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

Julie A. Christianson, PhD
Associate Professor and Director of Graduate Studies
Departments of Anatomy & Cell Biology and Anesthesiology
University of Kansas Medical Center

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

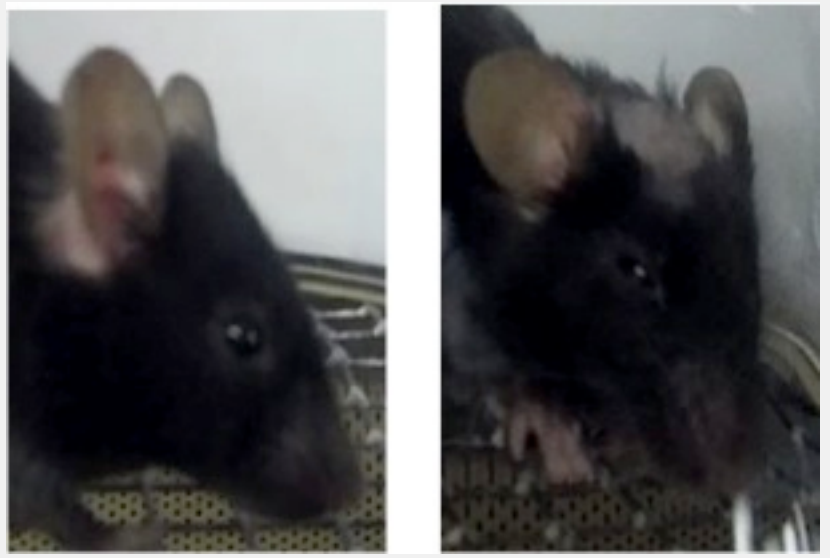
<u>Evoked measures</u>

- Withdrawal to a stimulus
  - Mechanical
  - Thermal

HOW "PAIN" IS MEASURED

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

Pa
se s
by
•

Centralized pain

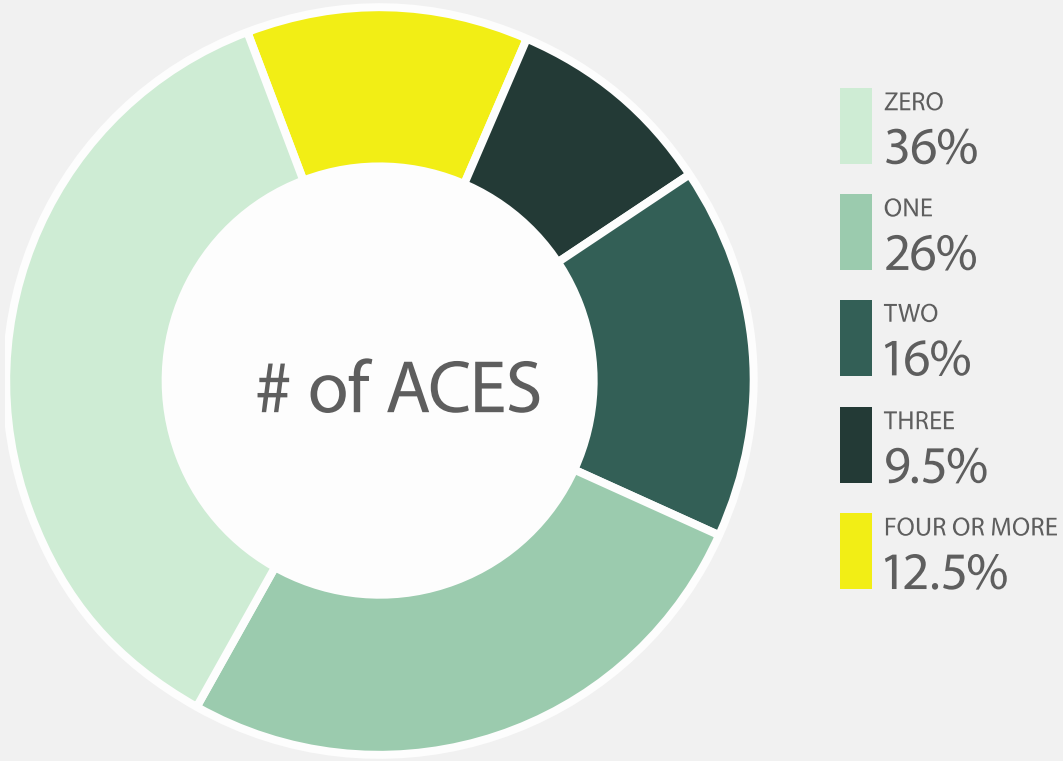
Pain is widespread and accompanied by fatigue, sleep, memory, and/or mood difficulties.

