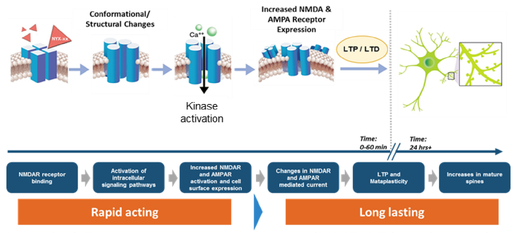
NYX-2925 re-normalizes synaptic-plasticity associated protein expression in rat mPFC: a potential mechanism for the compound's analgesic effect in neuropathic pain

G. Morrison[1], M. Asiedu[1], E. Colechio[1], J.M. Priebe[1], T.K. Bhattacharya[1], J. Aguado[1], N. Ghoreishi-Haack[1], R.A. Kroes[1,2], M.S. Bowers[1,2], A.L. Barth[1], C.N. Cearley[1], J.R. Moskal[1,2]

PSN 114

[1]Aptinyx Inc., Evanston, IL; [2]Falk Center For Molecular Therapeutics, Northwestern University, Evanston, IL

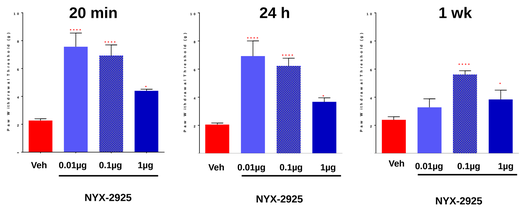
INTRODUCTION



N-methyl-D-aspartate receptor (NMDAR) dependent synaptic plasticity processes play an important role in the establishment and maintenance of chronic pain. Aptinyx, Inc. has developed a platform of orally bioavailable NMDAR modulators as potential therapeutics for challenging CNS disorders, including neuropathic pain.

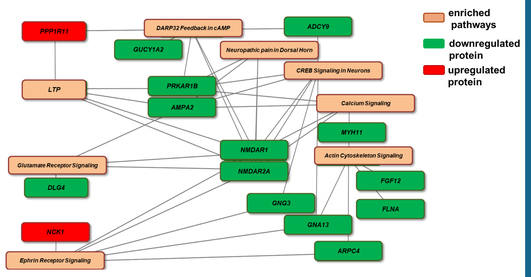
NYX-2925 is a NMDAR2A-2D modulator currently in Phase 2 clinical studies for painful diabetic peripheral neuropathy and fibromyalgia. Oral administration of NYX-2925 improves mechanical hypersensitivity in multiple rodent neuropathic pain models as early as 1 hour and up to at least 1 week post oral administration. Changes in the mPFC are likely critical for the maintenance of neuropathic pain. Direct infusion of NYX-2925 into the rat medial prefrontal cortex (mPFC) reverses mechanical hypersensitivity induced by the chronic constriction nerve injury (CCI). Thus, we investigated the effect of NYX-2925 in normalizing pain induced changes in the mPFC, to elucidate its mechanism underlying the alleviation of pain.

Direct injection of NYX-2925 into the mPFC alleviates neuropathic pain



Direct mPFC infusion of NYX-2925. Administration of 0.01 µg NYX-2925 significantly increased paw withdrawal threshold (PWT) at 20 min and 24 h post-administration, but not at 1 week vs. vehicle ($p < 0.05$). Administration of 0.1 and 1 µg significantly increased PWT at all time points vs. vehicle ($p < 0.05$). 0.1µg dose, significantly increased PWT at the 20 min and 24 h time points. N = 6-9 animals per group. Mean ± SEM, * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

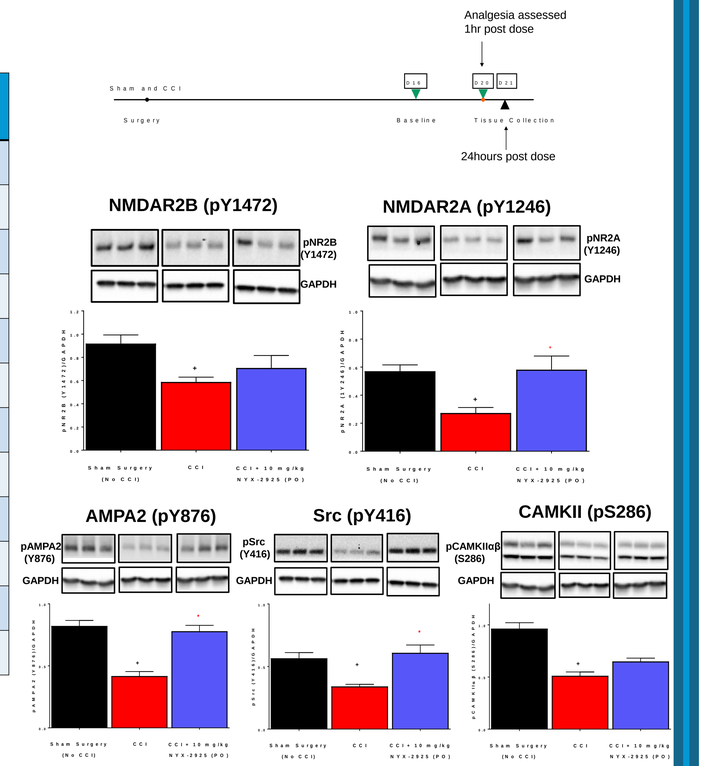
Plasticity related pathways are downregulated in the mPFC following CCI



