NYX-2925, a Novel NMDA Receptor Modulator, Shows Efficacy in Neuropathic Pain that Is NMDA and AMPA Receptor Dependent and Appears to Be Driven through Brain, but NOT Spinal Circuitry

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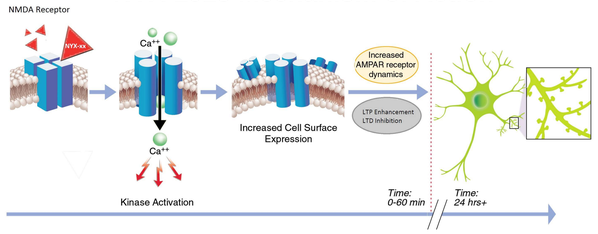
NYX-2925 Mechanism of Action



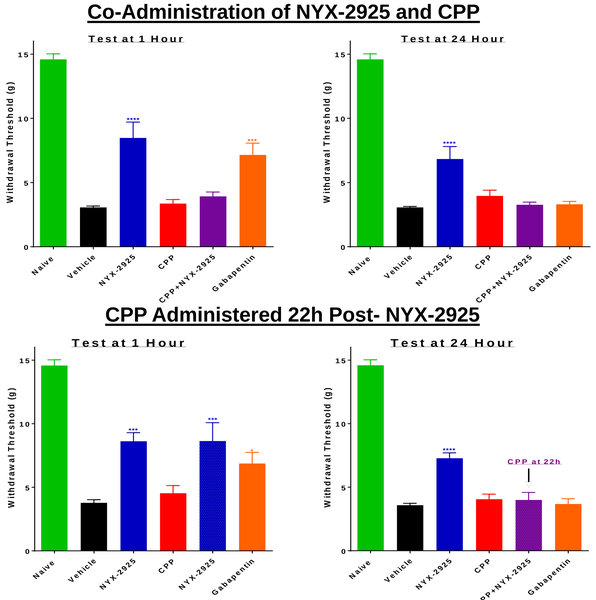
INTRODUCTION

Neuropathic pain (NP) is a highly prevalent condition caused by a primary lesion or dysfunction of the nervous system.[1] Current treatments for neuropathic pain are only modestly effective and in only a minority of patients. There is a high unmet medical need for more effective therapeutics for neuropathic pain conditions.

Glutamate *N-methyl-D-aspartate* receptor (NMDAR) dependent synaptic plasticity processes may play a role in the establishment of neuropathic pain and as such, the NMDAR is a potential therapeutic target.

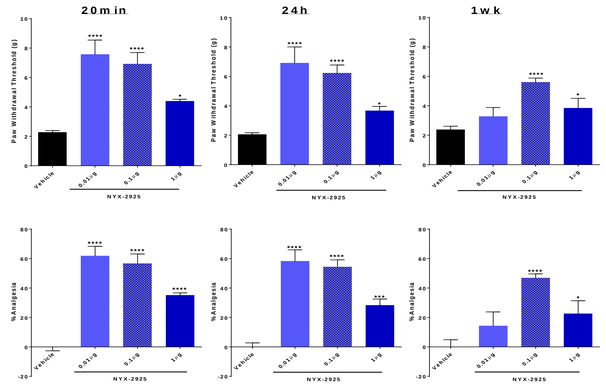
NYX-2925 is an orally bioavailable, small molecule NMDAR modulator with rapid-acting and long lasting efficacy in animal models of neuropathic pain. Previous studies have demonstrated the time course and dose response of the analgesic effects of NYX-2925 in the chronic constriction injury (CCI) and spared nerve injury (SNI) models. The preliminary data presented here evaluate the mechanism whereby, and the sites through which, NYX-2925 targets neuropathic pain. To evaluate the NMDAR- or AMPAR-dependent effect of NYX-2925 in the CCI model, the compound was given alongside the NMDAR antagonist CPP or the AMPAR antagonist NBQX. To evaluate the site of action of NYX-2925 in the CCI model, the compound was directly injected into the medial prefrontal cortex (mPFC) or onto spinal cord (between L5-L6). To evaluate the specificity of the effect to neuropathic vs. nociceptive pain conditions, NYX-2925 was assessed in the tail flick model.

