NYX-2925 facilitates auditory-evoked long-term potentiation in rats: a translational approach for measuring NMDA receptor-mediated synaptic plasticity

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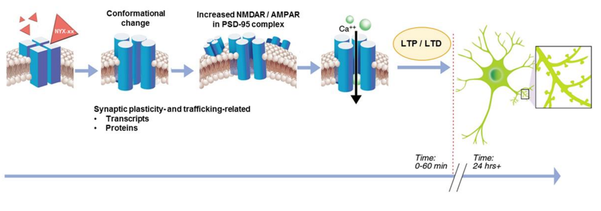


INTRODUCTION

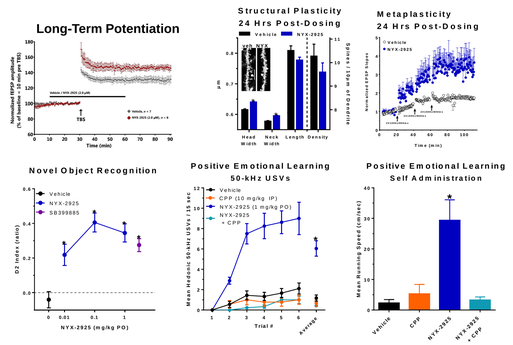
Aptinyx has developed a novel class of small molecule N-methyl-D-aspartate receptor (NMDAR) modulators with broad applicability across neurologic and psychiatric disorders. NYX-2925 is orally bioavailable and currently in Phase II studies for diabetic neuropathic pain and fibromyalgia, conditions in which sleep disruption is a core symptom. NYX-2925 facilitates synaptic plasticity, as measured by enhancement of long-term potentiation (LTP), both *in vitro* and *ex vivo* 1-7 days post-dosing (1-10 mg/kg, PO). In rats, NYX-2925 enhances novel object recognition, positive emotional learning, non-REM (NREM) sleep, and the day-night cycle of emotion, all of which are NMDAR-mediate processes.

The present studies examine the effects of NYX-2925 on NMDAR-mediate synaptic plasticity *in vivo* by measuring auditory-evoked potentials via EEG recording of the frontal cortex. NYX-2925 enhanced mismatch negativity 1 hr post-dosing (0.1, 1 mg/kg PO), as well as auditory-induced LTP (1, 10 mg/kg PO). In addition, NYX-2925 (0.1, 1, 10 mg/kg PO) enhanced resting theta-alpha power which also may have utility as a biomarker of drug activity.

CONCLUSIONS

1) NYX-2925 enhanced NMDAR-mediate mismatch negativity and auditory-evoked LTP.
2) NYX-2925 increased resting theta-alpha power.
3) Facilitation of auditory-evoked potentials may be a useful biomarker for drug development.
4) Altogether, these data demonstrate that NYX-2925 enhances NMDAR activation *in vivo.*

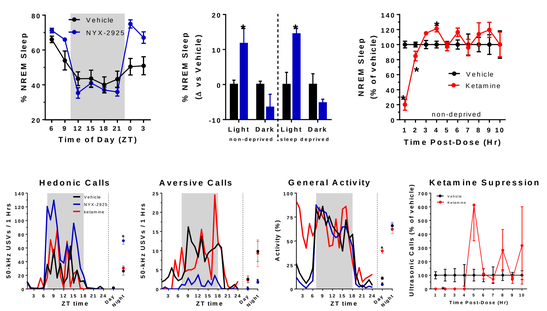
NYX-2925 enhanced synaptic plasticity, learning and memory



NYX-2925 enhanced NMDAR activity as measured by hippocampal LTP *in vitro*. One day after dosing *in vivo*, NYX-2925 increased spine head and neck diameter in rat hippocampal dentate gyrus primary dendrites and the magnitude of hippocampal LTP in slices. In behaving rats, NYX-2925 enhanced learning and memory in the novel object recognition task, as well as NMDAR-mediated positive emotional learning. The ability of NYX-2925 to facilitate learning and memory was blocked by co-administration of the NMDAR antagonist CPP. * P< .05

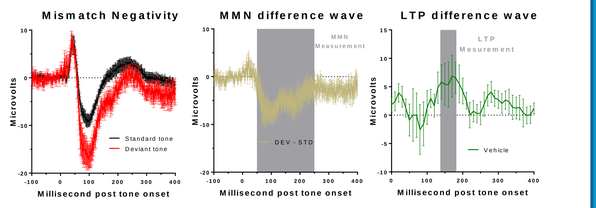
Data adapted from: Khan et al. 2018. NYX-2925 is a novel NMDA receptor-specific spirocyclic-beta-lactam that modulates synaptic plasticity processes associated with learning and memory. *Int J Neuropsychopharmacol* 21, 242-254.