Proteomic analysis of mPFC from CCI and Sham surgery rats. mPFC were isolated 21 days after surgery. All differentially expressed proteins were analyzed using Ingenuity pathway analysis. Majority of the differentially expressed proteins in these pathways were all decreased in the mPFC under CCI conditions. Samples were analyzed by nano LC-MS/MS (N=5-6 per group). Study was in collaboration with MS Bioworks (USA).

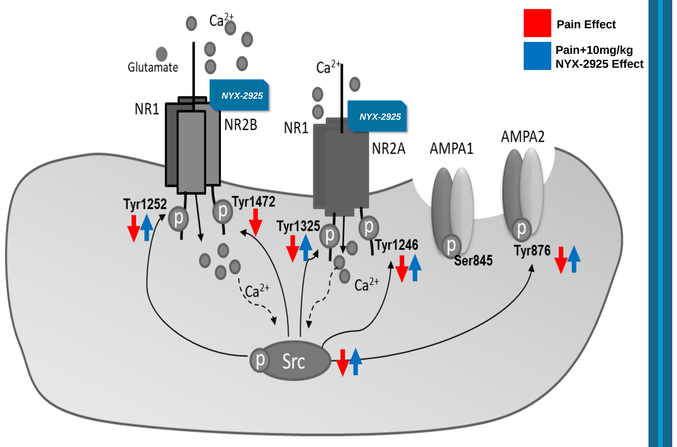
Phosphorylation of Src family kinase regulated proteins were selectively restored following treatment with NYX-2925 in the rat CCI model

phosphorylated proteins	Kinase	CCI relative to sham	CCI + NYX-2925 (10mg/kg) relative to CCI
NMDAR2B (Y1252)	SFK	Down	Restored
NMDAR2B (Y1472)	SFK	Down	No change
NMDAR2B (S1303)	CamKII / PKC	No change	No change
NMDAR2A (Y1246)	SFK	Down	Restored
NMDAR2A (Y1325)	SFK	Down	Restored
AMPA1 (S845)	PKA	No change	No change
AMPA1 (S831)	CamKII / PKC	No change	No change
AMPA2 (S876)	SFK	Down	Restored
Src (Y416)	PYK2 auto	Down	Restored
CAMKII (S286)	auto	Down	No change
mTOR (S2448)	PKA	Down	No change
CREB (S133)	MAPK, Ca^{2+}	Down	Restored



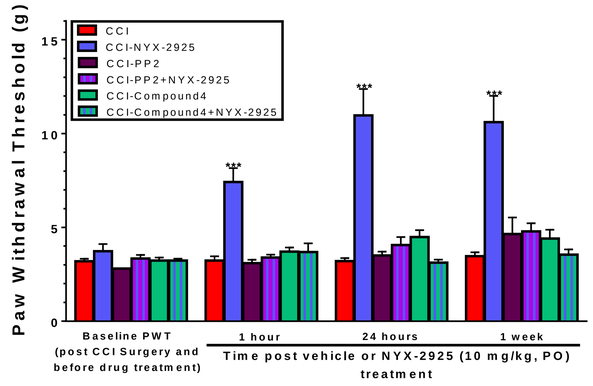
NYX-2925 administration restores pain induced decrease of Src-regulated proteins to baseline (sham) levels. Table represents multiple phosphoprotein sites in the mPFC analyzed by western blot for Sham and CCI rats (Vehicle or 10 mg/kg NYX-2925) at 24 hours post oral dosing. Western blot for phosphorylated proteins and GAPDH were analyzed in all groups. Significant Down and Restored changes were detected by one-way ANOVA followed by Dunnett's posthoc, $p < 0.05$. For select proteins from the table, graphs depict means ± SEM. +p<0.05 relative to sham, * p<0.05 relative to CCI.

