



**AALBORG UNIVERSITY**  
DENMARK

**Aalborg Universitet**

## **Does Threat Enlarge Nociceptive Reflex Receptive Fields?**

Lannon, Edward W.; Jure, Fabricio A.; Andersen, Ole Kæseler; Rhudy, Jamie L.

*Published in:*  
The Journal of Pain

*DOI (link to publication from Publisher):*  
[10.1016/j.jpain.2020.10.006](https://doi.org/10.1016/j.jpain.2020.10.006)

*Creative Commons License*  
CC BY-NC-ND 4.0

*Publication date:*  
2021

*Document Version*  
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*  
Lannon, E. W., Jure, F. A., Andersen, O. K., & Rhudy, J. L. (2021). Does Threat Enlarge Nociceptive Reflex Receptive Fields? *The Journal of Pain*, 22(5), 487-497. Advance online publication. <https://doi.org/10.1016/j.jpain.2020.10.006>

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

### **Take down policy**

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.

# The Journal of Pain

## Does Threat Enlarge Nociceptive Reflex Receptive Fields?

--Manuscript Draft--

<b>Manuscript Number:</b>	JPAIN-D-20-00256R2
<b>Article Type:</b>	Human Study
<b>Section/Category:</b>	Observational Study *
<b>Keywords:</b>	Pain; Reflex Receptive Fields; Anxiety; Nociception; Spinal Nociception
<b>Corresponding Author:</b>	Edward Lannon University of Tulsa UNITED STATES
<b>First Author:</b>	Edward Lannon
<b>Order of Authors:</b>	Edward Lannon
	Fabricio Jure
	Ole Andersen
	Jamie Rhudy
<b>Abstract:</b>	<p>Threat-induced pain modulation can increase survival by amplifying physiological and behavioral reactions towards danger. Threat can also modulate spinal nociception, suggesting engagement of endogenous top-down circuitry. A unique method to assess spinal nociception is via reflex receptive fields (RRF) associated with the nociceptive withdrawal reflex (NWR, a protective spinally-mediated reflex). The size of nociceptive RRFs can be modulated by top-down circuitry with the enlargement of RRFs related to increased spinal nociception. Threat has been previously shown to enhance pain and spinal nociception, but the relationship between threat and RRFs has not been investigated. The present study investigated this issue in 25 healthy individuals. RRFs were determined from NWRs measured by electromyography (EMG) of the tibialis anterior following electrocutaneous stimulations. RRFs and pain were assessed during periods in which participants were under threat of unpredictable painful abdominal stimulations and when they were not under threat. Results indicated that threat periods led to significantly higher pain, larger nociceptive RRFs and NWR magnitudes. These findings imply that threat produces changes in protective reflexes related to spinal nociceptive sensitivity and increased pain perception. This is likely mediated by top-down circuitry that enhances dorsal horn nociceptive neurons by enlarging RRFs and amplifying ascending pain signals.</p>

## Does Threat Enlarge Nociceptive Reflex Receptive Fields?

Edward W. Lannon, M.A.<sup>1,2</sup>, Fabricio A. Jure, M.Sc.<sup>2</sup>, Ole Kæseler Andersen, Dr. scient., Ph.D<sup>2</sup>, &  
Jamie L. Rhudy, Ph.D<sup>1</sup>.

<sup>1</sup>Department of Psychology, The University of Tulsa, 800 S Tucker Dr, Tulsa, OK 74104

<sup>2</sup>Center for Neuroplasticity and Pain (CNAP), Aalborg University, Fredrik Bajers Vej 7, 9220  
Aalborg Ø, Denmark

Number of text pages: 18

Number of figures and tables: 3 Figures and 1 table

Manuscript was developed at The University of Tulsa, Tulsa, OK and the Center for Neuroplasticity and Pain (CNAP), SMI<sup>®</sup>, Aalborg University, Aalborg Ø, Denmark.

Funding: Research reported in this publication was supported by the National Science Foundation Graduate [grant number 1546597] and the Graduate Research Opportunities Worldwide (GROW) Fellowship to E.L. Edward Lannon is a part of Center for Neuroplasticity and Pain (CNAP) which is supported by the Danish National Research Foundation (DNRF121). The content is solely the responsibility of the authors and does not necessarily represent the official views of The University of Tulsa, Center for Neuroplasticity and Pain (CNAP), or National Science Foundation. The authors have no conflicts of interest to report.

Correspondence concerning this article should be addressed to Edward Lannon, Ph.D., Department of Psychology, The University of Tulsa, 800 South Tucker Drive, Tulsa, OK 74104. Telephone: (619) 399-8872  
E-mail: edward-lannon@utulsa.edu

### Abstract

Threat-induced pain modulation can increase survival by amplifying physiological and behavioral reactions towards danger. Threat can also modulate spinal nociception, suggesting engagement of endogenous top-down circuitry. A unique method to assess spinal nociception is via reflex receptive fields (RRF) associated with the nociceptive withdrawal reflex (NWR, a protective spinally-mediated reflex). The size of nociceptive RRFs can be modulated by top-down circuitry with the enlargement of RRFs related to increased spinal nociception. Threat has been previously shown to enhance pain and spinal nociception, but the relationship between threat and RRFs has not been investigated. The present study investigated this issue in 25 healthy individuals. RRFs were determined from NWRs measured by electromyography (EMG) of the tibialis anterior following electrocutaneous stimulations. RRFs and pain were assessed during periods in which participants were under threat of unpredictable painful abdominal stimulations and when they were not under threat. Results indicated that threat periods led to significantly higher pain, larger nociceptive RRFs and NWR magnitudes. These findings imply that threat produces changes in protective reflexes related to spinal nociceptive sensitivity and increased pain perception. This is likely mediated by top-down circuitry that enhances dorsal horn nociceptive neurons by enlarging RRFs and amplifying ascending pain signals.

**Perspective:** This article presents the enlargement of reflex receptive fields (RRF) during periods of threat. The results from this study may help clarify the mechanism underlining emotional modulation of spinal nociception.

**Key Words:** Pain, Reflex Receptive Fields, Anxiety, Nociception, Spinal Nociception.

### Introduction

Pain modulation can increase survival. For example, during situations of possible threat, increased pain perception allows for early detection, and stronger reaction to, somatic danger [42; 49]. Consistent with this, several studies have noted an increase in pain perception when anticipating an unpredictable threat [27; 73; 76].

The changes in pain perception due to threat appear to be mediated by activation of descending pathways. Consequently, incoming nociceptive signals are facilitated at the spinal cord level [42; 49]. This is evidenced by a threat-induced increase in the magnitude of a spinally-mediated protective reflex, the nociceptive withdrawal reflex (NWR)[27; 73].

The NWR requires precise movements in order for the reflex to properly withdraw a limb from somatic danger [62; 69]. To achieve this, the NWR has a modular organization in which each muscle or muscle group has a specific cutaneous reflex receptive field (RRF) and corresponding spinal neurons in the dorsal horn that mediate the elicitation of the motor movement [2; 3; 67]. Stimulations within an RRF typically elicit a reflex (flexion or extension) from its respective muscle, but stimulations outside of the RRF do not [2]. Because skin areas have overlapping RRFs, the NWR is the net reflex-related muscle activity triggered by stimulations within a set of RRFs [3; 66].

