NYX-2925 REVERSES THE DECREASE IN HEDONIC ULTRASONIC VOCALIZATION AND THE INCREASE IN AVERSIVE VOCALIZATION OBSERVED WITH CCI-INDUCED NEUROPATHIC PAIN IN RATS

#PSN180

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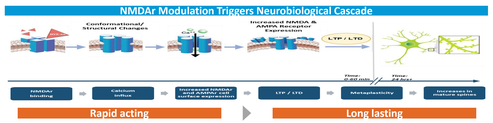
NYX-2925 Mechanism of Action



INTRODUCTION

Neuropathic pain impairs learning and memory, increases negative affect, and induces anhedonia, all of which reduce patient's quality of life. N-methyl-D-aspartate receptors (NMDARs) are ligand-gated ionotropic glutamate receptors that are predominately expressed in the central nervous system and are critical for learning and memory. Increasing evidence indicates that neuropathic pain induces aberrant NMDAR-dependent plasticity in mesolimbic regions, such as the medial prefrontal cortex (mPFC). Additionally, mPFC hypofunction is thought to underlie the impaired cognition and negative affect observed with chronic pain (Apkarian et al., 2013; Bushnel et al., 2013; and Kelly and Martina 2018).

Here, we sought to test the hypothesis that pain-induced impairments in cognition and affect would be normalized by increasing NMDAR activation in the mPFC. NYX-2925 is a novel NMDAR-specific modulator that facilitates synaptic plasticity and enhances learning and memory in rodents NMDA-dependent tasks (Khan et al., 2017). NYX-2925 also exhibits antidepressant-like effects in rats (Khan et al., 2017). To test our hypothesis, neuropathic pain was induced via chronic constriction injury (CCI), and the impact of NYX-2925 on cognition and affect was evaluated via heterospecific rough-and-tumble play. The rough-and-tumble play assay was used to measure affective components of neuropathic pain, as quantified by hedonic and aversive ultrasonic vocalizations (USVs).

METHODS

Chronic constriction injury (CCI)

Peripheral injury of the common sciatic nerve was performed, as previously described (Bennett and Xie, 1988; Ghoreishi-Haack et al., 2018). Briefly, rats were anesthetized using isoflurane. The sciatic nerve was isolated, exposed, and loosely ligated. The same procedure was performed for the sham surgery except the sciatic nerve was left intact and not ligated.

Three weeks following nerve injury, rats were tested for mechanical hypersensitivity via von Frey filaments. Any rat with 50% paw withdrawal threshold (PWT) greater than 5.0 grams was considered not to be allodynic and was excluded from the study.

Heterospecific rough-and-tumble play

Heterospecific rough-and-tumble play was conducted 3-4 weeks post CCI surgery, as previously described (Burgdorf et al., 2008; Ghoreishi-Haack et al. 2018). Briefly, heterospecific rough-and-tumble play stimulation was administered by the experimenter's right hand. Rats received 3 min of heterospecific rough-and-tumble play consisting of alternating 15 sec blocks of play and 15 sec of no-stimulation. The experimenter was blind to treatment condition of the animals. High frequency USVs (50 kHz and 20 kHz) were recorded (Avisoft UltraSoundGate, Germany) during the 15 sec no-stimulation blocks and analyzed by sonogram (Avisoft SASlab Pro, Germany) in a blind manner.

METHODS (continued)

Homecage recordings

Rats were housed 3 per cage by experimental condition in circular acrylic home cages (35.6 CM diameter X 30.5 CM high; Pinnacle, USA) with aspen wood chip bedding. Plexiglas® lids with 9 X 50 cm holes were outfitted with a microphone (Avisoft, Germany) that was suspended from the middle hole. Rats were maintained on a 12:12 hr light:dark cycle (lights on at 6 am), and given ad libitum access to Purina lab chow (USA) and tap water throughout the study. High frequency USVs were recorded (Avisoft UltraSoundGate, Germany) in 15 min bins for 24 hrs and analyzed via sonogram (Avisoft SASlab Pro, Germany) with high (R > 0.90) blinded inter-rater reliability. Spontaneous, aversive 20-kHz USVs were defined as bouts of calls that occurred immediately prior to at least 60 seconds of inactivity. Behavioral activation (e.g. locomotor activity, sniffing, eating, and drinking) were quantified by the total sound output of the microphone, which captures both sonic and ultrasonic sound. Inactivity (% of total time) was defined by the amount of time in which the sound intensity was similar to levels recorded from an empty cage. Activity was defined as 100 - inactivity.

