NYX-2925 Improves Both Pain and Its Affective States in the Rat Chronic Constriction Injury Model of Neuropathic Pain

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#PSN180

INTRODUCTION

In humans, neuropathic pain is associated with several comorbidities including anhedonia. N-methyl-D-aspartate receptors (NMDARs) are ligand-gated ionotropic glutamate receptors that are predominately expressed in the central nervous system. NMDARs are critical for emotions and synaptic plasticity. Here, we sought to establish if the affective components of neuropathic pain can be demonstrated following chronic constriction injury of the rat sciatic nerve. To do so, we measured USV vocalizations after heterospecific rough-and-tumble play as well as homecage recordings in rats that underwent chronic constriction injury. A clear deficit in USVs calls, and activity levels were seen in rats with neuropathic pain in both heterospecific rough-and-tumble play and homecage recordings. We then evaluated the potential effects of NYX-2925, a NMDA receptor modulator, to normalize those deficiencies in USVs and the self-administration of play.

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.

Two to 3 weeks following nerve injury, rats were tested for mechanical hypersensitivity via von Frey filaments and the Chaplan method (Chaplan et al. 1994). Any rat with a 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., 2001, and 2011; 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. High frequency USVs (50 kHz and 20 kHz) were recorded by Avisoft software (UltraSoundGate, Germany) during the 15 sec no-stimulation blocks and analyzed by sonogram (Avisoft SASlab Pro, Germany) in a blind manner.

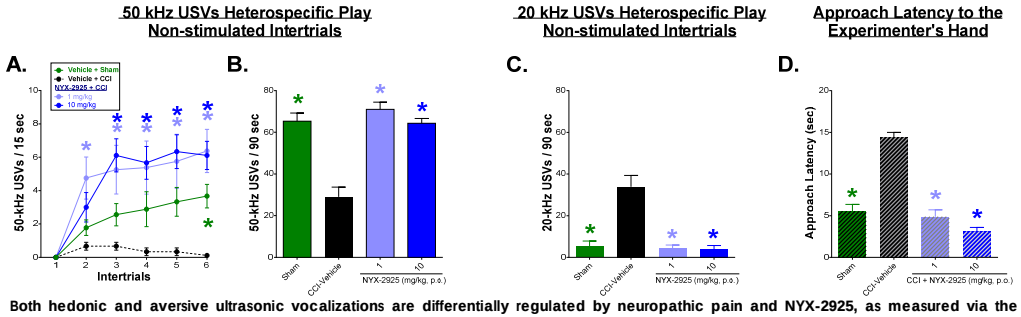
NYX-2925 administration

The impact of NYX-2925 administration on the affective components of neuropathic pain was evaluated in rats 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.

Homecage recordings.

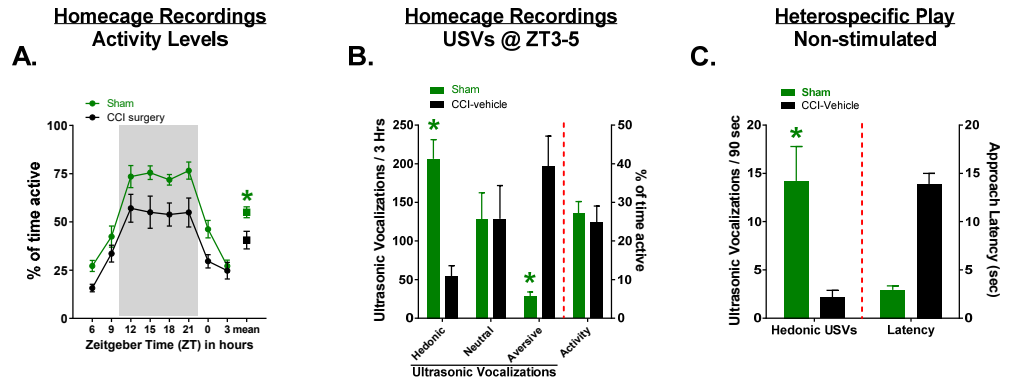
Rats were housed 3 per cage by experimental condition in circular acrylic home cages (35.6 cm diameter X 30.5 cm high; Pinnacle, USA) with aspen wood chip bedding. Plexiglas® lids with 9 X 50 cm holes were outfitted with a microphone (Avisoft, Germany) that was suspended from the center. Rats were maintained 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, hedonic (50 kHz), or aversive 20-kHz USVs were defined as bouts of calls that occurred immediately prior to at least 60 seconds of inactivity. Behavioral activity (e.g. locomotor activity, sniffing, eating, and drinking) was 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 time was defined as 100 - inactivity.

NYX-2925 Increases Hedonic Ultrasonic Vocalizations, Decreases Aversive Ultrasonic Vocalizations, and Reduces Approach Latency in the Rough-and-Tumble Play Assay in Rats with CCI-induced Neuropathic Pain



Both hedonic and aversive ultrasonic vocalizations are differentially regulated by neuropathic pain and NYX-2925, as measured via the heteospecific rough-and-tumble play. A. Fewer hedonic calls were made by rats that received CCI versus rats that underwent sham surgery. NYX-2925 increased the number of hedonic ultrasonic vocalizations in rats with CCI-induced neuropathic pain. Specifically, a single administration of NYX-2925 to CCI rats, 1 hr prior to the first trial, significantly increased average 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. **B.** Sum of hedonic 50 kHz calls in 90 sec intertrials per group for the data shown in (A). **C.** Rats with neuropathic pain produced significantly more 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 the number of 20-kHz ultrasonic vocalizations. **D.** 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 mean ± SEM. Refer to Ghoreishi-Haack et al., 2018.

Neuropathic Pain Reduces Hedonic Ultrasonic Vocalizations, Increases Aversive Ultrasonic Vocalizations, and Decreases Activity Levels in Homecage Recordings



Both spontaneous and evoked ultrasonic vocalizations are useful to quantify the affective component of neuropathic pain. A. Rats with neuropathic pain showed significantly lower average activity levels across the 24 hr homecage recordings. **B**. Rates of ultrasonic vocalizations during Zeitgeber time 3-5, in which activity levels were similar between groups, showed a significant decrease in hedonic (50-kHz) and an increase in aversive (20-kHz) calls in rats with neuropathic pain compared to the sham-operated rats. C. In heterospecific rough-and-tumble play, rates of hedonic 50-kHz USVs in running speed to self-administer additional stimulations was significantly reduced in rats with CCI-induced neuropathic pain when compared to sham-operated rats. * $p < 0.05$ ANOVA CCI-surgery vs. sham. $n = 5$ animals per group. Data are reported as mean ± SEM.

CONCLUSIONS

1. Rat ultrasonic vocalizations can be used to capture the affective components of neuropathic pain.
2. NYX-2925 normalized the lower rates of hedonic 50-kHz USVs seen in rats with CCI-induced neuropathic pain.
3. NYX-2925 normalized the higher rates of aversive 20-kHz USVs observed 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 affective components of pain.

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FINANCIAL DISCLOSURES

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

ADDITIONAL POSTERS

PSN114: NYX-2925, a Novel NMDA Receptor Modulator, Normalizes Synaptic Plasticity-associated Cortical Protein Expression in Rats with Chronic Constriction Nerve Injury