Because the spatial organization of the RRFs are mediated mostly by deep dorsal horn neurons, RRFs can act as a noninvasive correlate of dorsal horn nociceptive receptive fields [67]. Indeed, the size of RRFs can be modulated, with an increase in size related to increased spinal nociception and hyperalgesia. For instance, individuals with disorders associated with hyperalgesia (e.g., chronic neck pain, chronic low back pain, endometriosis) have larger RRFs

than healthy controls [38; 44]. Likewise, RRFs enlarge after procedures that are associated with sensitization and hyperalgesia (e.g., after the administration of capsaicin) [35], and after repeated stimulations of the same intensity (temporal summation) [4; 35]. Interestingly, cognitive factors (e.g., attention) can also modulate the size of RRFs through descending pathways [7].

According to Latremoliere and Woolf, enlarged nociceptive receptive fields of dorsal horn neurons are a marker of central sensitization [32]. Indeed, enlarged dorsal horn neuron receptive fields are seen in animal models of cancer bone pain [74], arthritis (inflammation) [11; 22; 28], postsurgical pain [30; 46], and spinal nerve ligation [11; 71]. Further, there is an expansion of dorsal horn receptive fields after repeated frequent stimulation (1Hz for 20 secs) [12], administration of mustard oil [79], injection of noxious agent into a muscle tissue [26], administration of noxious heat [40], noxious pinch [31], noxious stimulation of the viscera [10], and ischemia [14]. As such, measuring RRF expansion may provide insight into central sensitization mechanisms and the chronification of pain.

Previous studies indicate that NWR magnitudes are enhanced during periods of threat [27; 72], but the effect of threat on nociceptive RRFs is unknown. To address this gap in the literature, the present study will measure RRFs during periods in which unpredictable abdominal stimulations are delivered (threat periods) and periods in which they are not delivered (safety periods). Given that unpredictable threat increases NWR magnitudes and produces hyperalgesia [27; 73], and that hyperalgesia is related to expansion of RRFs, we hypothesize that threat will produce anxiety, and in turn increase pain and the size of RRFs.

Further, as an added manipulation check, analyses verified that NWR magnitudes (not RRF) were increased during threat, relative to safe, periods.

### **Methods**

In order to measure changes in RRFs elicited by threat, participants completed a procedure that consisted of the presentation of 8 threat and 8 safe periods. Unpredictable painful abdominal stimulations were delivered during threat periods, but never during safe periods. The RRF of the tibialis anterior (TA) was measured during the threat/safe paradigm by surface recording the TA-EMG in response to painful electrocutaneous stimulations to 10 sites on the sole of the foot. Participants were seated in a comfortable position with a slight flexion in the knee joint to reach approximately 75° throughout the experiment. Power analysis using effect sizes from prior studies investigating successful psychological modulation of RRFs [7], and an  $\alpha=.05$ , indicated that a sample of 15 was needed to achieve a power of .8. The current study targeted 25 in order to ensure adequate power and obtain a sample size large enough to produce a normally distributed sampling distribution of the mean (to meet the assumption of normality).

### **Participants**

26 participants were recruited from the community surrounding Aalborg University (located in Aalborg, Denmark) between November 2017 and January 2018, but 1 person did not complete the study due to discomfort with electrical stimulations. Thus, the final sample consisted of 25 healthy, pain-free individuals (8 women, 17 men, mean age=24.1 years,  $SD=3.41$ ). Participants were excluded if they endorsed any of the following via self-report: skin, neurological, cardiovascular, or circulatory problems; acute pain or chronic pain (pain that lasts

longer than 3 months); being under the influence of substances that influence the central nervous system (e.g., antidepressants, anxiolytics, beta blockers, cannabis, opioids, alcohol, pain medication); pregnancy or lactation; participation in other related studies within 1 week of the study; and age younger than 18. All procedures were approved by the local ethical committee of North Denmark Region (N-20150038) and the Declaration of Helsinki was respected.

### **Questionnaires**

Participants verbally rated their pain using a numerical rating scale (NRS) that ranged from 0 (no pain) to 10 (pain as bad as it could be)[47] . Participants verbally rated their anxiety on a 5-point Likert scale (1=not at all anxious, 2=a little anxious, 3=moderately anxious, 4=very anxious, 5=extremely anxious) [15].

### **Sensor Placement and EMG Recording**

Prior to any sensor placement, the area was shaved and cleaned using isopropyl alcohol. In order to ensure proper electrical impedances at the sole of the foot, the thick epidermal layers at the bottom of the foot were removed by soaking the foot in warm saline water (~ 10 mins), and then exfoliating using a foot exfoliator. Biological markers on the bottom of the foot were used as anchors to ensure proper placement of each electrode (e.g., sites 1, 2, and 3 on the balls of the feet; sites 4, 5, and 6 on the center of the foot, sites 9, and 10 on the heel of the foot and sites 7, and 8 between the heel and the center of the foot).

EMG activity was recorded from the belly of the TA with a double differential configuration using 3 electrodes (type 720, Ambu A/S, Denmark; interelectrode distance of 20 mm) and a ground (type 720, Ambu A/S, Denmark) placed on the fibula. Electrode placement

followed the recommendations of Surface EMG for Non-Invasive Assessment of Muscles (SEMIAN) for the electrode placement [25]. EMG signals were sampled at 2000 Hz, amplified, filtered (5-500 Hz), and stored on a hard drive.

### **Electrical stimulation**

Electrical stimulations were delivered by a grid of 10 self-adhesive Ag/AgCl surface electrodes (20 mm X 15 mm, type 700, Ambu A/S, Ballerup, Denmark) placed on the sole of the right foot (see Figure 1A for distribution of the electrodes). A common large anode (75 mm X 100 mm electrode, PALS Model #895340, Axelgaard Manufacturing Co. Ltd., Fallbrook, CA) was mounted on the top of the foot to ensure that stimulations were perceived to come from the sole (by allowing electron flow from the dorsum of the foot to the plantar side of the foot). Foot stimulations were 5 constant-current, monophasic 1 ms pulses delivered at 200 Hz which felt like one pulse. Electrodes were moved slightly if the electrocutaneous stimulations produced a radiating sensation in order to ensure that a nerve was not directly stimulated.

Abdominal stimulations were delivered to the lower right quadrant of the abdomen by an Ag/AgCl surface electrode (20 mm X 15 mm, type 700, Ambu A/S, Ballerup, Denmark) and a large anode (75 mm X 100 mm electrode, PALS Model #896240, Axelgaard Manufacturing Co. Ltd., Fallbrook, CA). Abdominal stimulations consisted of 75 monophasic 1 ms pulses at 100 Hz experienced as multiple stimulations. Stimulations, both to the abdomen and to the sole of the foot, were delivered by a Noxitest IES 230 stimulator (Aalborg University, Denmark). For participant safety, no abdominal or foot electrocutaneous stimulation exceeded 50 mA.