- Stress
- Sedentary caging
- Diet

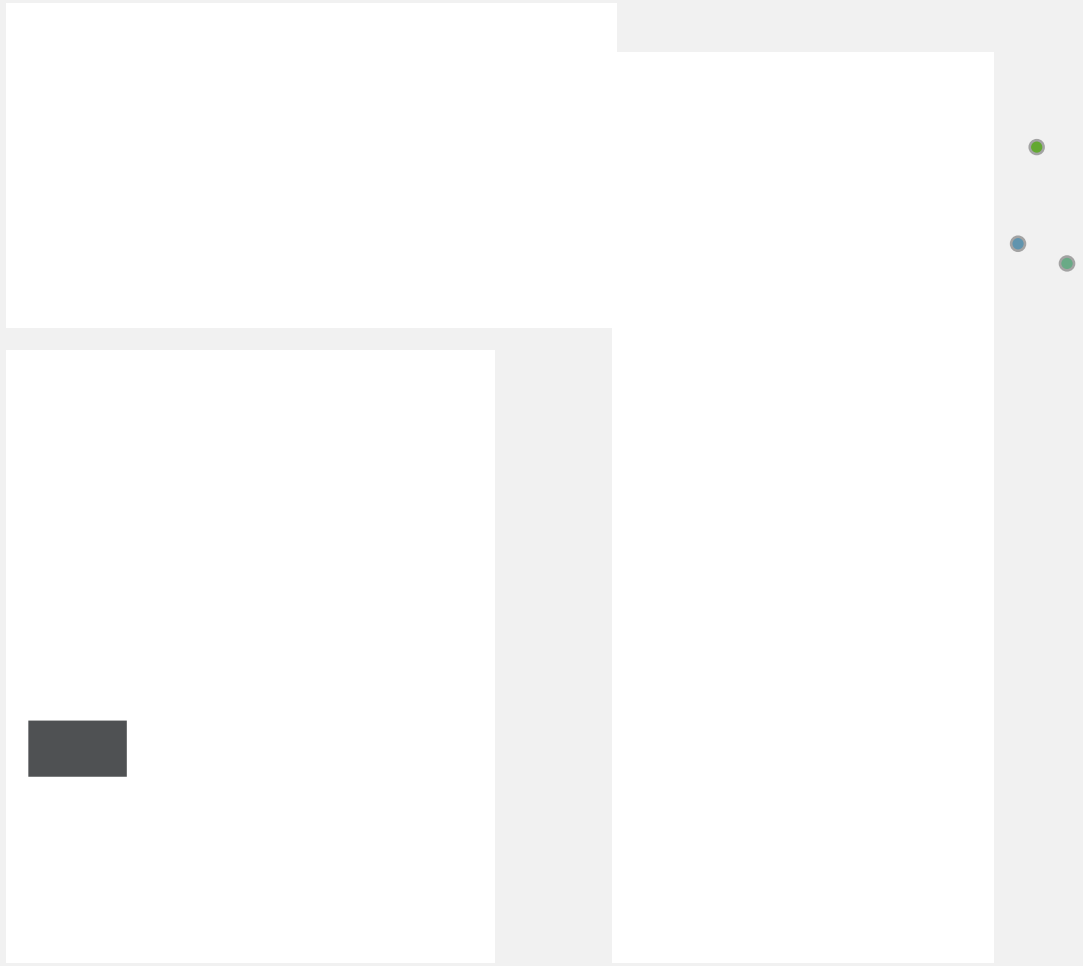
STRESS
PAIN

2012 KAISER ADVERSE CHILDHOOD EXPERIENCES (ACES) STUDY

Two-thirds reported at least 1 ACE



↑ ACE score ↑ Morbidity and mortality

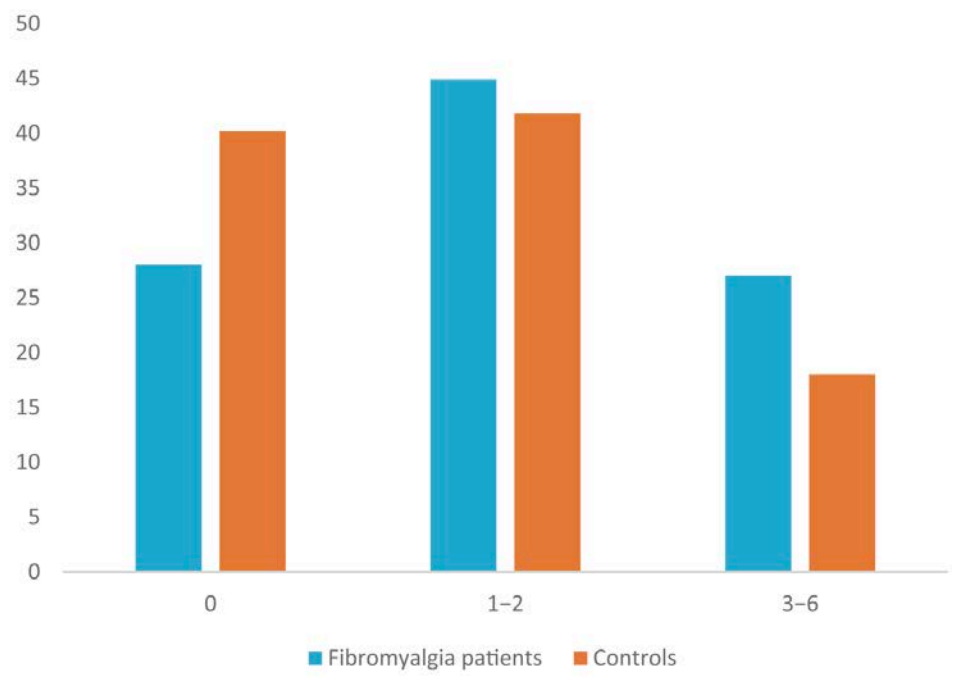


ACE study, *US Dept of HHS* 2012.