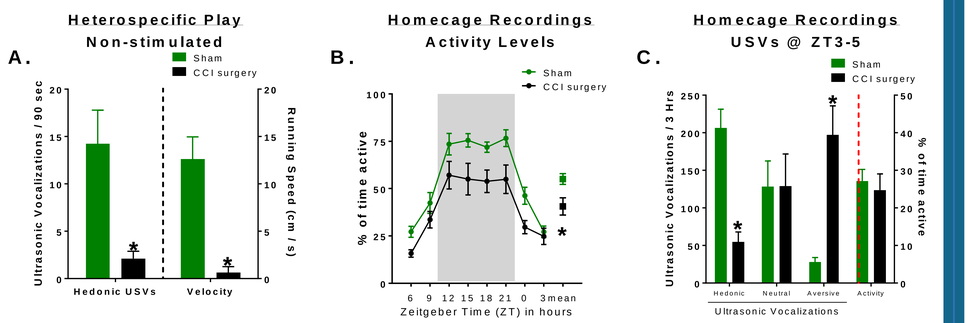
NYX-2925 administration

The impact of NYX-2925 administration on the affective components of neuropathic pain was evaluated in another rat cohort that underwent either sham or CCI surgery. NYX-2925 (1 or 10 mg/kg, p.o.) or vehicle was administered one hour prior to testing in the heterospecific rough-and-tumble play assay. Hedonic and aversive calls as well as the latency to run to the experimenter's hand to self-administer play were measured.

Statistical significance

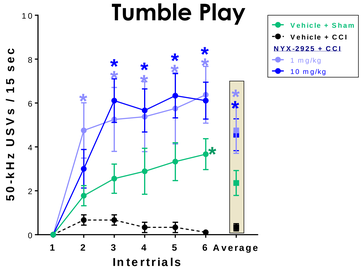
All data were analyzed by a 1-way ANOVA followed by a Fisher (panel 1) and Bonferroni (panels 2 - 4) post-hoc test. * $p < 0.05$ was considered statistically. significant.

1 Neuropathic Pain Reduces Positive Affect, Increases Negative Affect, and Decreases Homecage Activity Levels



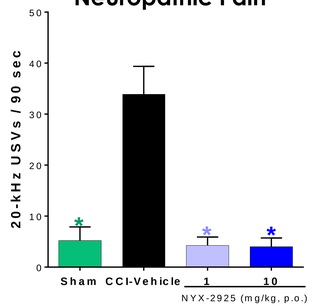
A. Rates of hedonic 50-kHz USVs and the running speed to self-administer additional stimulation was significantly reduced in rats with CCI-induced neuropathic pain when compared to the sham-operated rats. **B**. Rats with neuropathic pain showed significantly lower average activity levels across the 24 h homecage recordings. **C**. Rates of ultrasonic vocalizations during Zeitgeber time 3-5 (hrs), in which activity levels do not change, showed a significant decrease in hedonic and an increase in aversive calls in rats with neuropathic pain compared to the sham-operated rats. * $p < 0.05$ ANOVA CCI surgery vs. sham. Data are reported as mean ± SEM.

2 NYX-2925 Increases Hedonic Ultrasonic Vocalization in Rats with CCI-Induced Neuropathic Pain Measured via Rough-and-Tumble Play



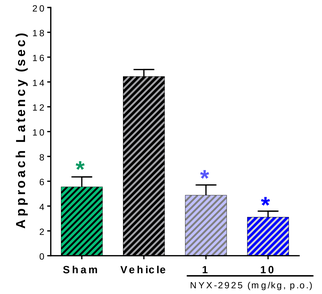
In the heterospecific rough-and-tumble play assay, deficits were seen in the hedonic calls made by rats that received CCI versus rats that underwent sham surgery. NYX-2925 reversed the CCI-induced deficits in hedonic ultrasonic vocalizations. Specifically, a single administration of NYX-2925 to CCI rats, 1 h prior to the first trial, significantly increased hedonic 50-kHz ultrasonic vocalizations between trials 2 through 6 and trials 3 through 6 for 1 mg/kg and 10 mg/kg, respectively, when compared to the CCI-vehicle control. A trend towards enhanced hedonic calls was seen in trials 1-5, and a significant difference was observed in trial 6. * $p < 0.05$ compared with the vehicle group at the same time point, n = 8 - 9 animals per group. Data represent means ± SEM.