### **Determining Stimulation Intensities**

In an attempt to reliably elicit NWRs, stimulation intensities were individually calibrated to each stimulation site. First, pain threshold (PTh) was determined at site 4 (See Figure 1A) using 3 ascending/descending staircases. The stimulations intensities began at 4 mA and increase in 2 mA steps until a NRS rating  $\geq 5$  was reached. After a NRS rating  $\geq 5$  was obtained, the stimulus was decreased in 1 mA steps until a NRS rating  $< 5$  was reached. The second and third ascending/descending staircases used 1 mA steps. PTh was defined as the average stimulus intensity (mA) of the last 2 peaks and troughs. Then, the stimulation intensity on site 4 was increases to 1.5 X PTh in order to reduce floor/ceiling effects. After increasing stimulation intensity of site 4, the pain ratings of the other sites were matched to site 4 in order to ensure equal nociceptive input during the threat/safe paradigm (See Figure 1). Stimulations were matched to site 4 by delivering a stimulation on a site then increasing/decreasing the stimulation intensity until the ratings matched. So, the 3 ascending/descending staircase method was only used on site 4.

Abdominal stimulation intensity was determined using the same 3 ascending/descending staircase methods used for site 4. However, the target rating was a NRS of 8 instead of a NRS of 5. Abdominal stimulation during the threat/safe paradigm intensity was set at the intensity that elicited a NRS rating of 8.

### **Threat/Safe Paradigm**

After stimulation intensities were determined, participants underwent a validated threat paradigm [27; 73](See Figure 2). The paradigm consisted of recording the RRF of the TA during 8 threat and 8 safe periods (all periods were 65 s in length). The threat/safe periods

were pseudorandomly ordered with the restrictions that first period was always a threat period and no more than 3 of the same periods could occur in sequence. During all threat periods, red text was displayed on a computer screen that read “DANGER: Abdominal Stimulation may be Given at Any Time.” During all safe periods, green text was displayed that read “SAFE: No Abdominal Stimulation will be Given.” As the text notes, no abdominal stimulation ever occurred during the safe periods.

Because the threat periods were longer (65s) than prior studies (~30s)[27; 72], the present study increased the number of abdominal stimulations to the abdomen in order to maintain threat. Abdominal stimulations (.75 s in length) were delivered between 1-5 s of every threat period and at the end (60-65s) of 4 out of the 8 threat periods (50% reinforcement). The rationale for presenting the second abdominal stimulation only 50% of the time was to increase the unpredictability of the threat. There was a short break (8 - 10 s) in between each period. At the end of each period, participants were asked to rate the average anxiety they felt during the period.

During each period, participants received 5 electrocutaneous stimulations at different stimulation sites to the sole of the foot (inter-stimulus interval = 5 - 10 s). Periods were presented for at least 5 s before any abdominal stimulation was delivered. Foot stimulations occurred between 5 and 60 s after the onset of the period, but never overlapped with an abdominal stimulation. In each safe period, participants received 5 foot stimulations whereas, in each threat period, participants received 5 foot stimulations and either 1 or 2 abdominal stimulations.

Within each condition (Threat vs. Safe), all foot electrode sites were stimulated (order randomized) before another stimulation in the same site could occur. Immediately following a foot or abdominal stimulation, participants rated the pain verbally using the NRS.

### Calculation of Reflex Receptive Fields

A previously validated procedure was used to calculate the size of the RRF [7; 29; 35-39; 44; 70]. For all foot stimulations, EMG signal from the TA was recorded for a 1 s period. The RRFs were calculated in the following way: 1) A z score was computed with the unrectified EMG from the 200 ms prestimulation period (baseline window) and the peak unrectified EMG that occurred in the 80-180 ms poststimulation period (Peak NWR window). If the z score was greater than 12, a reflex was detected. The z scores were computed in the following way.

$$Zscore = \frac{(|Peak NWR Window| - |Average Baseline Window|)}{SD\ of\ |Baseline\ Window|}$$

2) The probabilities of having a reflex were computed for each stimulation site. The formula used was as follows:

$$Reflex\ probability\ per\ site = \frac{number\ of\ reflexes\ obtained\ on\ site}{4}$$

3) The probabilities for all foot stimulation sites were then interpolated using Kriging algorithm for non-uniformly spaced data points and placed onto a schematic of a foot (See Figure 3).

4) The *RRF area* is the percentage of the foot in which the interpolated values were over 0.25 (i.e., the area of the foot in which the site produced an NWR at least 25% of the time). RRF area was used as the dependent variable in analyses. As a follow-up analysis, NWR magnitudes of the most pronounced site will be investigated.

### Calculation of NWR Magnitude for Validity Analysis

To determine whether NWR magnitudes were enhanced by threat, similar to previous studies [50], NWR magnitudes were calculated for site 4 (the site with the most probable NWR).

NWR magnitude was calculated as:

$$Zscore = \frac{(|Peak\ NWR\ Window| - |Average\ Baseline\ Window|)}{SD\ of\ |Baseline\ Window|}$$

### Data Analysis & Outlier Detection

Dependent samples t-tests were used to investigate group differences between the threat and safe conditions. Dependent variables were anxiety ratings, pain ratings, and RRF area. A 1-way ANCOVA MLM (with trial covaried) was used to examine the effect of threat on NWR magnitude. Zero-order-correlations using change scores (threat minus safe) were used to investigate the relationships between changes in anxiety and changes in pain/RRF.

**Data screening.** In order to allow for proper modulation of RRFs (i.e., to reduce ceiling and floor effects), participants with an RRF area = 1 or 0 were excluded from reflex-related analysis because these values could not be modulated up or down, respectively. Prior to any analysis, outliers were detected on the change scores of the dependent variables (threat minus safe) using robust estimations (median absolute deviation > 2.24) and excluded from analyses [75]. During analysis of NWR magnitude, outliers were detected and excluded from the analysis.

## Results

### Stimulus Parameters

No significant differences in pain ratings were found between stimulations sites after matching pain ratings to site 4 with the stimulation intensity set to 1.5 X PTh (*Grand Mean of all sites* = 5.78, *SD* = 0.07),  $F(9,216) = 1.48, p = 0.16$ ; see Figure 1B. The average stimulation

intensity of all the stimulating sites after the all sites were matched was 21.60 mA ( $SD=3.29$ ; See Figure 1C).

### **Threat Evoked Differences in RRF**

RRFs were determined for all 25 participants for the safe and threat conditions. Outliers were detected and excluded from analysis (4 for change scores in pain ratings, 1 for change scores in RRF area). 4 participants were excluded because of a RRF area of 1 or 0 in the threat or safe condition. No outliers in anxiety were noted. Hence, analyses using pain scores had  $N=21$  whereas analyses using EMG had  $N=20$ . Baseline EMG was not significantly different between threat ( $M=1.95 \mu V$ ;  $SD=.24 \mu V$ ) and safe periods ( $M=1.89 \mu V$ ;  $SD=.12 \mu V$ ),  $t(19) = 1.76$ ,  $p=.09$ . In both conditions, the highest reflex probability was exhibited near the medial and distal sections of the foot (See Figure 3). Results suggested significantly greater anxiety and pain ratings during threat periods than safe periods (See Table 1). Furthermore, RRF areas were significantly larger during threat periods than safe periods (See Table 1 & Figure 3).

### **Threat Evoked Differences in NWR Magnitude**

Site 4 had significantly large NWR magnitudes during the threat condition ( $M=58.11$ ,  $SE=11.92$ ) than the safe condition ( $M=44.214$ ,  $SE=11.91$ )  $F(1,120.1) = 5.06$ ,  $p = .03$ .