Normalization of Src-regulated proteins in the mPFC may underlie the analgesic effect of NYX-2925



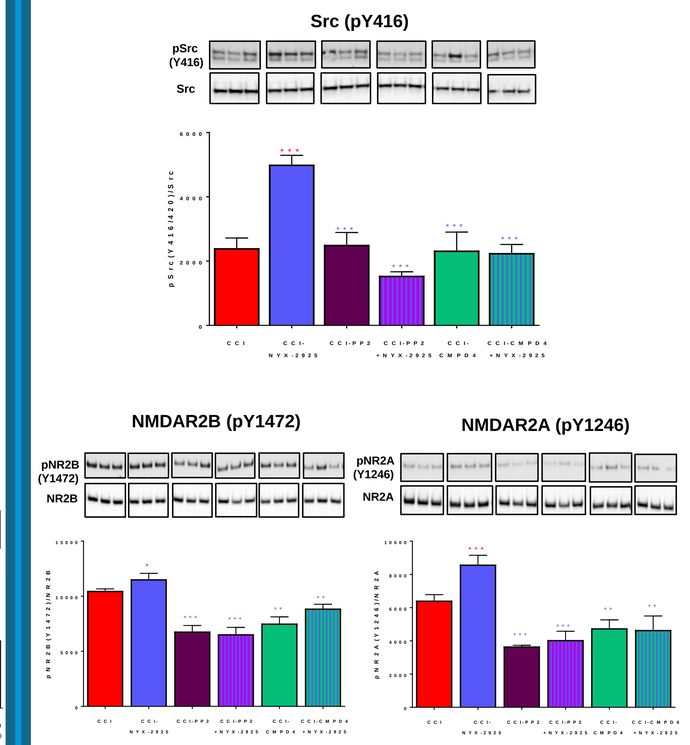
Src phosphorylation of several NMDAR and AMPA2 phosphorylation sites under pain and after NYX-2925 triggered effects. Pain caused a global decrease in the phosphorylation of several Src-dependent protein targets and NYX-2925 restored them to sham levels.

Direct injection of Src family kinase inhibitors into the prelimbic cortex blocks the analgesic effect of NYX-2925



Direct mPFC infusion of Src Kinase inhibitors reverses the analgesic effect of NYX-2925. Src family kinase inhibitors (10µM, compound 4 or PP2) were infused in the mPFC for 20 min prior to NYX-2925 (10 mg/kg, PO) and tested for mechanical hypersensitivity 1, 24 hrs, and 1 wk post-dose. Pretreatment with PP2 or Compound 4 blocked the effect of NYX-2925 at all time points. (***$p > 0.001$). N = 6-8 animals per group.

NYX-2925 upregulation of synaptic plasticity associated proteins may be Src-dependent



Inhibition of Src kinase, prevents the NYX-2925-driven upregulation of phosphorylated NR2B and NR2A. Enriched synaptosomes were prepared from the mPFC of CCI rats with direct injection of Src Family kinase inhibitors and oral dose of NYX-2925. Western blots for phosphorylated Src (pY416), NR2B (pY1472) and NR2A (pY1246) were analyzed. Values are expressed as means ± SEM and were analyzed by one-way ANOVA followed by Dunnett's posthoc. (p * <0.05, ** <0.01, *** <0.001).

CONCLUSIONS

- Pathways related to synaptic plasticity are downregulated in the mPFC following the development of neuropathic pain after CCI
- NYX-2925 specifically restores Src-dependent protein phosphorylation to sham levels in whole cell lysates and enriched synaptosomes in the mPFC of rats with CCI-induced neuropathic pain
- Inhibition of Src family kinase(s) inhibits the analgesic effect of NYX-2925 and also prevents the normalization of pain induced proteomic changes to sham levels
- The long-lasting effects of NYX-2925 in the treatment of neuropathic pain may, in part, require Src-dependent trafficking and stabilization of NMDAR and AMPAR at the cell surface

REFERENCES

Khan, M. A.,et al (2018). NYX-2925 Is a Novel NMDA Receptor-Specific Spirocyclic-β-Lactam That Modulates Synaptic Plasticity Processes Associated with Learning and Memory. *International Journal of Neuropsychopharmacology*, *21*(3), 242–254.

Ghoreishi-Haack, N.et al (2018), NYX-2925 is a novel NMDA receptor modulator that induces rapid and long-lasting analgesia in rat models of neuropathic pain. *JPET,* 118.249409.

FINANCIAL DISCLOSURES

GM, MA, , EC, JMP, TKB,, JA, NGH, RAK, MSB, AB, CC and JRM are employees of Aptinyx, Inc.