3 NYX-2925 Reduces Aversive Ultrasonic Vocalization in the Rough-and-Tumble Play Assay in Rats with CCI-Induced Neuropathic Pain



Rats with neuropathic pain had significantly higher aversive 20-kHz vocalizations across non-stimulation periods when compared to sham-surgery animals. A single administration of NYX-2925 (1 mg/kg or 10 mg/kg, p.o.) to rats with neuropathic pain significantly decreased 20-kHz ultrasonic vocalizations. * $p < 0.05$ compared with the CCI-vehicle group at the same time point, n = 8 - 9 animals per group. Data represent means ± SEM.

4 NYX-2925 Reduces Approach Latency in the Rough-and-Tumble Play Assay in Rats with CCI-Induced Neuropathic Pain



CCI-induced neuropathic pain significantly increased the latency to approach the experimenter's hand to self-administer heterospecific play when compared to the sham-surgery animals. A single administration of NYX-2925 (1 mg/kg or 10 mg/kg, p.o.) to rats with neuropathic pain significantly decreased this latency when compared to vehicle-administered animals. * $p < 0.05$ compared with the vehicle group at the same time point, n = 8 - 9 animals per group. Data represent means ± SEM.

CONCLUSIONS

1. Rat ultrasonic vocalizations can be used to capture the affective components of neuropathic pain.
2. NYX-2925 rescued the lower rates of hedonic 50-kHz USVs seen in rats with CCI-induced neuropathic pain.
3. NYX-2925 rescued the higher rates of aversive 20-kHz USVs seen in rats with CCI-induced neuropathic pain.
4. NYX-2925 **decreased** the latency to self-administer heterospecific play in rats with CCI-induced neuropathic pain.
5. Twenty-four hour homecage ultrasonic vocalization monitoring is a promising technique for measuring spontaneous pain.

REFERENCES

Apkarian AV, Baliki MN , and Farmer MA (2013) **Predicting transition to chronic pain**. Curr Opin Neurol 26:360-367.

Bennett, G.J. and Xie, Y.-K. 1988. **A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man**. Pain 33:87-107.

Burgdorf J, Kroes RA, Weiss C, Oh MM, Disterhoft JF, Brudzynski SM, Panksepp J , and Moskal JR (2011a) **Positive emotional learning is regulated in the medial prefrontal cortex by GluN2B-containing NMDA receptors**. Neuroscience 192:515-523.

Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, Brudzynski SM, Panksepp J. **Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. Journal of comparative psychology** 2008; 122 (4):357-367.

Bushnell MC, Ceko M , and Low LA (2013) **Cognitive and emotional control of pain and its disruption in chronic pain.** Nat Rev Neurosci 14:502-511.

Kelly CJ , and Martina M (2018) **Circuit-selective properties of glutamatergic inputs to the rat prelimbic cortex and their alterations in neuropathic pain.** Brain Struct Funct. 2018 Jul;223(6):2627-2639.

Khan MA, Houck DR, Gross AL, Zhang XL, Cearley C, Madsen TM, Kroes RA, Stanton PK, Burgdorf J, Moskal JR. **NYX-2925 is a novel NMDA receptor-specific spirocyclic-beta-lactam that modulates synaptic plasticity processes associated with learning and memory**. Int J Neuropsychopharmacol. 2018 Mar 1;21(3):242-254

Ghoreishi-Haack N, Priebe JM, Aguado JD, Colechio EM, Burgdorf JS, Bowers MS, Cearley CN, Khan MA, and Moskal JR. **NYX-2925 Is a Novel N-Methyl-d-Aspartate Receptor Modulator that Induces Rapid and Long-Lasting Analgesia in Rat Models of Neuropathic Pain.** J Pharmacol Exp Ther. 2018 Sep;366(3):485-497

FINANCIAL DISCLOSURES

NGH, JSB, JMP, JDA, RAK, CNC, & JRM receive financial compensation and stocks from Aptinyx, Inc.