### **Relationships between Anxiety, RRF, and pain**

Changes in anxiety were not significantly correlated with changes in pain,  $r(21) = 0.27$ ,  $p = 0.24$ , or changes in RRF area,  $r(20) = 0.002$ ,  $p = 0.99$ . Moreover, anxiety scores during the safe condition were not related to RRF safe area  $r(20)=.413$ ,  $p=.07$  or mean pain ratings during the safe condition  $r(21)=.17$ ,  $p=.46$ . Anxiety ratings during the threat condition were not related to

RRF threat area  $r(20)=.37$ ,  $p=.10$  or mean pain ratings  $r(21)=.17$ ,  $p=.45$  during the threat condition.

### Discussion

The current study investigated the relationship between threat, pain, and spinal nociceptive processing (as measured by NWR RRF). In the present study, the threat condition resulted in increased anxiety, as well as increased pain ratings and RRFs areas, relative to the safe condition. The increase in pain ratings (albeit small) is consistent with previous studies noting that anticipation of an unpredictable threat enhances pain and spinal nociceptive processes [27; 53; 73; 76]. This can be partly explained by the top-down facilitation of dorsal horn neurons that amplify the nociceptive signals at the spinal cord level [49].

Previously, Terry et al. [72] and Hubbard et al. [27] used a similar threat/safe paradigm to induce an increase in NWR magnitudes and hyperalgesia. The results from the present study replicate these results to show that NWR magnitudes at site 4 were enhanced by threat, but also expand on this research by indicating that RRFs expand during threat.

As is suggested by Schouenborg, weighted connections between interneurons and motor neurons mediate RRF areas [63; 65]. In essence, there is a greater synaptic connection between the foci of a RRF and specific motor neurons which diminishes as you move towards the edges of the RRF area. As such, stimulations occurring within a RRF elicit a reflex response from the specific muscle while stimulations outside the RRF do not [2; 21]. Furthermore, stimulations occurring at the center of a RRF produce a greater (and more probable) reflex response than stimulations occurring at the edges [2].

Additionally, stimulations occurring near, but outside, the reflex receptive fields provide a subthreshold input into the reflex encoder [78]. However, if the reflex encoder is in a sensitized state, then this input may elicit a reflex [12; 64] reflecting an expansion of the receptive field of the encoder neuron. The present study suggest that threat sensitizes dorsal horn neurons (possibly through activation of the descending pathway) which leads to enlargement of RRF areas (and NWR magnitudes). Indeed, deep dorsal horn neurons receive continuous descending excitatory/inhibitory input from supraspinal structures [24; 34; 35].

Evolutionarily speaking, having RRFs that change in size due to environmental stimuli is highly adaptive. For example, having enlarged RRFs in threatening situations could increase withdrawal reactions to, and detection of, somatic danger. This would increase the chance of survival and/or limit somatic damage. Hence, having healthy RRFs that can modulate in size are likely advantages for survival.

A perceptual consequence of threat is anxiety/fear [16]. However, anxiety is typically defined as a future-oriented emotion associated with unpredictable threat, whereas fear is defined as an emotion focused on a present (typically severe) threat and usually evokes active fight-or-flight [45; 51; 52; 54; 68]. Moreover, anxiety elicits hyperalgesia, whereas fear elicits hypoalgesia. Given these distinctions, we designed the threat paradigm to evoke anxiety, because the periods were long (65s) and the abdominal stimulations were unpredictable. Moreover, we were interested in studying hyperalgesia. Consistent with this, prior studies have used this threat paradigm to elicit hyperalgesia and NWR facilitation [27; 73]. We found that self-reported anxiety was significantly greater during threat periods compared to safe periods;

therefore, it can be inferred that the unpredictable abdominal stimulations elicited anxiety in our participants.

The labels on the Likert scale indicated that the anxiety level, on average, for people during the threat condition was between “a little anxious” and “moderately anxious” whereas scores during the safe condition endorsed being between “not at all anxious” and “a little anxious.” The score in the safe condition are as expected (slight anxiety due to the foot stimulations). However, the scores in the threat condition are a lower than expected. This may reflect comfortability with the experimental setting and trust in the experimenter.

Interestingly, correlations between the changes in RRF size, pain intensity, and anxiety ratings were not significant. This could signify that the perceptual consequences of the threat paradigm (i.e., the subjective experience of anxiety) were not related to changes in pain or spinal nociception *per se*, but rather the biological consequences of the threatening situation itself (see further discussion below).

### **Modulation of Spinal Nociception by Threat**

Pain typically begins by the activation of primary nociceptors following a noxious event. The signals are then transmitted to the spinal cord and up to different supraspinal structures related to pain processing (e.g., prefrontal cortex [PFC], anterior cingulate cortex [ACC], primary somatosensory cortex [SI], secondary somatosensory cortex [SII], parabrachial area [PB], periaqueductal gray [PAG], hypothalamus, pons and medulla)[5; 6; 18]. Incoming nociceptive signals can be modulated at the spinal cord level through supraspinal activation of the descending, top-down, pathway. The structures implicated in the descending pathways include

the PFC, ACC, SI, SII, hypothalamus, amygdala, basal ganglia; midbrain structures such as the PB, and PAG; and hindbrain structures such as the pons and the medulla [6; 8; 13].

The amygdala and the bed nucleus of the stria terminalis [BNST] are intimately involved in the processing of threat [1; 17; 24; 33]. Importantly, the amygdala and the extended amygdala have projections to the PAG and the rostral ventromedial medulla [RVM] [43]. The RVM is known to play a pivotal role in the descending control and thereby modulation of incoming nociceptive signals at the spinal cord level [23; 24]. Given the extensive literature noting descending modulation of protective reflexes and pain through descending pathways [51; 54-61], we believe the current findings suggest a role of descending facilitation. However other mechanisms (e.g., motor neuron facilitation) cannot be ruled out.

Interestingly, different structures and pathways process threat at the conscious versus nonconscious level. For example, Carlsson and colleagues [9] used a masking paradigm with pictures of phobic and feared stimuli and investigated positron emission tomography (PET) changes related to the conscious and nonconscious processing of threatening stimuli. The results suggested activation of the amygdala in the nonconscious processing of the phobic stimuli, but amygdala, ACC, anterior insula, orbital frontal cortex (OFC), and PAG in the processing of conscious threatening stimuli. Further, a study using masked and unmasked threatening stimuli with participants that had “blindsight” revealed an increase in connectivity between the right amygdala, pulvinar, and superior colliculus in masked conditions [41]. The results from our correlations suggest that the presence of threat, not the perceptual consequence (i.e., anxiety), enlarged RRFs. Hence, it is possible that the activation of the

amygdala, and midbrain pathways are related to the enlargement of RRFs (i.e. modulation of the top-down pathways). However, this hypothesis has to be explicitly tested.

### **Implications**

The results from the present study have several implications. First, because pain ratings were greater in the threat condition than during the safe condition, it further supports the notion that threat has enhancing effects on the pain system (albeit a small effect in the current study)[73]. Second, because there was an enlargement of RRFs in the context of increased sensitivity, it strengthens the argument that enlarged RRF areas are related to increased spinal nociception. Third, our results suggest that in healthy individuals, RRFs enlarge under threatening situations. This implies that RRF modulation can help protect from somatic threats, when needed. And finally, these results provide additional support for the relationship between environmental forces and amplification of pain processing, thus highlighting the significance of the biopsychosocial model [20] in the conceptualization of pain and its impact on human suffering.