ACES AND CHRONIC PAIN

Fibromyalgia

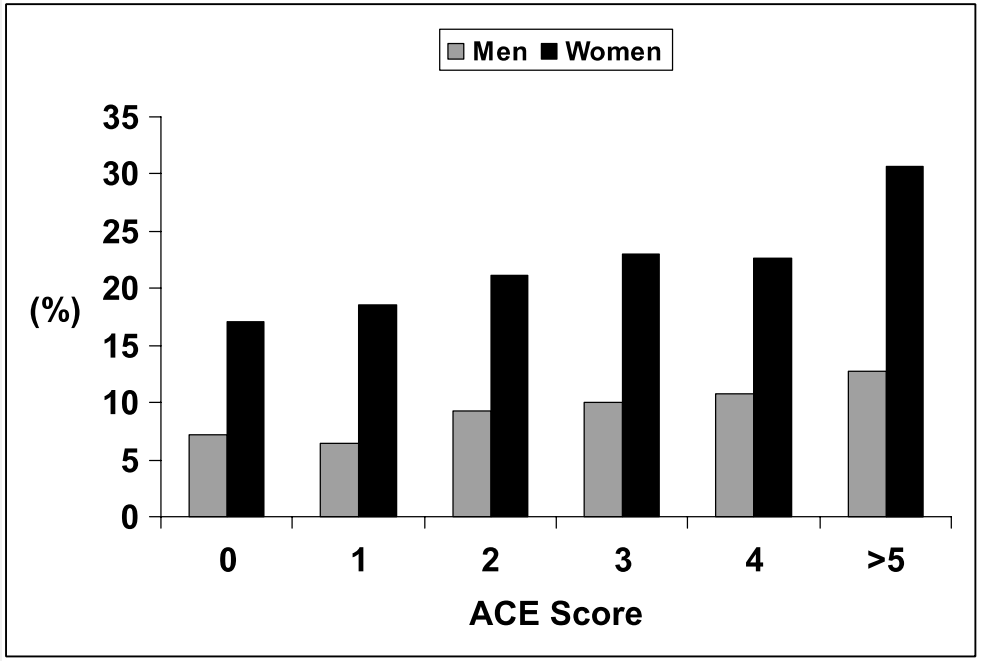
Significant correlation between the number of ACEs and occurrence of fibromyalgia



Varinen et al., *J Psychosom Res*, 2017.

Headache

Prevalence and risk of frequent headache increased with ACE score.

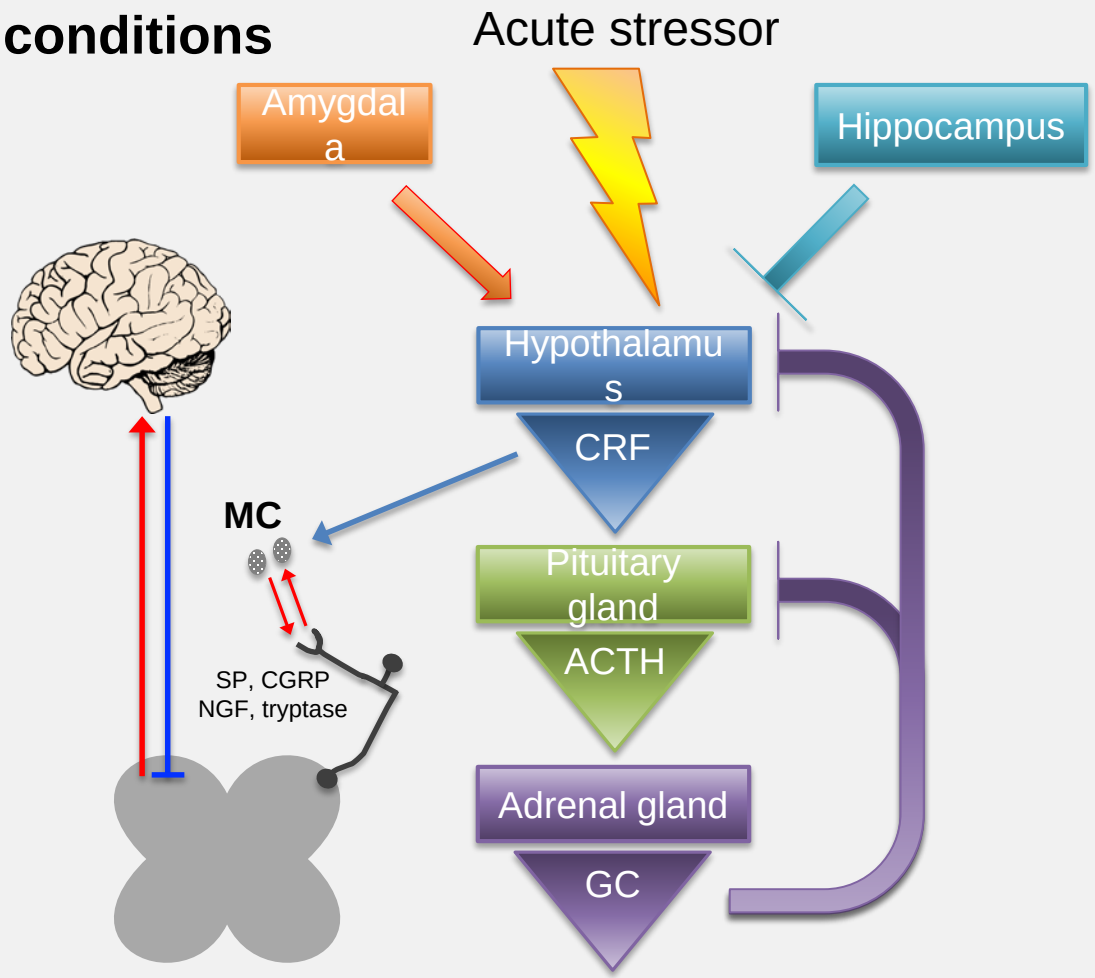


Anda et al., *Headache*, 2010.

