ANRE patient IgG (1:50) incubation measured by HEK cell β-lactamase assay, n=6-12. Untx=un-transfected HEK cells. Significance by ANOVA (Dunnett's) compared to buffer (vehicle), ** *p≤0.01*, *** *p ≤ 0.001*, **** *p≤0.0001.*

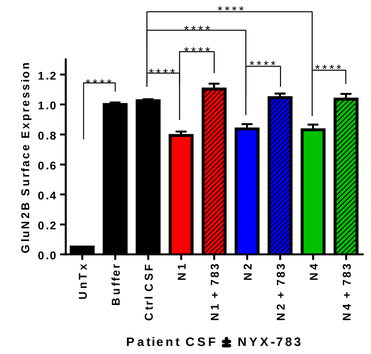
7 NYX-783 restores GluN2B-NMDAR surface expression following ANRE patient antibody incubation in GluN1/GluN2B HEK cells.



A.



B.



NYX-783 restores GluN2B surface expression following incubation with ANRE patient IgG or CSF. Patient IgG or CSF was applied to cells for 45min at 37°C then washed off. GluN2B-NMDAR surface expression was measured by HEK cell β-lactamase assay, n=9-12. Untx=un-transfected HEK cells. Significance by ANOVA (Tukey's) multiple comparisons, *p≤0.05, **p≤0.01, ***p ≤ 0.001, ****p≤0.0001. **A.** NYX-783 concentration curve following ANRE patient IgG (1:50) incubation **B.** 1 pM NYX-783 following incubation with CSF (1:10) from 3 individual ANRE patients (**N1**, **N2**, **N4**). Ctrl CSF=average of 7 control non-ANRE patient CSF samples.

CONCLUSIONS

- NYX-783 improved recognition memory, enhanced *ex vivo* LTP, and increased NMDARs at the synapse *in vivo* and at the cell surface *in vitro*.
- In NMDARs exposed to ANRE patient IgG or CSF, NYX-783 restored lost NMDARs to the surface *in vitro*.
- Altogether, these data show that NYX-783 enhance cognition and synaptic plasticity processes in the brain, likely through an increase in NMDAR trafficking. This supports continued investigation of NYX-783 treatment in disorders involving loss of NMDAR expression and function, such as ANRE.

FINANCIAL DISCLOSURES

MES, SUS, RAK, EC, TB, CC, and JRM received financial compensation and stock from Aptinyx, Inc. NASDAQ : APTX
MB is a consultant for Aptinyx Inc.