Administration of the NMDAR Antagonist CPP Blocks the NYX-2925 Analgesic Effect in the Rat CCI Model



Testing for both studies occurred approximately 2 weeks post CCI/Bennett surgery. In both studies, vehicle (0.5% Na-CMC in 0.9% sterile saline), NYX-2925 at 10 mg/kg and gabapentin at 150 mg/kg were dosed by oral gavage. CPP at 10 mg/kg was injected IP either an hour prior to NYX-2925 (co-administration) or 22h post NYX-2925 (post-administration). Mechanical hypersensitivity was measured using Von Frey filaments and Dixon's up-down method (*Chaplan* et al., 1994) both at 1h and 24h post dosing). Mean ± SE, n = 5 - 6 , * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

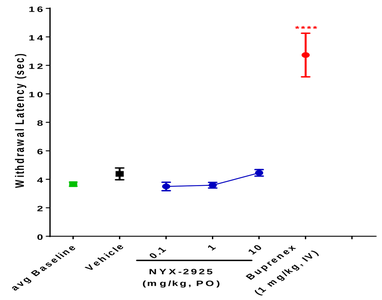
Direct MPFC Infusion of NYX-2925 Is Analgesic in the Rat CCI Model



Rats were anesthetized with 5% isoflurane gas. CCI / Bennett surgery was performed as previously described (*Bennett* and *Xie*, 1988). Bilateral guide cannulae (22 gauge, 1.5 mm center-to-center distance; Plastics One, Roanoke, VA) were implanted in the mPFC (2.7 mm rostral and 0.5 mm lateral to bregma) in a level-skull preparation. Each guide cannula was inserted to a depth of 3 mm ventral to the surface of the skull and secured in a dental acrylic headcap for the duration of the experiment. After surgery, internal dummy cannulae were inserted into the guide cannulae. Animals recovered for 2 weeks following surgery prior to behavioral testing. 0.5 µl of NYX-2925 or saline was delivered into the mPFC (R & L) via an infusion pump using a 5-µl Hamilton (Reno, NV) syringe connected to polyethylene tubing. Infusions were administered over a 60-s period, and the injection needles (28 gauge, 15 mm insertion length) were left in the guide cannula for an additional 120 s to allow for diffusion of the drug from the needle tip.

Mechanical hypersensitivity was measured using von Frey filaments and Dixon's up-down method (*Chaplan* et al., 1994) both at 20min, 24h, and 1wk post dosing.). Mean ± SE, n = 6 – 7 , * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

NYX-2925 Does Not Show an Analgesic Effect in the Rat Tail Flick Model of Acute Pain

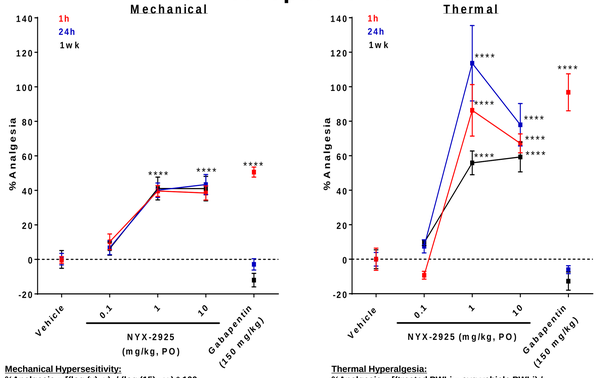


Each rat was restrained and placed on the tail flick apparatus (Ugo Basile Tail Flick Cat#: 37360) such that the tip of the tail (at 2-2.5 cm) was placed on top of the mounted window above the IR heat source. IR frequency was set at 50 and the cut off time at 15 sec. The baseline latency (sec) at which the rat removed its tail from the heat source was measured 15 minutes prior to dosing. Rats were then dosed with NYX-2925 (0.1, 1, 10 mg/kg, PO), vehicle (0.5% Na-CMC, PO) or the positive control (buprenorphine 1 mg/kg, IV). Withdrawal latencies were measured at 1 h post dosing.). Mean ± SE, n = 7 , * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

CONCLUSIONS

- NYX-2925 analgesic effect in neuropathic pain is rapid and long lasting.
- NYX-2925 analgesic effect is dependent on NMDA receptor and AMPA receptor function.
- NYX-2925 analgesic effect appears to be through a brain, but not spinal cord site of action.
- NYX-2925 analgesic effect is specific to neuropathic and not nociceptive pain.