frontiers
in Cellular Neuroscience

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Normal conditions

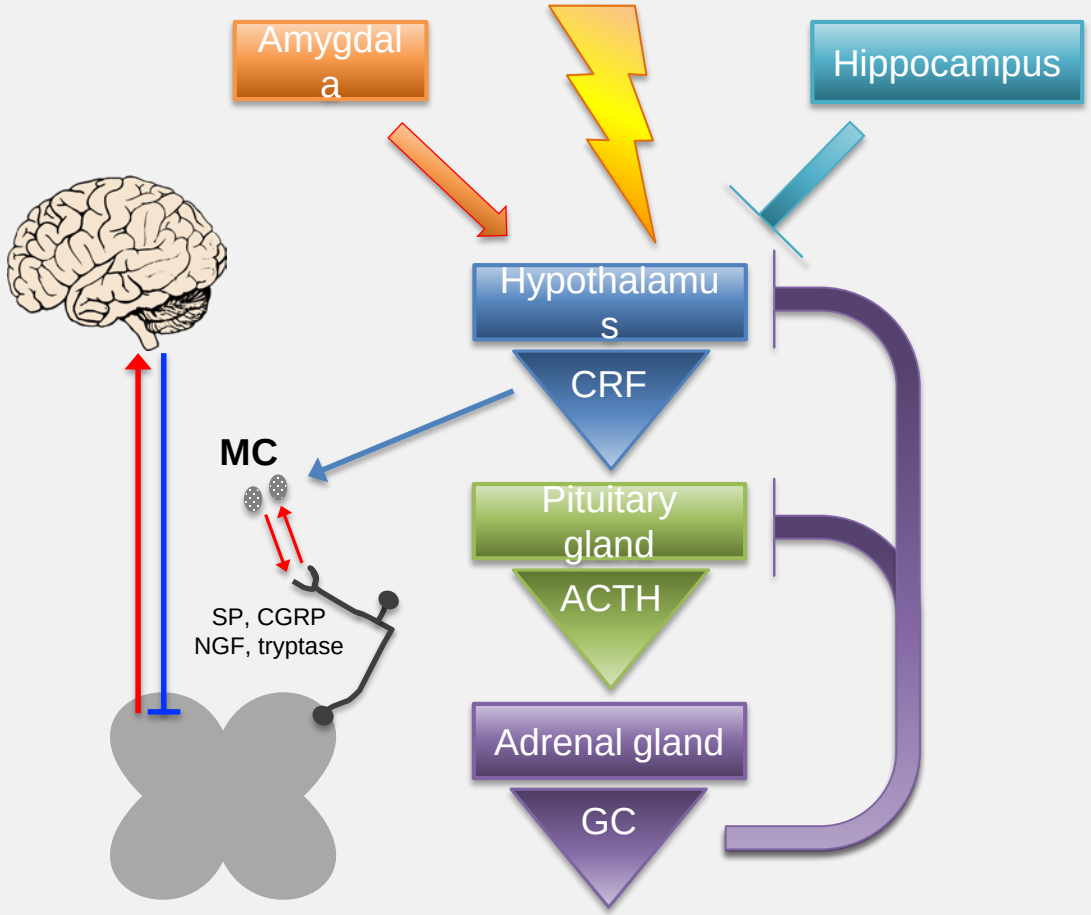


Eller-Smith, Nicol, & Christianson, *Front Cell Neurosci*, 2018

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

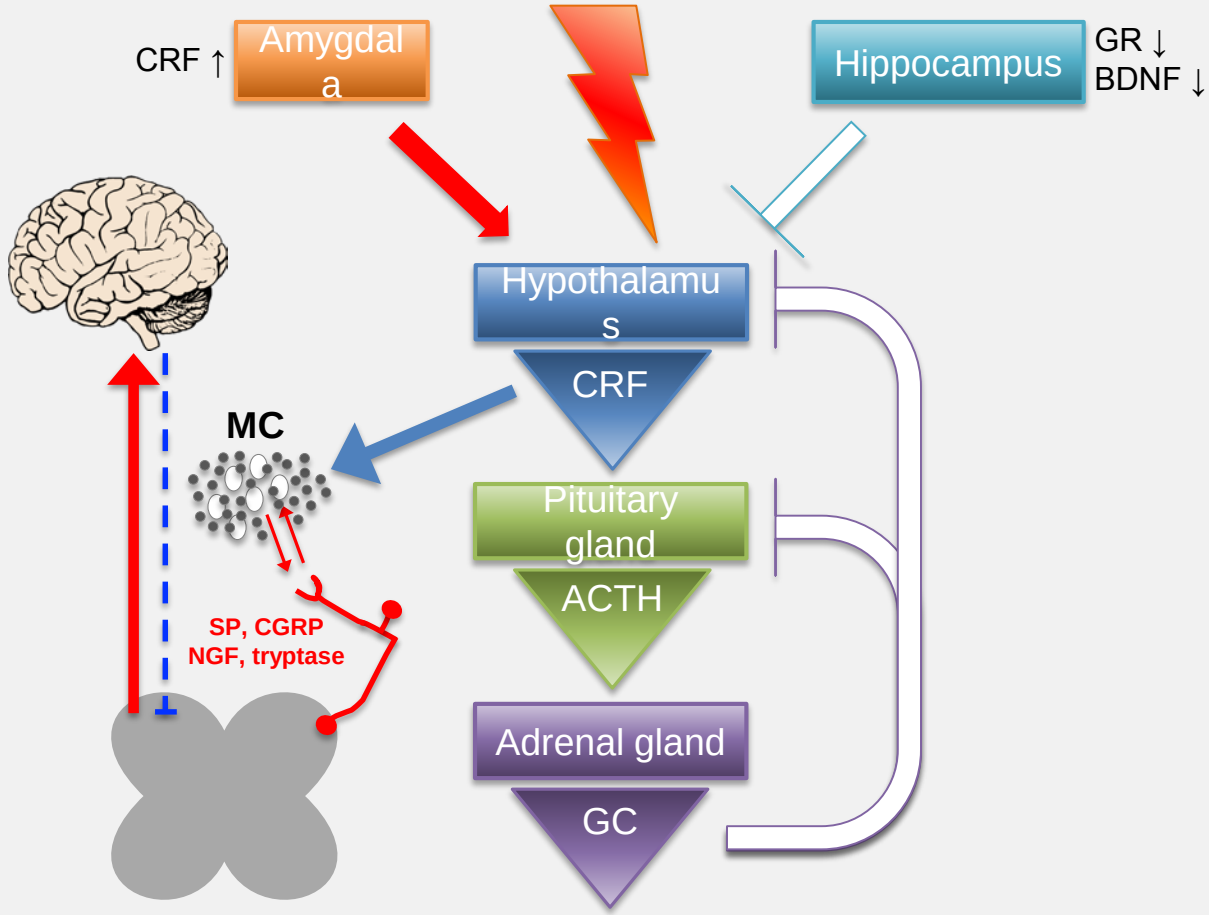