### **Strengths & Limitations**

The present study has several strengths. First, this is the first study investigating emotional modulation of RRFs, thus further linking top-down modulation with protective reflexes [49; 62]. Second, a within-subject experimental design was used to reduce confounding variables and the effect of individual variability. Third, EMG signals were recorded using a double differential configuration that reduces muscle crosstalk helping to isolate the activity of tibialis anterior muscle so that RRFs can be more accurately measured [29]. Fourth, data

analysis was conducted with the support of modern outlier detection methods that maximize statistical power within small samples [75].

Nonetheless, several limitations must be noted. For one, the study was conducted on young, healthy pain-free individuals. Hence, the results may not translate to unhealthy populations such as people with chronic pain or those with affective disturbance. Future research should investigate whether the findings generalize to other populations.

Second, the effect size of the RRF change was small. Past research investigating changes in RRFs elicited by psychological constructs, resulted in effect sizes (Cohen's  $d$ ) between 0.40 and 0.71 [7]. However, past studies investigating psychological constructs that modulate the size of RRF used an experimental design in which the control condition was always before the active condition. In the current study, the control condition and the active condition were pseudorandomly ordered in such a way that the pain system, theoretically, was moving between an unsensitized and sensitized state within very short time periods (i.e., 65 s). This may have decreased the amount of change seen in the size of RRFs between the threat and safe conditions. By contrast, the small effect sizes may indicate that our threat manipulation was less effective than expected. For example, it is possible that the effect of abdominal stimulation threat waxed and waned across each period. Future studies could add a continuous measure of anxiety (e.g., using an electronic VAS) in order to detect covariation between pain, threat, and RRFs at a more specific level.

Third, although it was found that threat enlarged RRFs, it is unknown if other environmental stimuli would also modulate the size of RRFs (e.g., pleasant stimuli). It is possible that, in the current study, unmeasured psychological factors contributed to the change of RRF

sizes. Therefore, future studies should focus on investigating whether other situations modulate RRFs. Further, several studies have noted how individual difference factors (e.g., pain catastrophizing) can contribute to individual differences in affective reactions and pain modulation [19; 77]. Hence, future studies should investigate the role of these factors in threat-evoked changes in RRF.

Fourth, even though we recruited more participants than was suggested by our power analysis, this study still had a small sample. Hence, these results should be interpreted with caution until replicated.

Fifth, during threat periods participants received painful abdominal stimulations before the measurement of RRF areas. There is a possibility that CPM-like ('pain inhibits pain') mechanisms may have been activated and thus reduced the facilitating effect of threat on RRFs. Although, this may be possible, it is unlikely because CPM typically requires a tonic, long-lasting, conditioning stimulus to activate descending inhibition. In the current study the abdominal stimulations were very brief and thus unlikely to activate CPM-like inhibition [48].

In conclusion, the present study was the first to investigate the hyperalgesic effects of threat on nociceptive withdrawal reflex receptive fields (RRF). Our results indicated that threat enlarged RRF sizes, increased NWR magnitudes, and produced hyperalgesia. Enlarged RRF sizes may be a novel explanation for the emotional modulation of spinal nociception and how threatening contexts may lead to central sensitization.

### References

- [1] Adolphs R. The biology of fear. *Current Biology* 2013;23(2):R79-R93.
- [2] Andersen OK, Sonnenborg FA, Arendt-Nielsen L. Modular organization of human leg withdrawal reflexes elicited by electrical stimulation of the foot sole. *Muscle & nerve* 1999;22(11):1520-1530.
- [3] Andersen OK, Sonnenborg FA, Arendt-Nielsen L. Reflex receptive fields for human withdrawal reflexes elicited by non-painful and painful electrical stimulation of the foot sole. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* 2001;112(4):641-649.
- [4] Andersen OK, Spaich EG, Madeleine P, Arendt-Nielsen L. Gradual enlargement of human withdrawal reflex receptive fields following repetitive painful stimulation. *Brain Research* 2005;1042(2):194-204.
- [5] Apkarian AV, Bushnell MC, Schweinhardt P. Representation of pain in brain. In: SB McMahon, M Koltzenburg, I Tracey, D Turk, editors. *Wall and Melzack's Textbook of Pain*. Philadelphia, PA: Elsevier, 2013.
- [6] Apkarian AV, Bushnell MC, Treede R-D, Zubieta J-K. Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain* 2005;9(4):463-484.
- [7] Bjerre L, Andersen AT, Hagelskjær MT, Ge N, Mørch CD, Andersen O. Dynamic tuning of human withdrawal reflex receptive fields during cognitive attention and distraction tasks. *European Journal of Pain* 2011;15(8):816-821.
- [8] Bushnell MC, Čeko M, Low LA. Cognitive and emotional control of pain and its disruption in chronic pain. *Nature Reviews Neuroscience* 2013;14(7):502-511.
- [9] Carlsson K, Petersson KM, Lundqvist D, Karlsson A, Ingvar M, Öhman A. Fear and the amygdala: manipulation of awareness generates differential cerebral responses to phobic and fear-relevant (but nonfeared) stimuli. *Emotion* 2004;4(4):340.
- [10] Cervero F, Laird JM, Pozo MA. Selective changes of receptive field properties of spinal nociceptive neurones induced by noxious visceral stimulation in the cat. *Pain* 1992;51(3):335-342.
- [11] Chu KL, Faltynek CR, Jarvis MF, McGaraughty S. Increased WDR spontaneous activity and receptive field size in rats following a neuropathic or inflammatory injury: implications for mechanical sensitivity. *Neuroscience letters* 2004;372(1-2):123-126.
- [12] Cook AJ, Woolf CJ, Wall PD, McMahon SB. Dynamic receptive field plasticity in rat spinal cord dorsal horn following C-primary afferent input. *Nature* 1987;325(6100):151-153.
- [13] Cordero-Erausquin M, Inquimbert P, Schlichter R, Hugel S. Neuronal networks and nociceptive processing in the dorsal horn of the spinal cord. *Neuroscience* 2016;338:230-247.
- [14] Crews JC, Cahall MA. An investigation of the neurophysiologic mechanisms of tourniquet-related pain: changes in spontaneous activity and receptive field size in spinal dorsal horn neurons. *Regional anesthesia and pain medicine* 1999;24(2):102-109.
- [15] Davey HM, Barratt AL, Butow PN, Deeks JJ. A one-item question with a Likert or Visual Analog Scale adequately measured current anxiety. *Journal of clinical epidemiology* 2007;60(4):356-360.
- [16] Davis M, Lang PJ. Emotion. In: M Gallagher, RJ Nelson, editors. *Handbook of psychology: Biological psychology, Vol 3*. New York, NY: John Wiley & Sons, Inc, 2003. pp. 405-439.
- [17] Davis M, Walker DL, Miles L, Grillon C. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology* 2010;35(1):105-135.
- [18] Dostrovsky JO, Craig AD. Ascending Projection System. In: SB McMahon, M Koltzenburg, I Tracey, D Turk, editors. *Wall and Melzack's Textbook of Pain*. Philadelphia, PA: Elsevier, 2013. pp. 182-210.
- [19] Fillingim RB. Individual differences in pain responses. *Current rheumatology reports* 2005;7(5):342-347.

