NYX-2925 is a NMDA receptor modulator with glycine site partial agonist-like properties: *in vitro* and *in vivo* characterization.

Poster # 223

J. Burgdorf[1], A. Gross[2], X. Zhang[3], E. Colechio[2], N. Haack[2], M. Schmidt[2], S. Sahu[2], P. Kansara[2], E. Trahey[2], E. Pollard[2], T. Madsen[2], P. Stanton[3], M. Khan[2], R. Kroes[2], D. Houck[2], C. Cearley[2], J. Moskal[1,2]

[1]Northwestern University, Evanston, IL, USA; [2]Aptinyx Inc., Evanston, IL, USA; [3]New York Medical College, Valhalla, NY, USA

ABSTRACT

Aim of Investigation: NYX-2925 is an orally bioavailable small molecule NMDA receptor modulator. The present studies detail the *in vitro* and *in vivo* characteristics of NYX-2925.

Methods: Functional glycine-site partial agonist effects were measured using an *in vitro* [3H]MK-801 binding assay in membrane extracts prepared from human NMDAR2A-2D subtype-expressing HEK cells. Pharmacologically isolated NMDA currents were measured in Schaffer collateral-evoked EPSCs in CA1 pyramidal neurons using whole-cell patch clamp recordings. Long-term potentiation (LTP) was measured at Schaffer collateral CA1 synapses in rat hippocampal slices following acute bath application of NYX-2925 as well as 24 hours post-dosing *in vivo*. Learning and memory as well as antidepressant like effects were measured in the rat positive emotional learning, Morris water maze, and Porsolt tests. Sedative / ataxic effects were measured in the rat Rota-Rod test. Oral bioavailability was measured in rat plasma by LC-MS/MS.

Results: In recombinant human NMDAR2A-2D -expressing HEK cells, partial agonist activity of NYX-2925 was demonstrated at all four receptor subtypes, with greater potency at NMDAR2B (for NMDAR2A, NMDAR2B, NMDAR2C, NMDAR2D, respectively, EC_{50} was 55 pM, 28 fM, 11 pM, 55 pM and % maximal activation relative to glycine was 41%, 47%, 63%, and 58%). NYX-2925 also increased NMDA current in hippocampal slices at concentrations between 100-500 nM and but not at 5 µM. Similarly, in the hippocampal LTP assay, NYX-2925 facilitated LTP at concentrations between 100-500 nM but not at 2µM. NYX-2925 (1 mg/kg PO) facilitated ex vivo hippocampal LTP 24 hrs post-dosing. NYX-2925 (1 mg/kg PO) facilitated Morris water maze learning from 1 h to at least 5 days post-dosing. NYX-2925 also produced an antidepressant-like effect in the Porsolt test (EC_{50} = 6 µg/kg PO) and facilitated positive emotional learning (EC_{50} = 3 µg/kg PO) 1 h post-dosing. NYX-2925 (1-100 mg/kg PO) did not show sedative / ataxic effects in the Rota-Rod test when measured up to 2 h post-dosing. NYX-2925 showed 50% oral bioavailability (IV vs PO plasma AUC).

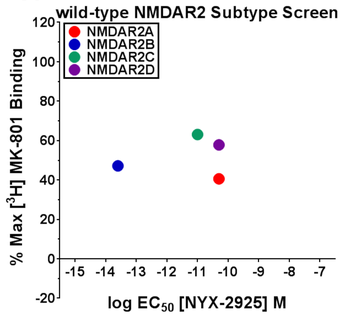
Conclusions: These data demonstrate that NYX-2925 is an orally bioavailable compound that facilitates LTP and NMDA current through its interaction with the NMDA receptor where it has affinity for all four NR2 subtypes. NYX-2925 shows efficacy in multiple learning and memory models as well as animal models of depression without sedative or ataxic effects.

Note: see poster # 221 for additional in vivo efficacy of NYX-2925 in neuropathic pain models

FINANCIAL DISCLOSURES

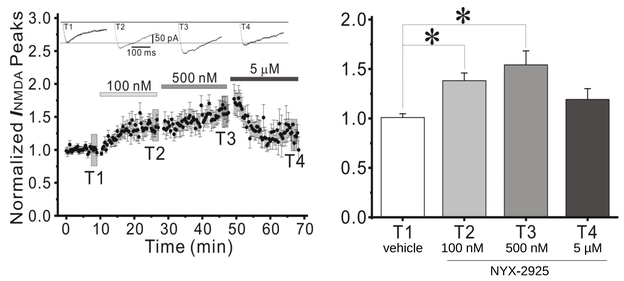
JB and PS are consultants for Aptinyx Inc.
NH, EC, AG, JP, JD, TM, MK, RK, DH, CC and JM are employees of Aptinyx Inc.

1 NYX-2925 Binds to All Four NMDA Receptor Subtypes



Potentiation of [3H]MK-801 binding under non-equilibrium conditions in NMDAR2A-2D expressing HEK cells. Membrane extract protein was preincubated in the presence of saturating concentrations of glutamate (50 µM) and varying concentrations of NYX-2925 (1 fM-100nM), or 1 mM glycine. 0.3 µCi of [3H]MK-801 (22.5 Ci/mmol) was added and reactions were incubated for 15 min (nonequilibrium). Bound and free [3H]MK-801 were separated. The % maximal [3H]MK-801 binding was calculated relative to that in the presence of glycine and glutamate.