Normal conditions

Acute stressor



Sensitized condition

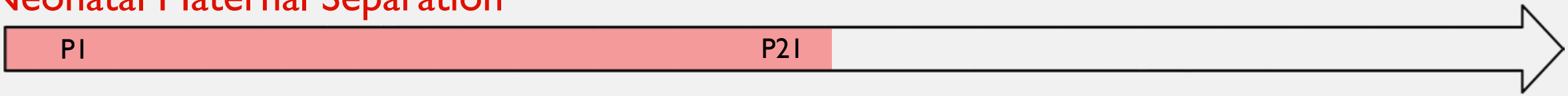
Chronic Stress

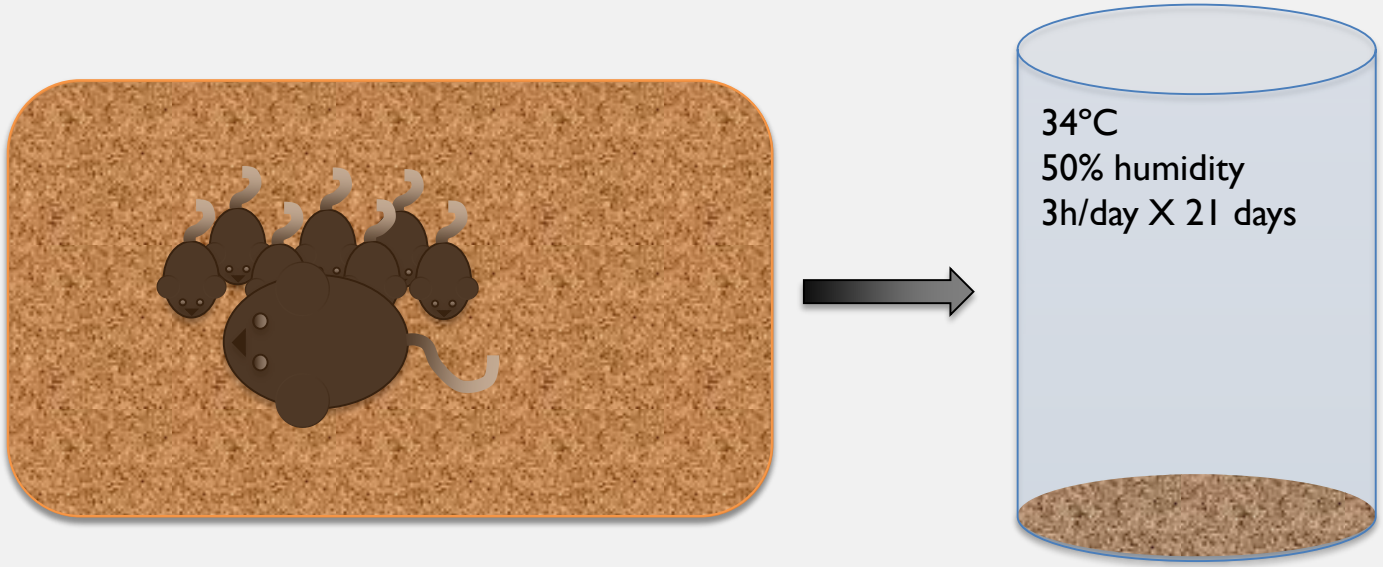


Eller-Smith, Nicol, & Christianson, *Front Cell Neurosci*, 2018

EARLY LIFE STRESS PARADIGM

Neonatal Maternal Separation





Fuentes et al., *JoVE*, 2015.