- [20] Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychological bulletin* 2007;133(4):581.
- [21] Grimby L. Normal plantar response: integration of flexor and extensor reflex components. *Journal of neurology, neurosurgery, and psychiatry* 1963;26(1):39.
- [22] Grubb B, Stiller R, Schaible H-G. Dynamic changes in the receptive field properties of spinal cord neurons with ankle input in rats with chronic unilateral inflammation in the ankle region. *Experimental brain research* 1993;92(3):441-452.
- [23] Hans J. *Clinical neuroanatomy: brain circuitry and its disorders*: Springer Science & Business Media, 2011.
- [24] Heinricher M, Fields H. Central nervous system mechanisms of pain modulation. In: SB McMahon, M Koltzenburg, I Tracey, D Turk, editors. *Wall and Melzack's Textbook of Pain*. Philadelphia, PA: Elsevier, 2013. pp. 129-219.
- [25] Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of electromyography and Kinesiology* 2000;10(5):361-374.
- [26] Hoheisel U, Mense S. Long-term changes in discharge behaviour of cat dorsal horn neurones following noxious stimulation of deep tissues. *Pain* 1989;36(2):239-247.
- [27] Hubbard CS, Ornitz EM, Gaspar JX, Smith S, Amin J, Labus JS, Kilpatrick LA, Rhudy JL, Mayer EA, Naliboff B. Modulation of nociceptive and acoustic startle responses to an unpredictable threat in men and women. *PAIN* 2011;152:1632-1634.
- [28] Hylden JL, Nahin RL, Traub RJ, Dubner R. Expansion of receptive fields of spinal lamina I projection neurons in rats with unilateral adjuvant-induced inflammation: the contribution of dorsal horn mechanisms. *Pain* 1989;37(2):229.
- [29] Jensen MB, Manresa JB, Andersen OK. A new objective method for acquisition and quantification of reflex receptive fields. *Pain* 2015;156(3):555-564.
- [30] Kawamata M, Koshizaki M, Shimada SG, Narimatsu E, Kozuka Y, Takahashi T, Namiki A, Collins JG. Changes in response properties and receptive fields of spinal dorsal horn neurons in rats after surgical incision in hairy skin. *Anesthesiology: The Journal of the American Society of Anesthesiologists* 2005;102(1):141-151.
- [31] Laird J, Cervero F. A comparative study of the changes in receptive-field properties of multireceptive and nociceptive rat dorsal horn neurons following noxious mechanical stimulation. *Journal of neurophysiology* 1989;62(4):854-863.
- [32] Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The journal of pain : official journal of the American Pain Society* 2009;10(9):895-926.
- [33] Ledoux J, Phelps E. Emotional Networks in the Brain. In: M Lewis, J Haviland-Jones, L Barrett, editors. *Handbook of Emotions*. New York: The Guilford Press, 2010. pp. 159-179.
- [34] Lundberg A. Multisensory control of spinal reflex pathways. *Progress in brain research*, Vol. 50: Elsevier, 1979. pp. 11-28.
- [35] Manresa J, Finnerup NSB, Johannesen IL, Biering-Sørensen F, Jensen TS, Arendt-Nielsen L, Andersen OK. Central sensitization in spinal cord injured humans assessed by reflex receptive fields. *Clinical Neurophysiology* 2014;125(2):352-362.
- [36] Manresa J, Jensen MB, Andersen OK. Introducing the reflex probability maps in the quantification of nociceptive withdrawal reflex receptive fields in humans. *Journal of Electromyography and Kinesiology* 2011;21(1):67-76.
- [37] Manresa J, Mørch CD, Andersen OK. Long-term facilitation of nociceptive withdrawal reflexes following low-frequency conditioning electrical stimulation: a new model for central sensitization in humans. *European Journal of Pain* 2010;14(8):822-831.

- [38] Manresa J, Neziri AY, Curatolo M, Arendt-Nielsen L, Andersen OK. Reflex receptive fields are enlarged in patients with musculoskeletal low back and neck pain. *PAIN*® 2013;154(8):1318-1324.
- [39] Manresa J, Nguyen GP, Curatolo M, Moeslund TB, Andersen OK. Probabilistic model for individual assessment of central hyperexcitability using the nociceptive withdrawal reflex: a biomarker for chronic low back and neck pain. *BMC neuroscience* 2013;14(1):110.
- [40] McMahan S, Wall P. Receptive fields of rat lamina 1 projection cells move to incorporate a nearby region of injury. *Pain* 1984;19(3):235-247.
- [41] Morris JS, Öhman A, Dolan RJ. A subcortical pathway to the right amygdala mediating “unseen” fear. *Proceedings of the National Academy of Sciences* 1999;96(4):1680-1685.
- [42] Naliboff B, Rhudy JL. Anxiety and functional pain disorders. In: EA Mayer, MC Bushnell, editors. *Functional pain syndromes: Presentation and pathophysiology*. Seattle: IASP Press, 2009.
- [43] Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. *The Neuroscientist* 2004;10(3):221-234.
- [44] Neziri AY, Haesler S, Petersen-Felix S, Müller M, Arendt-Nielsen L, Manresa J, Andersen OK, Curatolo M. Generalized expansion of nociceptive reflex receptive fields in chronic pain patients. *PAIN*® 2010;151(3):798-805.
- [45] Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, Wise R, Matthews PM, Rawlins JNP, Tracey I. Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *Journal of Neuroscience* 2001;21(24):9896-9903.
- [46] Pogatzki EM, Vandermeulen EP, Brennan TJ. Effect of plantar local anesthetic injection on dorsal horn neuron activity and pain behaviors caused by incision. *Pain* 2002;97(1-2):151-161.
- [47] Price D, Bush FM, Long S, Harkins SW. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. *Pain* 1994;56(2):217-226.
- [48] Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. *Pain* 2009;144(1-2):16-19.
- [49] Rhudy JL. Emotional Modulation of Pain. In: M al' Absi, MA Flaten editors. *The Neuroscience of Pain, Stress, and Emotion*. San Diego, CA: Academic Press, 2016. pp. 51-75.
- [50] Rhudy JL, France CR. Defining the nociceptive flexion reflex (NFR) threshold in human participants: A comparison of different scoring criteria *Pain* 2007;128:244-253.
- [51] Rhudy JL, Grimes JS, Meagher MW. Fear-induced hypoalgesia in humans: Effects on low intensity thermal stimulation and finger temperature. *Journal of Pain* 2004;5(8):458-468.
- [52] Rhudy JL, Meagher MW. Fear and anxiety: divergent effects on human pain thresholds. *Pain* 2000;84(1):65-75.
- [53] Rhudy JL, Meagher MW. The role of emotion in pain modulation. *Current Opinion in Psychiatry* 2001;14(3):241-245.
- [54] Rhudy JL, Meagher MW. Individual differences in the emotional reaction to shock determine whether hypoalgesia is observed. *Pain Medicine* 2003;4(3):244-256.
- [55] Rhudy JL, Williams AE, Dubbert PM, Parker JD, Burke RS. Affective modulation of pain in substance dependent veterans. Program No 635 2004 Abstract Viewer/Itinerary Planner 2004:Washington, DC: Society for Neuroscience, 2004. Online.
- [56] Rhudy JL, Williams AE, McCabe K, Nguyen MA, Rambo P. Affective modulation of nociception at spinal and supraspinal levels. *Psychophysiology* 2005;42:579-587.
- [57] Rhudy JL, Williams AE, McCabe KM, Maynard LJ, Russell JL. Affective modulation of spinal nociception and pain: Valence and arousal contribute. *Psychophysiology* 2006;43:S80.
- [58] Rhudy JL, Williams AE, McCabe KM, Rambo PL, Nguyen MV, Russell JL. Modulation of spinal nociception and pain by emotion: Does predictability of the noxious stimulus disengage modulation at spinal levels? American Pain Society National Conference. San Antonio, TX, 2006.

