JY-515,317 (NYX-2925) is a NMDA receptor modulator with glycine site partial agonist-like properties: *in vitro* and *in vivo* pharmacology

A.L. Gross[1], J.S. Burgdorf[1], X-L. Zhang[3], E.M. Colechio[1], N. Ghoreishi-Haack[1], M.E.Schmidt[1], S. Sahu[1], P.P. Kansara[1], E.C. Rodriguez[1], E.A. Pollard[1], T.M. Madsen[1], P.K. Stanton[3], M.A. Khan[1], R.A. Kroes[1], J.R. Moskal[1,2]

[1]Aptinyx Inc., Evanston, IL; [2]Biomed. Engin., Northwestern Univ., Evanston, IL, [3]Cell Biol. & Anat., New York Med. Col., Valhalla, NY

609.08 / CC4

INTRODUCTION

Modulating N-methyl D-aspartate receptor (NMDAR)–mediated synaptic activity is a growing focus for the development of pharmaceuticals that have wide applicability across CNS disorders. The NMDAR plays a critical role in learning and memory. Decline in NMDAR function is observed in humans and other mammals and is directly correlated with impaired performance in learning-dependent tasks.

Aptinyx Inc. has developed a platform of NMDAR modulators that are orally bioavailable and work via a novel mechanism: functional glycine-site partial agonist modulation of the NMDAR. Unlike NMDAR antagonists, the Aptinyx compounds appear to be both highly efficacious and tolerable. The goal of the present study was to specifically characterize the *in vitro* and *in vivo* pharmacology of one of those molecules: JY-515,317 (NYX-2925).

CONCLUSIONS

- JY-515,317 preferentially binds to NMDAR2B, but has an affinity for all 4 NMDAR2A-2D subtypes.
- JY-515,317 is an orally bioavailable compound that facilitates LTP and NMDA current, likely through its interaction with the NMDA receptor
- JY-515,317 shows efficacy in multiple learning and memory models without sedative or ataxic effects.

ADDITIONAL *IN VIVO* STUDIES

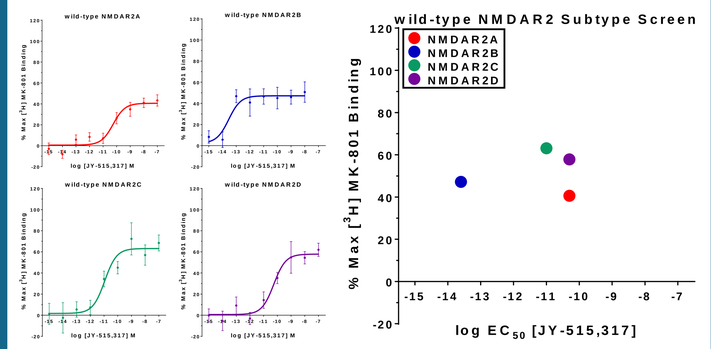
- JY-515,317 has also shown neuroprotective effects in a rat model of blast-induced brain injury (**see poster #609.12 / CC8**).
- JY-515,317 has also shown rapid-acting and long-lasting therapeutic potential for the treatment of neuropathic pain (**see poster #609.03 / BB17**).

FINANCIAL DISCLOSURES

X-LZ, and PKS are consultants for Aptinyx Inc.

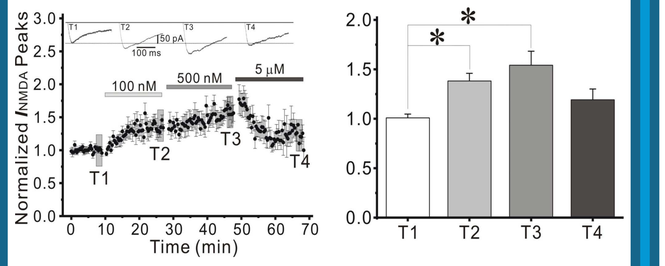
ALG, JSB, EMC, NG-H, MES, SS, PPK, ECR, EAP, TMM, MAK, RAK and JRM are all employees of Aptinyx Inc.

JY-515,317 binds to NMDAR2A-2D



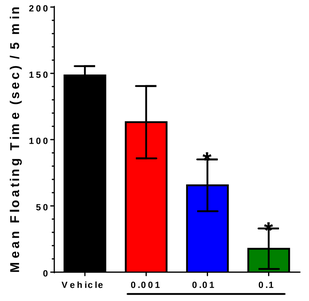
Potentiation of [³H]MK-801 binding under non-equilibrium conditions in NMDAR2A-2D expressing HEK cells. Membrane extract protein was preincubated in the presence of saturating concentrations of glutamate (50 µM) and varying concentrations of NYX-2925 (1 fM-100nM), or 1 mM glycine. 0.3 µCi of [³H]MK-801 (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.

JY-515,317 enhances whole cell NMDA current in CA1 pyramidal neurons



Dose-dependent effect of JY-515,317 on the pharmacologically-isolated NMDA component of Schaffer collateral-evoked EPSCs in CA1 pyramidal neurons. JY-515,317 enhanced NMDA current at low doses (100 nM, 500 nM) an effect that was reversed at a higher concentrations (5 µM). Whole-cell patch clamp recordings were obtained at 30°C, at a holding potential of -60 mV, in 10 µM bicuculline, 20 µM NBQX, Mg2+-free ACSF to enhance NMDAR conductance. Data are mean ± SEM of EPSC peak amplitude. *p<0.05 compared to T1 (vehicle).