EARLY LIFE STRESS PARADIGM

NMS

Exercise

P1 P21 P22 P28 ⇢

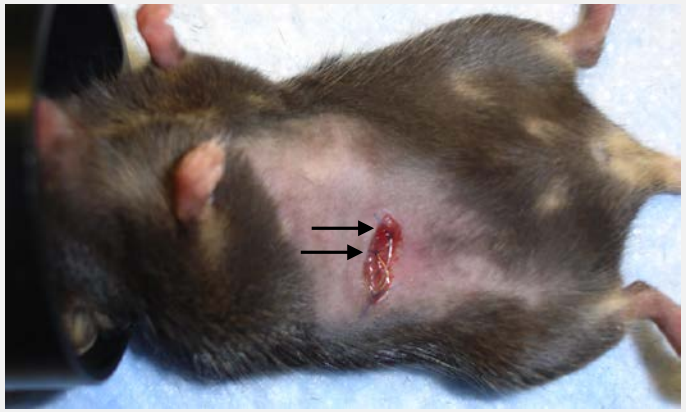
- Pelvic organ sensitivity
- Comorbidities
- HPA axis regulation

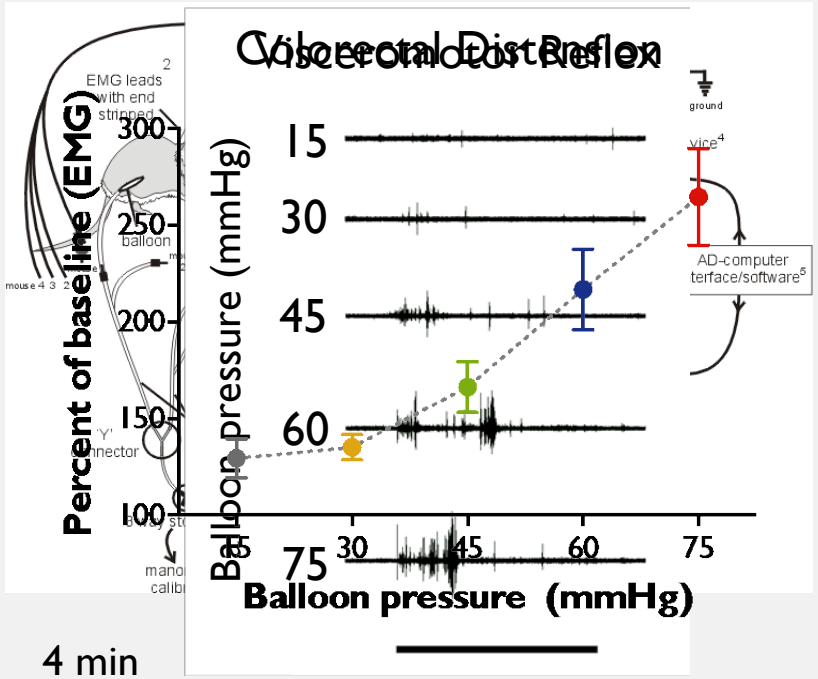
Fuentes et al., *JoVE*, 2015.

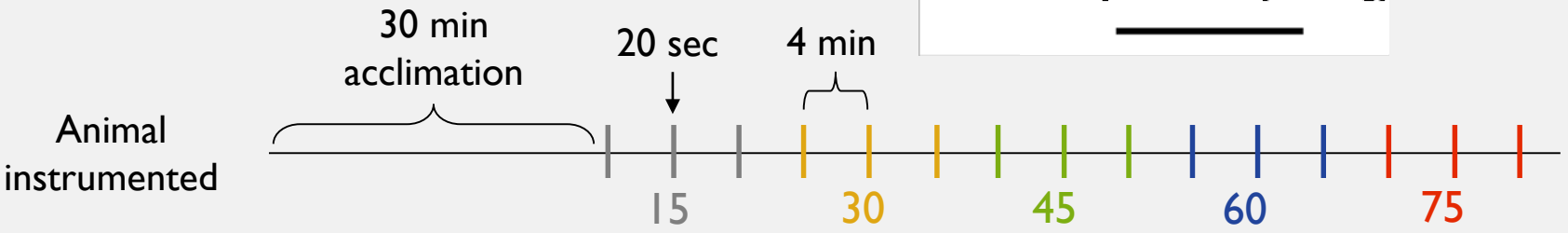
PELVIC ORGAN SENSITIVITY

Pelvic organ distension

- Electromyographic (EMG) recordings of abdominal muscles.
- Reflexive contraction termed the visceromotor reflex (VMR).







Perigenital mechanical sensitivity

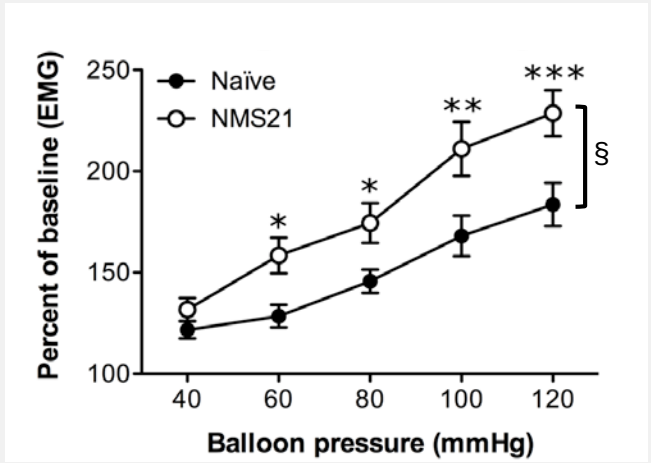
- Calculate the mechanical threshold required to elicit a positive response.