NYX-2925 facilitated NREM sleep and the circadian rhythm of behavior



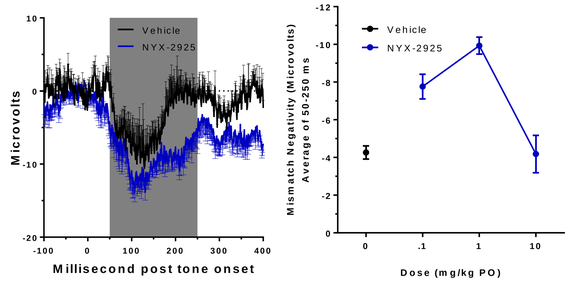
Top Panel– *NYX-2925 facilitates NREM sleep during the light phase whereas the NMDAR antagonist ketamine inhibits NREM*. Non-deprived or sleep-deprived (ZT0-6) rats received either NYX-2925 (1 mg/kg PO) or vehicle at ZT5 and sleep EEG was recorded for 24 hrs post-dosing.
Bottom panel – *NYX-2925 facilitates the circadian rhythm of both activity and emotional expression whereas the noncompetitive NMDAR blocker, ketamine, is inhibitory.* 24 hr homecage recordings were obtained from rats treated with NYX-2925 (10 mg/kg PO), ketamine (10 mg/kg IV) or vehicle. Rats were housed 3 per cage. NYX-2925 enhanced hedonic 50-khZ USVs during the night (6-12 hrs post-dosing) and decreased aversive calls during the whole day. Ketamine eliminated USVs during the first 3 hrs post-dosing. NYX-2925 increased sleep (decreased activity) during the day, and increased relative activity at night. In contrast, ketamine acutely suppressed sleep and increased locomotor activity for the first 6 hrs post-dosing. * P< .05

Mismatch negativity (MMN) and auditory-evoked LTP methods



Data acquisition and analysis. Auditory evoked potentials were recorded from frontal cortex skull screws using a cerebellum skull screw as a ground/reference. Data was acquired using a A&M (USA) amplifier and Data Wave (USA) acquisition software. Data was analyzed using Brain Products Analyzer 2 software (Germany).
Mismatch Negativity (MMN). Testing consisted of standard (6 kHz) or deviant (8 kHz) tone pips (50 ms duration) presented randomly, with 10% of the tones being deviant. One trial consisted of 200 deviant tones and 1800 standard tones (ie. a total of 2000 stimuli). The difference wave was obtained by subtracting the standard from the deviant waveform, and was used as the measure of MMN.
Auditory-evoked LTP. LTP was induced by an auditory tetanus (6-kHz, 50 ms in duration), presented 10 times per second for 5 min (total of 3,000 tones). MMN testing occurred immediately before tetanus (pre-tetanus) and 1 hr after tetanus (post-tetanus). Post-pre tetanus difference waves were generated to determine the range (in milliseconds) in which LTP occurred.

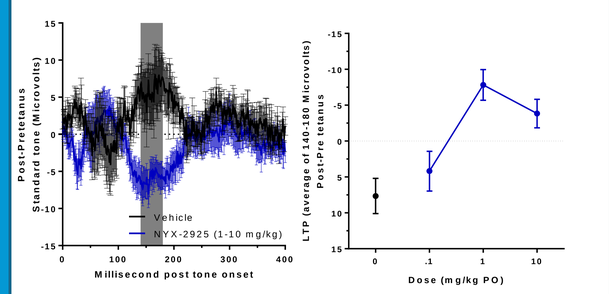
NYX-2925 enhanced MMN



Mismatch negativity was enhanced by NYX-2925 (0.1,1 mg/kg PO) 1 hr post-dosing, as measured by the deviant-standard difference wave 50-250 milliseconds after the tone onset. Using a similar methodology[i], NMDAR antagonists have been shown to decrease MMN, suggesting that NYX-2925 enhances NMDAR activation.

[i]Ehrlichman et al. (2008) Deviance-elicited changes in event-related potentials are attenuated by ketamine in mice. J Cogn Neurosci 20:1403-1414.

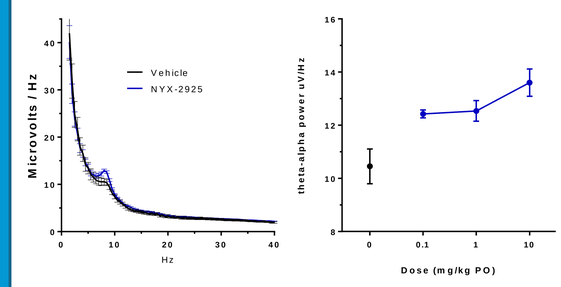
Auditory-evoked LTP is enhanced by NYX-2925



Auditory-evoked LTP was enhanced by NYX-2925 (1,10 mg/kg PO) 1 hr post dosing, as measured by the post-pre tetanus difference wave 140-180 milliseconds after the tone onset. Using a similar methodology[i], an NMDAR antagonist has been shown to inhibit sensory-induced LTP. This suggests that NYX-2925 enhances NMDAR activation.

[i]Clapp et al. (2006) Rapid visual stimulation induces N-methyl-D-aspartate receptor-dependent sensory long-term potentiation in the rat cortex. Neuroreport 17:511-515.

NYX-2925 increased resting theta-alpha power



Resting EEG theta-alpha power (8 & 8.5 Hz) was enhanced by NYX-2925 (0.1,1,10 mg/kg PO) 1 hr post-dosing, as measured by a Fast Fourier Transform analysis (2 sec Hanning window with a 10% taper, 1 second overlap, 0.5 Hz bins).

FINANCIAL DISCLOSURES

JSB, KL, EMC, TMM, MAK, RAK, and JRM are employees of Aptinyx Inc. PKS is a consultant for Aptinyx Inc. XLZ is supported in part by a grant from Aptinyx Inc. to PKS.