WHEN YOU GIVE A MOUSE A CENTRALIZED PAIN SYNDROME: THE PERIL AND POWER OF ANIMAL MODELS

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PAIN

An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.*

*The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. Biologists recognize that those stimuli which cause pain are liable to damage tissue. Accordingly, pain is that experience we associate with actual or potential tissue damage. It is unquestionably a sensation in a part or parts of the body, but it is also always unpleasant and therefore also an emotional experience. Experiences which resemble pain but are not unpleasant, e.g., pricking, should not be called pain. Unpleasant abnormal experiences (dysesthesias) may also be pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons (BS). There is usually no way to distinguish their experience from that due to tissue damage if we take the subjective report. If they regard their experience as pain, and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition avoids tying pain to the stimulus. Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause.
MOUSE PAIN SCALE

0 1 2 3 4 5 6 7 8 9 10
HOW “PAIN” IS MEASURED

Evoked measures

• Withdrawal to a stimulus
  • Mechanical
  • Thermal
Evoked measures

- Withdrawal to a stimulus
  - Mechanical
  - Thermal
- Behavioral responses
  - Guarding
  - Physiological
  - Preference
  - Grimace
HOW “PAIN” IS MEASURED

**Evoked measures**
- Withdrawal to a stimulus
  - Mechanical
  - Thermal
- Behavioral responses
  - Guarding
  - Physiological
  - Preference
  - Grimace

**Passive measures**
- Preference
- Exercise
- CPP
- Reward
HOW "PAIN" IS INDUCED

Neuropathic pain
Pain that arises from damage to the nerve, rather than from activation of pain receptors.
- Nerve injury
- Chemical
  - CIPN
  - DN

Inflammatory pain
Pain that arises from sensitization of nociceptors by immune mediators.
- Chemical

Centralized pain
Pain is widespread and accompanied by fatigue, sleep, memory, and/or mood difficulties.
- Stress
- Sedentary caging
- Diet
STRESS

PAIN
Two-thirds reported at least 1 ACE

↑ ACE score  ↑ Morbidity and mortality

# of ACES

- ZERO: 36%
- ONE: 26%
- TWO: 16%
- THREE: 9.5%
- FOUR OR MORE: 12.5%

**ACES AND CHRONIC PAIN**

**Fibromyalgia**

Significant correlation between the number of ACEs and occurrence of fibromyalgia


**Headache**

Prevalence and risk of frequent headache increased with ACE score.

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Normal conditions

Acute stressor

Hypothalamus

CRF

Pituitary gland

ACTH

Adrenal gland

GC

Hippocampus

Sp, CGRP

NGF, tryptase

MC

SP, CGRP

NGF, tryptase

Hypothalamic-Pituitary-Adrenal Axis

Eller-Smith, Nicol, & Christianson, Front Cell Neurosci, 2018
HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Normal conditions

Acute stressor

Sensitized condition

Chronic Stress

Hippocampus

CRF

Hypothalamus

Pituitary gland

Adrenal gland

GC

SP, CGRP

NGF, tryptase

MC

GR ↓

BDNF ↓

CRF ↑

MC

Hippocampus

GR ↓

BDNF ↓

CRF ↑

MC

SP, CGRP

NGF, tryptase

Adrenal gland

GC

SP, CGRP

NGF, tryptase

Adrenal gland

Eller-Smith, Nicol, & Christianson, Front Cell Neurosci, 2018
EARLY LIFE STRESS PARADIGM

Neonatal Maternal Separation

P1 - P21

34°C
50% humidity
3h/day × 21 days

Fuentes et al., JoVE, 2015.
EARLY LIFE STRESS PARADIGM

NMS  

• Pelvic organ sensitivity
• Comorbidities
• HPA axis regulation

Exercise

P1 P21 P22 P28 →

Fuentes et al., JoVE, 2015.
Pelvic organ distension
- Electromyographic (EMG) recordings of abdominal muscles.
- Reflexive contraction termed the visceromotor reflex (VMR).

Perigenital mechanical sensitivity
- Calculate the mechanical threshold required to elicit a positive response.

PELVIC ORGAN SENSITIVITY

**Female mice**

**Vagina**

![Graph showing sensitivity in female vagina](image)

**Bladder**

![Graph showing sensitivity in female bladder](image)

**Colorectum**

![Graph showing sensitivity in female colorectum](image)

**Male mice**

**Perigenital**

![Graph showing sensitivity in male perigenital](image)

**Colorectum**

![Graph showing sensitivity in male colorectum](image)


Brackets: NMS ($p < 0.05$), two-way RM ANOVA; *, **, *** $p < 0.05, 0.01, 0.001$ vs. naïve, Bonferroni posttest.
Female mice

Early exercise prevents the onset of urinary bladder hypersensitivity.

Pierce et al., Neurourol Urodyn, 2018.
Female mice

Void frequency was increased at 8 weeks in sedentary NMS mice. Exercise increased void size only in naïve mice.
Voluntary exercise prevents perigenital mechanical hypersensitivity in male NMS mice.

Fuentes et al., manuscript in preparation.
Bracket: exercise († p<0.05), NMS x exercise (& p<0.05), two-way ANOVA; **p<0.01 vs. naïve, # p<0.05 vs. sedentary; Fisher's LSD posttest.
Void frequency and total output was increased at 8 weeks in sedentary NMS male mice.

No difference between exercised naïve and NMS mice.