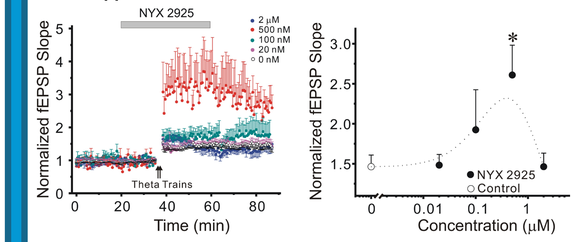
2 NYX-2925 (100-500 nM) Enhances Whole Cell NMDA Current in CA1 Pyramidal Neurons



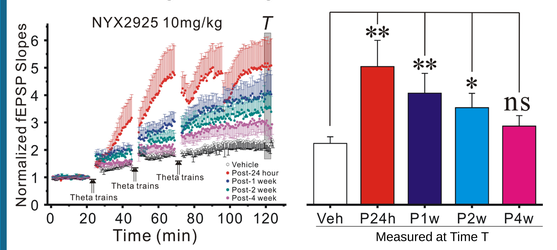
Dose-dependent effects of NYX-2925 on the pharmacologically-isolated NMDA component of Schaffer collateral-evoked EPSCs in CA1 pyramidal neurons. NYX-2925 enhanced NMDA current at low doses (100 nM, 500 nM) and the effect was reversed at a higher concentration (5 µM). Whole-cell patch clamp recordings were obtained at 30ºC, at a holding potential of -60 mV, in Mg2+-free ACSF to enhance NMDAR conductances. Data are mean ± SEM of EPSC peak amplitude. *p<0.05 compared to T1 (vehicle).

3 NYX-2925 Facilitates Hippocampal LTP

Bath application:



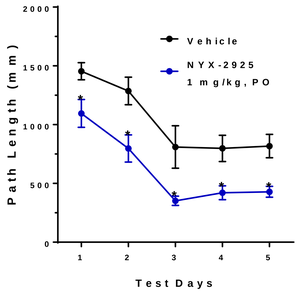
Ex-Vivo following oral dosing:



NYX-2925 bath application (0.02-2 µM) or oral dosing (10 mg/kg; *ex vivo* recordings 24 h – 4 weeks post-dosing) enhanced the magnitude of long-term potentiation of synaptic transmission at Schaffer collateral-CA1 synapses compared to control. LTP was induced by theta burst stimulation (arrows) at 32ºC. Data are mean ± SEM. *p< 0.05, **p<0.01 compared to aCSF control slices.

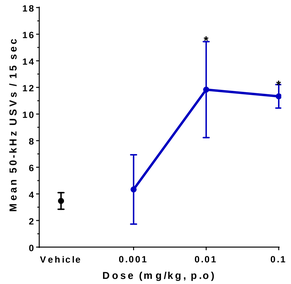
4 NYX-2925 Facilitates Spatial Learning and Positive Emotional Learning

Spatial Learning: Morris Water Maze



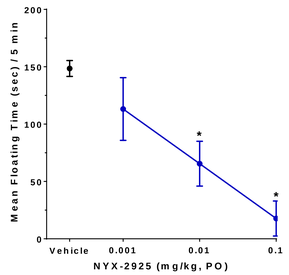
Mean (± SEM) path length to find the hidden platform in the movable platform version of the Morris water maze test. Male 2-3 month old Sprague Dawley rats were pretreated with a single dose of NYX-2925 (1 mg/kg PO) or sterile saline vehicle (0.9%, 1 ml/kg PO) 1 h before the first of 5 daily test sessions (4 trials/ session). N = 8-9 rats per group. * p < 0.05 (Fisher's PLSD post hoc test) NYX-2925 vs. vehicle for each test day.

Positive Emotional Learning: Ultrasonic Vocalization assay



Mean ± SEM hedonic (50-kHz) ultrasonic vocalizations (USVs) were measured in adult male Sprague Dawley rats. Animals were treated with NYX-2925 (0.001, 0.01 or 0.1 mg/kg PO) or sterile saline vehicle (0.9%, 1 ml/kg PO) 1 hr before testing. Positive emotional learning was measured during the last 2 conditioned stimulus (CS) trials preceding the tickle unconditioned stimulus (UCS) trials. Animals received 15 second trials consisting of 6 CS and 6 UCS trials each (3 min total). N = 3 per group for NYX-2925 and N=17 for the vehicle group. P < .05 Fisher's PLSD post hoc test 0.01 or 0.1 mg/kg vs. vehicle.

5 NYX-2925 Produces Antidepressant-like Effects in the Porsolt / Forced Swim Assay



Mean ± SEM floating time in the rat Porsolt test in adult male Sprague Dawley rats that were dosed with NYX-2925 (1 – 100 mg/kg PO), or 0.9% saline vehicle (1 ml/kg PO; black circles) 1 h before the 5 min test session. Animals received a 15 min Porsolt habituation session 24 hrs before testing. N = 3 per group for NYX-2925 and N=18 for the vehicle group. *p < 0.05 (Fisher's PLSD post hoc test) at 0.01 and 0.1 mg/kg vs. vehicle.

6 NYX-2925 (1-100 mg/kg PO) Does Not Show Sedative/Ataxic Side Effects in the Rat Rota-Rod Test

See Poster #221

7 NYX-2925 Has High Oral Bioavailability

	Dog Plasma		Rat Plasma	
Dose (mg/kg)	0.2 (IV)	2 (PO)	1 (PO)	10 (PO)
C_{max} (ng/mL)	311	1960	210	2403
AUC_{0-t} (ng*h/mL)	353	3660	494	5707
T_{max} (h)	0.25	0.5	1.0	0.5
$T_{1/2}$ (h)	1.06	1.26	6.81	4.33

- NYX-2925 is rapidly absorbed after PO administration and has a bioavailability of 50% in the rat and 100% in the dog
- NYX-2925 is detected at significant concentrations in the brain, as measured in CSF (10 mg/kg PO)