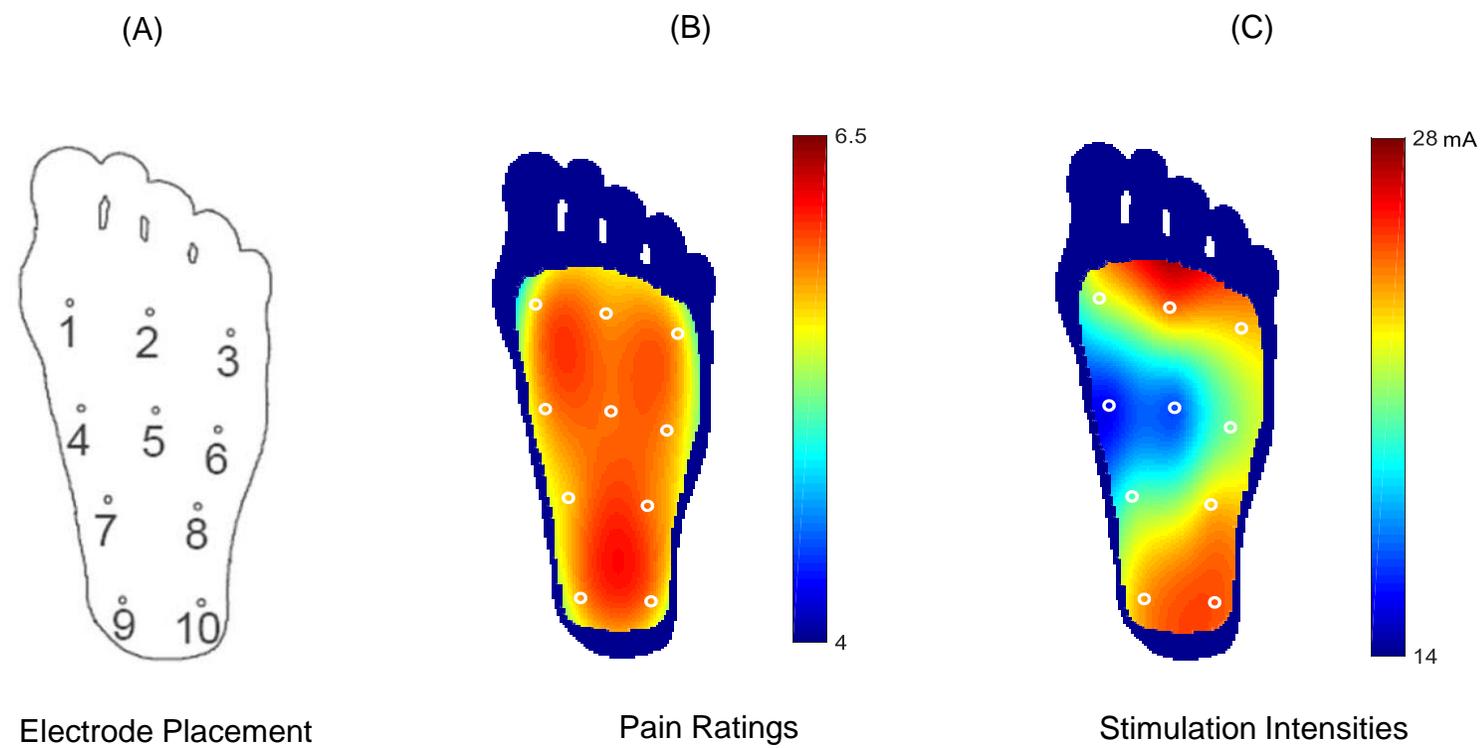
- [59] Rhudy JL, Williams AE, McCabe KM, Rambo PL, Russell JL. Emotional modulation of spinal nociception and pain: The impact of predictable noxious stimulation. *Pain* 2006;126:221-233.
- [60] Rhudy JL, Williams AE, McCabe KM, Russell JL. Emotional control of nociceptive reactions (ECON): Affective valence and arousal have independent effects across multiple response systems. *Proceedings of the American Pain Society*, 2007.
- [61] Rhudy JL, Williams AE, McCabe KM, Russell JL, Maynard LJ. Emotional control of nociceptive reactions (ECON): Do affective valence and arousal play a role? *Pain* 2008;136(3):250-261.
- [62] Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC. The lower limb flexion reflex in humans. *Progress in neurobiology* 2005;77(6):353-395.
- [63] Schouenborg J. Modular organisation and spinal somatosensory imprinting. *Brain research reviews* 2002;40(1):80-91.
- [64] Schouenborg J, Holmberg H, Weng H. Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. *Experimental brain research* 1991;90(3):469-478.
- [65] Schouenborg J, Weng H-R. Sensorimotor transformation in a spinal motor system. *Experimental brain research* 1994;100(1):170-174.
- [66] Schouenborg J, Weng H-R, Holmberg H. Modular Organization of Spinal Nociceptive Reflexes: A New Hypothesis. *Physiology* 1994;9(6):261-265.
- [67] Schouenborg J, Weng H-R, Kalliomäki J, Holmberg H. A survey of spinal dorsal horn neurones encoding the spatial organization of withdrawal reflexes in the rat. *Experimental brain research* 1995;106(1):19-27.
- [68] Schumacher R, Velden M. Anxiety, pain experience, and pain report: a signal-detection study. *Perceptual and motor skills* 1984;58(2):339-349.
- [69] Sherrington CS. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J Physiol* 1910;40(1-2):28-121.
- [70] Spaich EG, Arendt-Nielsen L, Andersen OK. Repetitive painful stimulation produces an expansion of withdrawal reflex receptive fields in humans. *Artificial Organs* 2005;29(3):224-228.
- [71] Suzuki R, Kontinen V, Matthews E, Williams E, Dickenson A. Enlargement of the receptive field size to low intensity mechanical stimulation in the rat spinal nerve ligation model of neuropathy. *Journal of the Peripheral Nervous System* 2000;5(4):248-248.
- [72] Terry EL, Thompson K, Rhudy JL. Threat-evoked pain facilitation is not influenced by experimental reductions in pain catastrophizing. *The Journal of Pain* 2015;16(4):S51.
- [73] Terry EL, Thompson K, Rhudy JL. Does pain catastrophizing contribute to threat-evoked amplification of pain and spinal nociception? *Pain* 2016;157(2):456-465.
- [74] Urch C, Donovan-Rodriguez T, Dickenson A. Alterations in dorsal horn neurones in a rat model of cancer-induced bone pain. *Pain* 2003;106(3):347-356.
- [75] Wilcox R. Numerical and Graphical Summaries of Data. *Modern Statistics for the Social and Behavioral Sciences: A Practical Introduction*. Boca Raton, FL: CRC Press, 2012.
- [76] Willer JC, Boureau F, Albe-Fessard D. Supraspinal influences on nociceptive flexion reflex and pain sensation in man. *Brain Research* 1979;179:61-68.
- [77] Winter KA, Kuiper NA. Individual differences in the experience of emotions. *Clinical psychology review* 1997;17(7):791-821.
- [78] Woolf CJ, King AE. Subthreshold components of the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat lumbar spinal cord. *Journal of neurophysiology* 1989;62(4):907-916.
- [79] Woolf CJ, King AE. Dynamic alterations in the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat spinal cord. *Journal of Neuroscience* 1990;10(8):2717-2726.