NYX-2925 Produces Long-lasting Analgesia in the CCI Model (Mechanical) and SNI (Thermal) Models of Neuropathic Pain



Mechanical Hypersesitivity:
%Analgesia = [(log (x) -y) / (log (15) - y) * 100
Where: x = paw withdrawal threshold (g)
y = the average of the log (x) values for the vehicle treated group

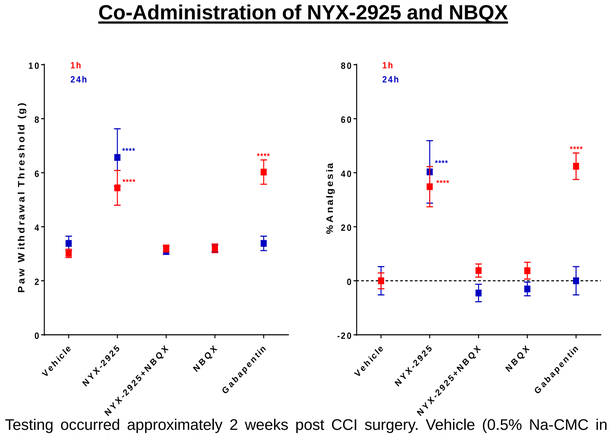
Thermal Hyperalgesia:
%Analgesia = [(treated PWLi – avg vehicle PWLi) / (avg vehicle PWLc – avg vehicle PWLi)] * 100.
Where: i = ipsilateral paw withdrawal latency (sec) c = contralateral paw withdrawal latency (sec)

For the mechanical analgesia studies, chronic constriction injury surgery was performed as previously described (*Bennett* and *Xie*, 1988). For the thermal analgesia studies, a spared nerve injury surgery was performed as previously described (*Decosterd* and *Woolf*, 2000). Testing for both studies occurred 1-2 weeks post-surgery. Testing occurred 1h, 24 h and 1wk post-dosing with NYX-2925 (0.1-10 mg/kg PO), gabapentin (150 mg/kg PO), or vehicle (0.5% Na-CMC in 0.9% sterile saline).

Mechanical analgesia was measured using Von Frey filaments and Dixon's up-down method (*Chaplan* et al., 1994). Mean ± SE, n = 6 – 24.

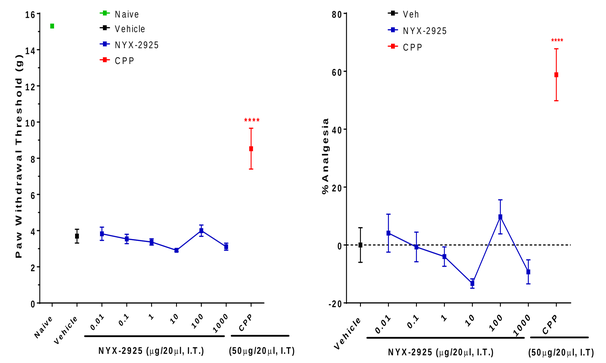
Thermal analgesia was measured using the Plantar Test (Hargreaves Apparatus; Ugo Basile, Italy). Mean ± SE, n = 6 - 7, * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Co-Administration of the AMPAR Antagonist NBQX Blocks the Analgesic Effects of NYX-2925 in the Rat CCI Model



Testing occurred approximately 2 weeks post CCI surgery. Vehicle (0.5% Na-CMC in 0.9% sterile saline), NYX-2925 at 10 mg/kg and gabapentin at 150 mg/kg were dosed by oral gavage. NBQX at 10 mg/kg was injected IP 10 min prior to NYX-2925. Mechanical hypersensitivity was measured using von Frey filaments and Dixon's up-down method (*Chaplan* et al., 1994) both at 1h and 24h post dosing. Mean ± SE, n = 6, * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Direct Injection of NYX-2925 onto the Spinal Cord Is NOT Analgesic in the Rat CCI Model



Chronic constriction injury surgery was performed as previously described (*Bennett* and *Xie* 1988). About 14 days post surgery and after baselining, rats were anesthetized by 5% isoflurane gas. The lumbar region of each rat was then shaved and cleaned with 70% ethanol. The rats was kept anesthetized under a nose cone. A 50ml Falcon tube was used to elevate the lumbar region. The L5-L6 region was marked. I.T injections were given between L5-L6 to the anesthetized rats using a 25 µl Hamilton syringe and 30G needle. Vehicle (20 µl saline), NYX-2925 (0.01-1000 µg in 20 µl saline) or CPP (50 µg/20 µl saline) was given over 1 min. Testing was done 30-45 min post injections.). Mean ± SE, n = 6 – 12 , * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

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