**Methods**

Methods: Inter-individual and sex differences have been well documented with regard to anxiety- and depression-like conditions and in pain. However, neural mechanisms and biomarkers related to pain vulnerability and resilience, including potential sexual dimorphisms, have yet to be fully elucidated. Fear learning and extinction networks have been implicated in neuropsychiatric disorders such as anxiety disorders, post-traumatic stress disorder (PTSD), and obsessive compulsive disorder (OCD). Vulnerability to these disorders has been predicted using fear extinction (FE) learning ability as a biomarker for inter-individual differences in the preclinical and clinical settings.

Bilateral studies are a crucial tool for the validation of pain mechanisms and for the assessment of potential pharmacological therapies. Higher integrated pain behavior at supraspinal levels has been assessed using vocalizations, an important method of communication among rodents. Inter-individual and sex differences in auditory and ultrasonic vocalizations, particularly in the context of pain and fear interactions, have not been determined. The purpose of this study was to examine the predictive value of fear extinction (FE) learning ability for inter-individual differences in pain-related behavioral responses, particularly emotional-affective pain aspects, with regard to sex. We subjected adult male and female rats to cues for fear learning and FE tests and correlated inter-individual differences with pain responses in models of acute pain and chronic neuropathic pain. We also investigated sex differences in FE phenotypes for measures of sensory (mechanical withdrawal thresholds) and emotional-affective (open field tests for anxiety-like behaviors and auditory and ultrasonic components of vocalizations) pain-related behaviors.

**Results**

**Figure 1** FE phenotypes

![FE phenotypes](image1)

**Figure 2** Arthritis pain behaviors

![Arthritis pain behaviors](image2)

**Figure 3** Neuropathic pain behaviors

![Neuropathic pain behaviors](image3)

**Figure 4** Representative vocalizations

![Representative vocalizations](image4)

**Conclusions**

**Inter-individual and sex differences in pain-related behaviors**

- The population of the FE+ phenotype was larger and the population of the FE- phenotype was smaller in female compared to male rats.
- Inter-individual and sex differences in pain behaviors
  - Emotional response to arthritis and neuropathic pain developed in all groups but emerged most prominently for female FE- rats.
  - Females of both phenotypes showed greater baseline anxiety levels than males.
- The data may suggest that sexual dimorphisms in FE learning ability have a predictive value for pain-related behavioral changes, particularly among emotional-affective pain aspects in both acute and a chronic pain model. The increased correlation between FE learning ability and affective pain-related behaviors in female compared to male rats warrant further investigation into sex-specific synaptic and cellular neurobiological mechanisms.

**Introduction**

Inter-individual and sex differences have been well documented with regard to anxiety- and depression-like conditions and in pain. However, neural mechanisms and biomarkers related to pain vulnerability and resilience, including potential sexual dimorphisms, have yet to be fully elucidated. Fear learning and extinction networks have been implicated in neuropsychiatric disorders such as anxiety disorders, post-traumatic stress disorder (PTSD), and obsessive compulsive disorder (OCD). Vulnerability to these disorders has been predicted using fear extinction (FE) learning ability as a biomarker for inter-individual differences in the preclinical and clinical settings.

Bilateral studies are a crucial tool for the validation of pain mechanisms and for the assessment of potential pharmacological therapies. Higher integrated pain behavior at supraspinal levels has been assessed using vocalizations, an important method of communication among rodents. Inter-individual and sex differences in auditory and ultrasonic vocalizations, particularly in the context of pain and fear interactions, have not been determined. The purpose of this study was to examine the predictive value of fear extinction (FE) learning ability for inter-individual differences in pain-related behavioral responses, particularly emotional-affective pain aspects, with regard to sex. We subjected adult male and female rats to cues for fear learning and FE tests and correlated inter-individual differences with pain responses in models of acute pain and chronic neuropathic pain. We also investigated sex differences in FE phenotypes for measures of sensory (mechanical withdrawal thresholds) and emotional-affective (open field tests for anxiety-like behaviors and auditory and ultrasonic components of vocalizations) pain-related behaviors.

**Methods**

**Animals:** Male and female Sprague-Dawley rats (150-350g) were housed in a temperature-controlled room and maintained on a 12-hour daytime cycle with water ad libitum and food and water.

**Experimental protocol:** Naive rats were subjected to fear conditioning and FE trials. Rats were then randomly assigned to the acute arthritis pain model (chronic pain) or the chronic neuropathic pain model (spinal nerve ligation, SNL). Behavioral studies were performed 4 weeks after surgery or 6 hours after arthritis induction.

**Fear conditioning and extinction:** Auditory fear conditioning and fear extinction (FE) learning were tested using two chambers of a Video Fear Conditioning System (Med Associates Inc.). On day 1, rats were habituated to context A followed by fear conditioning by 2 CS-US pairings, inter-trial interval, ITI 240 sec (CS: 80 dB, 4.5 kHz, 30s white noise), US: 0.7 mA foot shock, 2s. On day 2, rats were habituated to context B followed by extinction training (30 CSs).

**Arthritis pain model:** Rats were briefly anesthetized with isoflurane (2-3%); precision vaporizer, Harvard Apparatus) and a kaolin suspension (4% in sterile saline. 100 μL) was slowly injected into the left knee joint cavity followed by repetitive flexions and extensions of the leg for 15 min. A carrageenan solution (2% in sterile saline, 100 μL) was then injected into the knee joint cavity and the leg was flexed and extended for another 5 min. Naïve rats that underwent similar handling but did not receive intraarticular injections were used as a control group.

**Neuropathic pain model:** The well-established SNL model of neuropathic pain was used. Rats were anesthetized with isoflurane (2-3%); precision vaporizer, Harvard Apparatus) and underwent sterile surgery where the left L5 spinal nerve was exposed and tightly ligated using 6-0 sterile silk. This was compared to a sham-operated control group where the nerve was exposed but not ligated.

**Pain-related behavioral tests:** Behavioral assays were performed 6 h after arthritis induction in the acute pain group or 4 weeks after SNL or sham surgery in the chronic neuropathic pain group.

**Mechanical withdrawal thresholds** were measured by briefly anesthetizing rats (isoflurane, 2-3%) and placing them in a slightly restrained recording chamber that permitted movement in the hindlimb. Hindlimb withdrawal thresholds were evaluated using calibrated forceps with a force transducer whose output was displayed in grams on an LED screen. The calibrated forceps were used to gradually compress the left knee joint (arthritis pain model) or the left hindpaw (neuropathic pain model) with a continuously increasing intensity until a withdrawal reflex was evoked.

**Vocalizations in the audible (20Hz–18kHz):** Supraspinally organized nociceptive responses and ultrasonic (22kHz: limbic-driven negative emotional responses) were measured with our patented computerized recording system. Brief (10 s) noxious stimuli were applied to the left hind paw (arthritis pain model, stimulus: 1500 g/30 mm²) or to the left hind paw (neuropathic pain model, stimulus: 500 g/6 mm²) of rats using a calibrated forceps. Vocalizations were recorded for 1 min and analyzed using Ultraview XT 3.2 software (Noldus).

**Anxiety-like behavior** was measured using the open field test as decreased number of entries into the central arena (70 cm x 70 cm) for 15 min with a computerized videotaping system (Noldus, EthoVision XT).

**Statistical analysis:** Significance was accepted at the level P<0.05. All averaged values represent means ± SEM.

**Supported by NIH R01 NS038261, R01 NS106902, R01NS118731, R01 NS120395, and R01 NS109255**