**Figure Legends**

*Figure 1.* Pre-experiment characteristics. (A) Distribution of electrodes. (B) Distribution of mean pain ratings for matched stimulation intensities. (C) Distribution of stimulation amplitudes after matching pain ratings of each site to pain ratings of 1.5 X PTh reported on site 4.

*Figure 2.* Experimental procedures to examine threat induction on nociceptive reflex receptive fields (RRF). The paradigm included 8 threat and 8 safe periods (represented by #1-16). The figure above depicts one possible sequence of periods, but periods were pseudorandomly ordered (inter-period interval 8 - 10s). Example screenshots from the threat and safe periods are shown. Participants saw text presented on a computer screen that indicated the period type (threat vs. safe). The horizontal bar at the bottom of the screen filled left-to-right as time passed in the 65 s period. Five foot stimulations were given during each period. Abdominal stimulations were given during the first 5 s of each threat period and during the last 5 s of 4 randomly chosen threat periods in order to evoke unpredictable threat. No abdominal stimulations were ever given during safe periods. Pain and nociceptive withdrawal reflexes (NWR) were measured in response to all foot stimulations. Ab=abdominal. Stim=stimulation.

*Figure 3.* Depiction of nociceptive reflex receptive fields (RRFs) during threat and safe conditions. Warmer colors represent a higher probability of having a reflex while cooler color represent a lower probability. RRFs (depicted as the areas within the black lines) are larger in the threat condition (left) than in the safe condition (right;  $p < .05$ ).



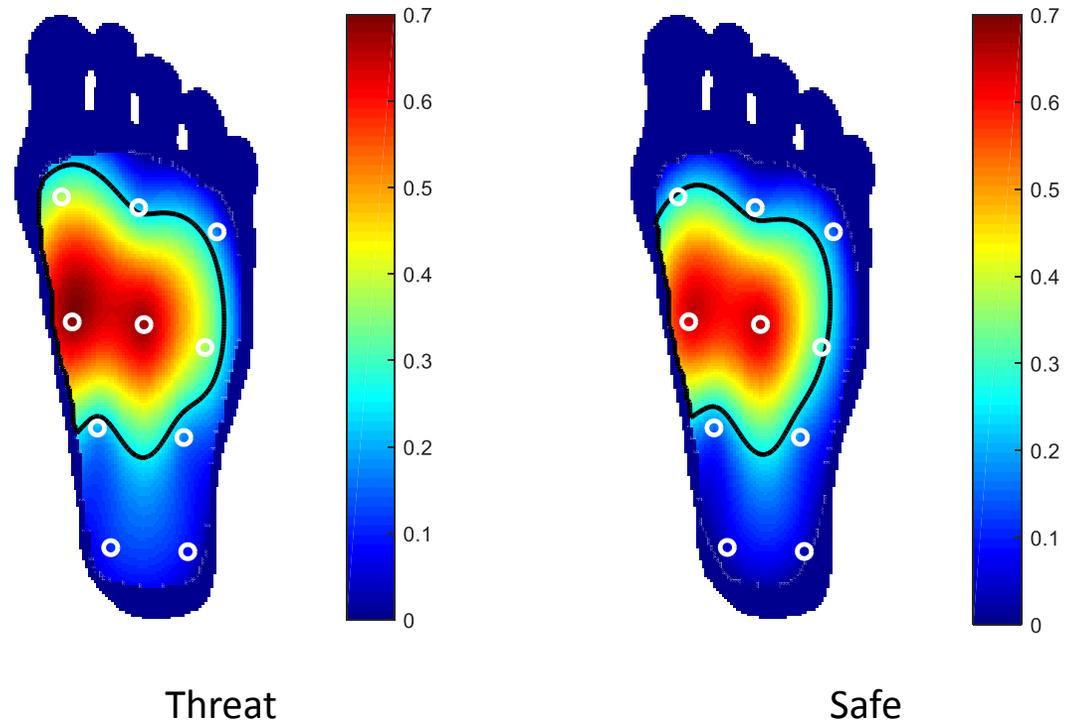
*Figure 1.* Pre-experiment characteristics. (A) Distribution of electrodes. (B) Distribution of mean pain ratings for matched stimulation intensities. (C) Distribution of stimulation amplitudes after matching pain ratings of each site to pain ratings of 1.5 X PTh reported on site 4.

- 16 periods (8 Threat/8 Safe)
- 80 foot stims
- 5 foot stim per period

- 1 abdom stim at beginning of every threat period
- 1 abdom stim at end of 4 (randomly chosen) threat periods



*Figure 2.* Experimental procedures to examine threat induction on nociceptive reflex receptive fields (RRF). The paradigm included 8 threat and 8 safe periods (represented by #1-16). The figure above depicts one possible sequence of periods, but periods were pseudorandomly ordered (inter-period interval 8 - 10s). Example screenshots from the threat and safe periods are shown. Participants saw text presented on a computer screen that indicated the period type (threat vs. safe). The horizontal bar at the bottom of the screen filled left-to-right as time passed in the 65 s period. Five foot stimulations were given during each period. Abdominal stimulations were given during the first 5 s of each threat period and during the last 5 s of 4 randomly chosen threat periods in order to evoke unpredictable threat. No abdominal stimulations were ever given during safe periods. Pain and nociceptive withdrawal reflexes (NWR) were measured in response to all foot stimulations. Ab=abdominal. Stim=stimulation.



*Figure 3.* Depiction of nociceptive reflex receptive fields (RRFs) during threat and safe conditions. Warmer colors represent a higher probability of having a reflex while cooler color represent a lower probability. RRFs (depicted as the areas within the black lines) are larger in the threat condition (left) than in the safe condition (right;  $p < .05$ ).

Table 1. Means and SDs for Anxiety, Pain, and RRFs during the Threat and Safe Periods

	<u>Threat</u> Mean ( <i>SD</i> )	<u>Safe</u> Mean ( <i>SD</i> )	<i>t</i>	<i>df</i>	<i>p</i>	Cohen's <i>d</i>
Anxiety (1-5; Ratings)	2.51 (1.00)	1.51 (0.53)	6.19	24	<.001	1.31
Pain Ratings (0-10; Ratings)	4.86 (1.04)	4.71 (0.95)	2.31	20	0.03	0.14
RRF Area (0-1; Percent)	0.47 (0.29)	0.41 (0.29)	2.78	19	0.01	0.20

*Note.* RRF=Reflex receptive field