Adapted from Christianson and Gebhart, Nature Protocols, 2007.

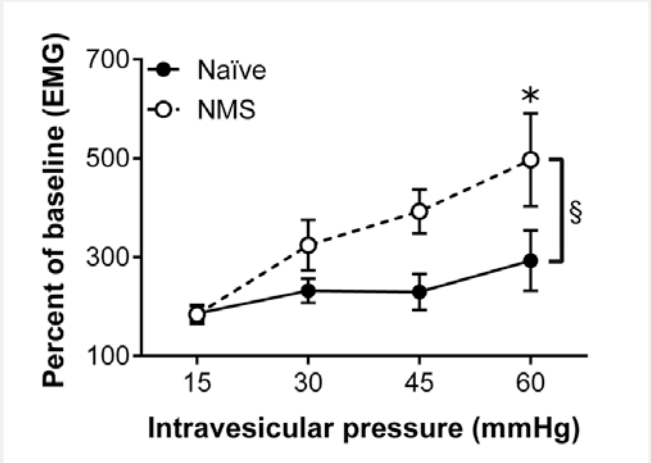
PELVIC ORGAN SENSITIVITY

Female mice

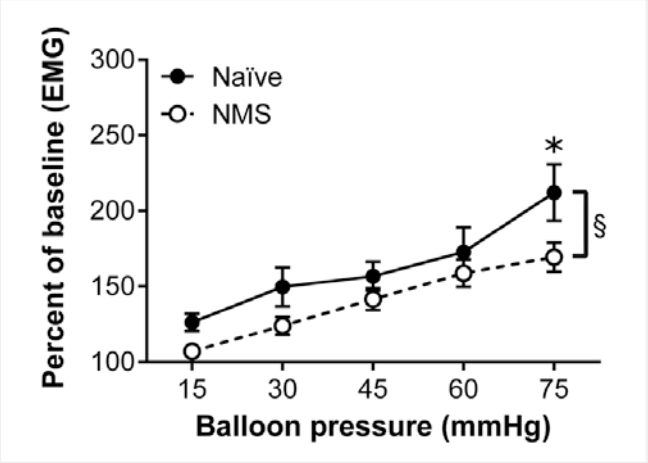
Vagina



Bladder

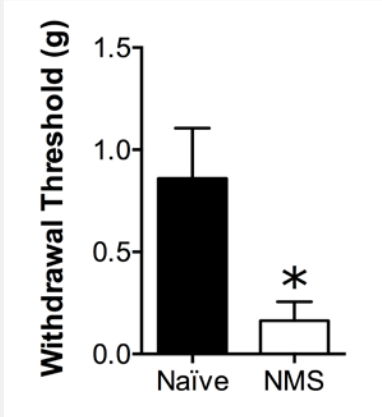


Colorectum

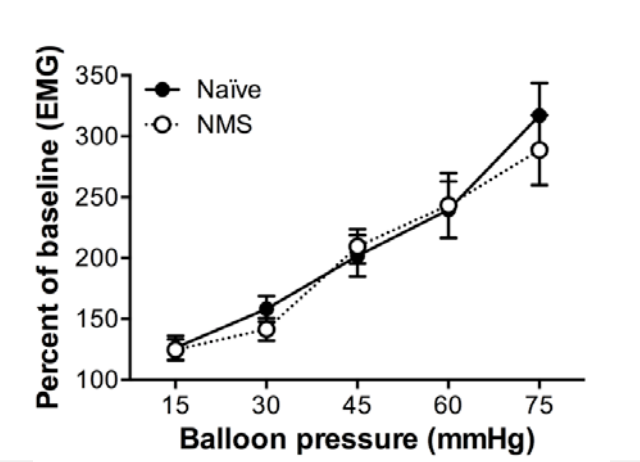


Male mice

Perigenital



Colorectum

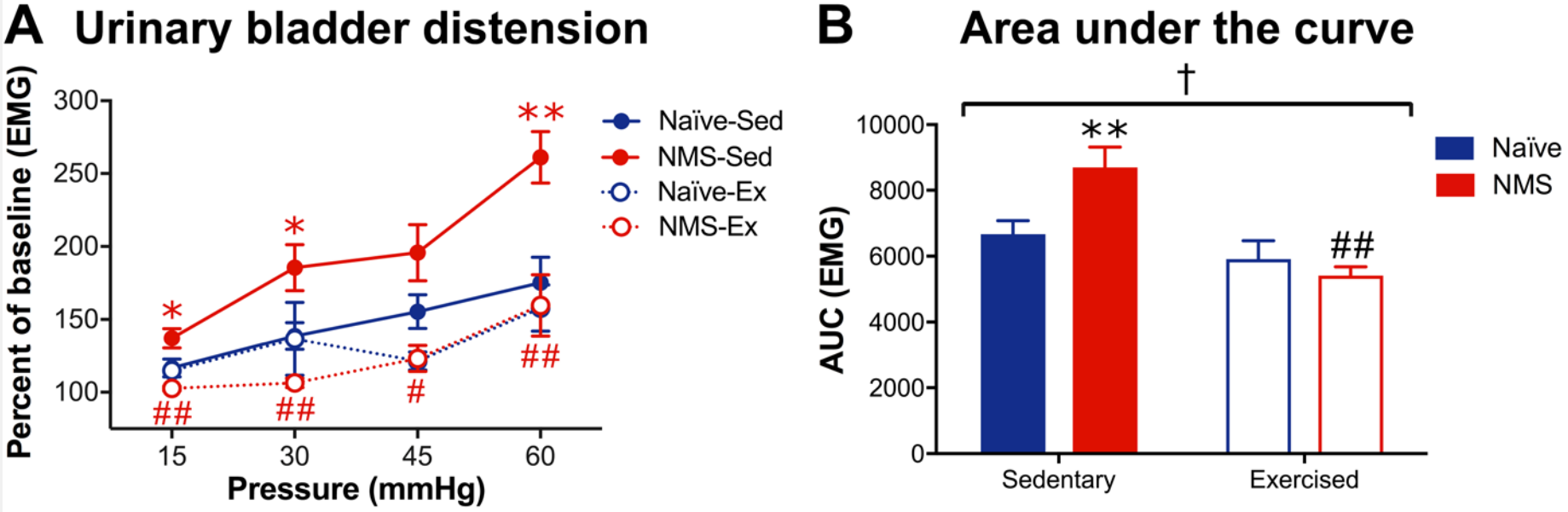


Pierce et al., 2014, 2016; Fuentes et al., 2015, 2016, 2018.

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