Fuentes et al., manuscript in preparation. Bracket: NMS x exercise (& $p<0.05$), two-way ANOVA; *$p<0.05$ vs. naïve, Fisher's LSD posttest.
COMORBIDITIES

Mood disorder
- Anxiety
- Panic disorder
- Depression

Widespread pain
- Fibromyalgia
- Migraine
- Temporomandibular disorder

Metabolic disorder
- Obesity
- Glucose intolerance
- Low aerobic capacity
MOOD DISORDER

Sucrose preference test

Mice given two identical water bottles (drinking water and 1% sucrose) to measure pleasure-seeking behaviors: Anhedonia.
Female mice

Sedentary NMS females have higher sucrose preference at 8 weeks of age than naïve. Decreased sucrose preference correlates with increased micturition frequency (within a “normal” range).

Pierce et al., Neurourol Urodyn, 2018.

Two-way ANOVA; * p<0.05 vs. naïve; Fisher’s LSD posttest. NMS-Sed (<5): r²=-0.8190, p=0.0069; NMS-Ex: r²=-0.8715, p=0.0237; Pearson’s correlation.

ANHEDONIA
Both female and male NMS mice run a shorter distance than naïve mice. Anhedonia or low aerobic capacity?

Pierce et al., *Neurourol Urodyn*, 2018; Fuentes et al., manuscript in preparation.

Brackets: time († *p* < 0.01), two-way RM ANOVA; *p* < 0.05 vs. naïve, Bonferroni posttest.
Sedentary NMS females display significant anxiety prior to VBD. Pelvic organ distension decreases anxiety-like behavior in NMS male and female mice.
Female NMS mice display thermal and mechanical hypersensitivity.

Male NMS mice display stress-dependent thermal and mechanical hypersensitivity.

Pierce et al., Neurosci, 2014; Fuentes et al., IBRO Rep, 2016.

Brackets: NMS (*, ** p<0.05, 0.01), NMSxCRD (+ p<0.05), two-way ANOVA; *, **, ***, **** p<0.05, 0.01, 0.001, 0.0001 vs. naïve; Bonferroni's posttest.
Exercise normalizes forepaw mechanical sensitivity in female NMS mice.

Sedentary behavior may contribute towards mechanical allodynia.

Eller et al., manuscript in preparation.

Brackets: exercise († p < 0.0001), time x exercise (& p < 0.05), two-way RM ANOVA;

***p < 0.001 vs. naïve, #, #### p 0.01, 0.0001 vs. sedentary, ‡‡, ‡‡‡‡ p 0.01, 0.0001 vs. BL, Bonferroni posttest.
Female sedentary NMS mice are significantly heavier and have more body fat than naïve or exercised NMS mice.
Female sedentary NMS mice have heavier fat pads than naïve or exercised NMS mice.

Eller-Smith, manuscript in preparation.
Male sedentary NMS mice trend toward greater body weight and fat, exercise significantly lowers both.
3h/day × 21 days
34°C
50% humidity

Weaned and group housed with littermates

NMS

High fat/high sucrose vs. control chow

Exercise wheels vs. sedentary

Body weight and composition
Perigenital sensitivity

P1  P21
4 wk  14 wks  16 wks  27 wks
All mice gained more weight on the Western diet.

Exercise lowered body fat in control-fed, greater effect on naïve mice.

Exercise was not protective of NMS HF-fed mice.

Eller-Smith, manuscript in preparation.

Brackets: exercise (†, †††† p<0.05, 0.0001), diet (δδδδ p<0.0001), exercise x diet (& p<0.05), two-way ANOVA; * p<0.05 vs. naïve; σ p<0.05 vs. sedentary; # p<0.01 vs. control; Fisher’s LSD posttest.
Glucose tolerance

Stratified effect of stress, exercise, and diet on glucose tolerance.

Exercise was not as protective in NMS mice.

Eller-Smith, manuscript in preparation.

Brackets: NMS (§§§§ p<0.0001), exercise (†††† p<0.0001), diet (δδδδ p<0.0001), interaction (& p<0.05), two-way ANOVA; 
* p<0.05 vs. naïve; σ p<0.05 vs. sedentary; # p<0.01 vs. control; Fisher’s LSD posttest.
Unlike metabolic measures, exercise was protective ONLY for NMS.

This is why animal models are perilous!!

Eller-Smith, manuscript in preparation.

Brackets: NMS (§ p<0.05), diet (δ p<0.05), interaction (& p<0.05), two-way ANOVA; * p<0.05 vs. naïve; σ p<0.05 vs. sedentary; # p<0.01 vs. control; Fisher’s LSD posttest.
**Therapeutic Interventions**

**Exercise**
- Cognitive Behavioral Therapy

**Hypothalamus**
- CRF
- ACTH
- SP, CGRP
- NGF, tryptase

**Pituitary gland**
- ACTH

**Adrenal gland**
- GC

**Hippocampus**
- Dendritic complexity ↑
- BDNF ↑
- Gray matter ↑

**Gray matter ↑**
- Somatosensory cortex
- PFC
- Amygdala

**iFC**
- MC

**THERAPEUTIC INTERVENTION**

Eller-Smith, Nicol, & Christianson, *Front Cell Neurosci*, 2018
• Keep in mind the etiology
  • The same endings don’t necessarily have the same beginning
• Don’t over-interpret the behavior
  • Allodynia ≠ ongoing pain
  • Grimace is a mystery
    • (Exercise gives mice RBF)
• Look for clinically-relevant comorbidities
  • More is good
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