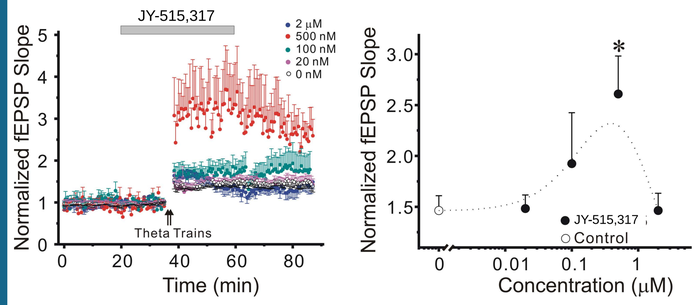
JY-515,317 reduces floating time in the Porsolt forced swim test



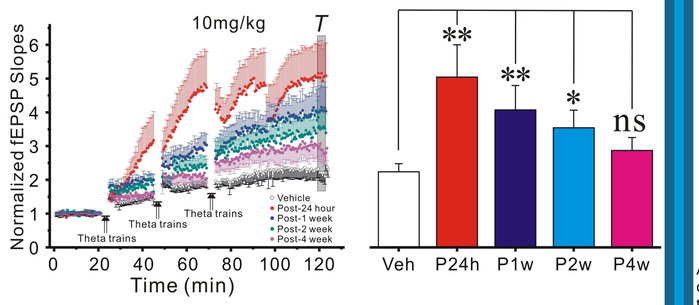
Dose-dependent effect of JY-515,317 on mean floating time in the Porsolt forced swim assay. Mean ± SEM floating time in the rat Porsolt test in adult male Sprague Dawley rats were dosed with JY-515,317 (1 – 100 mg/kg PO), or 0.9% saline vehicle (1 ml/kg PO; black bar) 1 hr before the 5 min test session. Animals received a 15 min Porsolt habituation session 24 hrs before testing. N = 3 per group for JY-515,317 and N=18 for the vehicle group. P < .05 Fisher's PLSD post hoc test 0.01 or 0.1 mg/kg vs. vehicle.

JY-515,317 enhances the magnitude of hippocampal LTP both in the presence of drug *in vitro* and up to 2 weeks following oral dosing

Bath application:

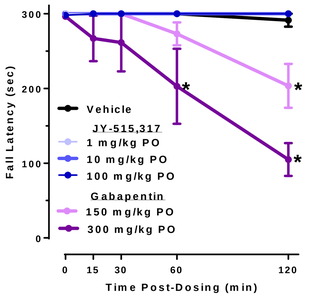


Ex-Vivo LTP following oral dosing:



JY-515,317 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; two trains 1 min apart of 10 x 100 Hz / 5 pulse bursts, 200 ms interburst interval) at 32°C. Data are mean ± SEM. *p< 0.05, **p<0.01 compared to untreated control slices.

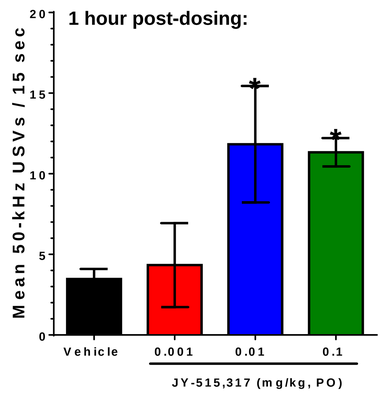
JY-515,317 does not show sedative/ataxic effects in the Rota-Rod test



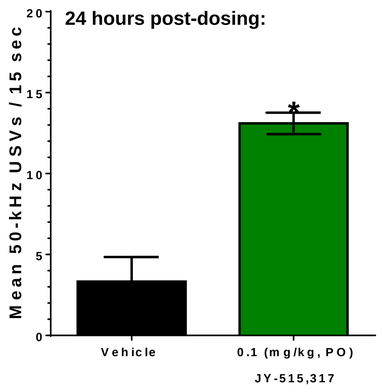
Animals were habituated 3 times before the start of testing with a fourth habituation session occurring right before dosing (0 min timepoint). A fixed speed version (16 rpm) of the Rota-rod test was used. Adult male Sprague Dawley rats were dosed with gabapentin (150,300 mg/kg PO), JY-515,317 (1-100 mg/kg PO) or 0.5% Na-CMC in 0.9% saline vehicle (1 ml/kg PO) 15 min before the first test session. Animals were re-tested 30, 60, 120 min post-dosing. Mean (± SEM) latency to fall of the Rota-Rod was recorded. N = 6-11 per group. * P < .05 Fisher's PLSD post hoc test gabapentin (150 or 300 mg/kg) vs. vehicle for each timepoint.

JY-515,317 enhances positive-emotional learning in the ultrasonic vocalization assay at 1 and 24 hrs

1 hour post-dosing:



24 hours post-dosing:



Mean ± SEM hedonic (50-kHz) ultrasonic vocalizations (USVs) were measured in adult male Sprague Dawley rats. Rats were treated with JY-515,317 (0.001, 0.01 or 0.1 mg/kg PO) or sterile saline vehicle (0.9%, 1 ml/kg PO) 1 or 24 hrs before testing. Positive emotional learning was measured during the conditioned stimulus (CS) trials preceding the tickle unconditioned stimulus (UCS) trials. Animals received 15 sec. trials consisting of 6 CS and 6 UCS trials each (3 min total). N = 3 per group.

JY-515,317 has high oral bioavailability and is CNS penetrant

	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

- JY-515,317 is rapidly absorbed after PO administration and has a bioavailability of 50% in rat and 100% in dog
- JY-515,317 is detected at significant concentrations in the brain, as measured in CSF