PELVIC ORGAN SENSITIVITY

Female mice



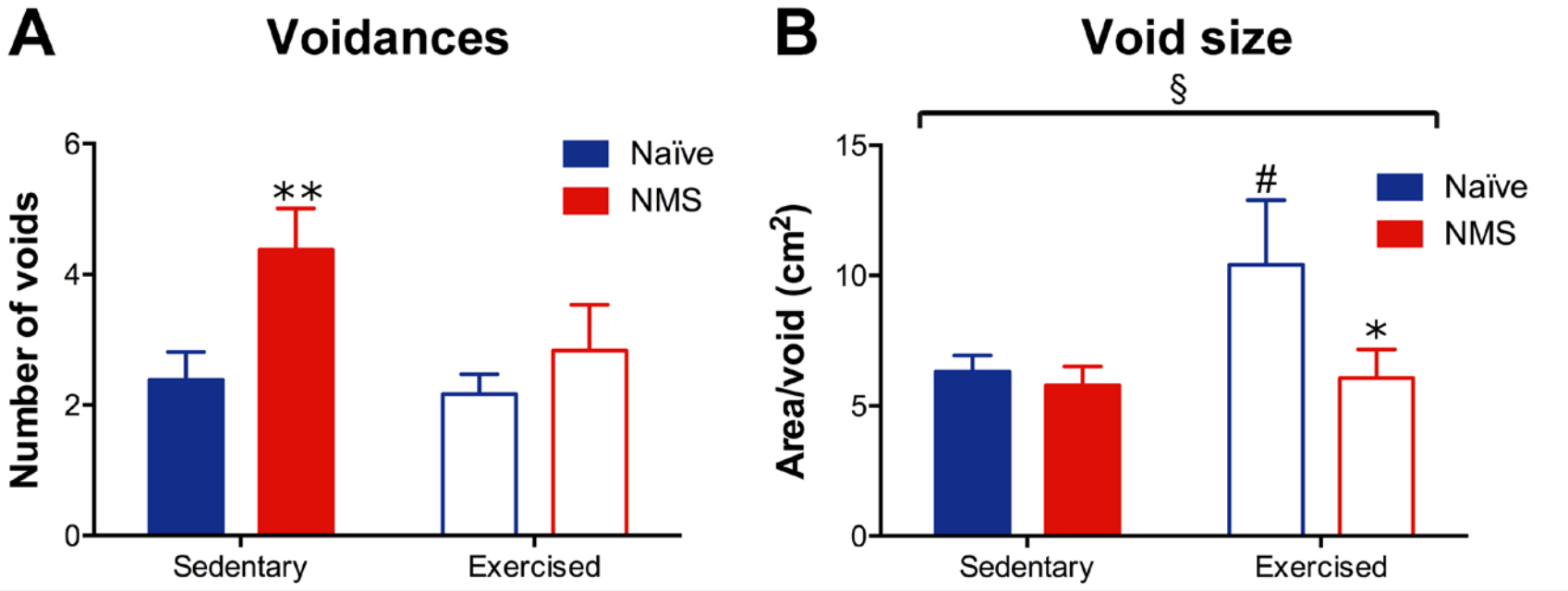
Early exercise prevents the onset of urinary bladder hypersensitivity.

Pierce et al., *Neurourol Urodyn,* 2018.

Brackets: exercise († $p<0.05$), two-way ANOVA. *, **, *** $p<0.05, 0.01, 0.001$ vs. naïve; #, ## $p<0.05, 0.01$ vs. sedentary; Fisher's LSD posttest

PELVIC ORGAN FUNCTION

Female mice



Void frequency was increased at 8 weeks in sedentary NMS mice.

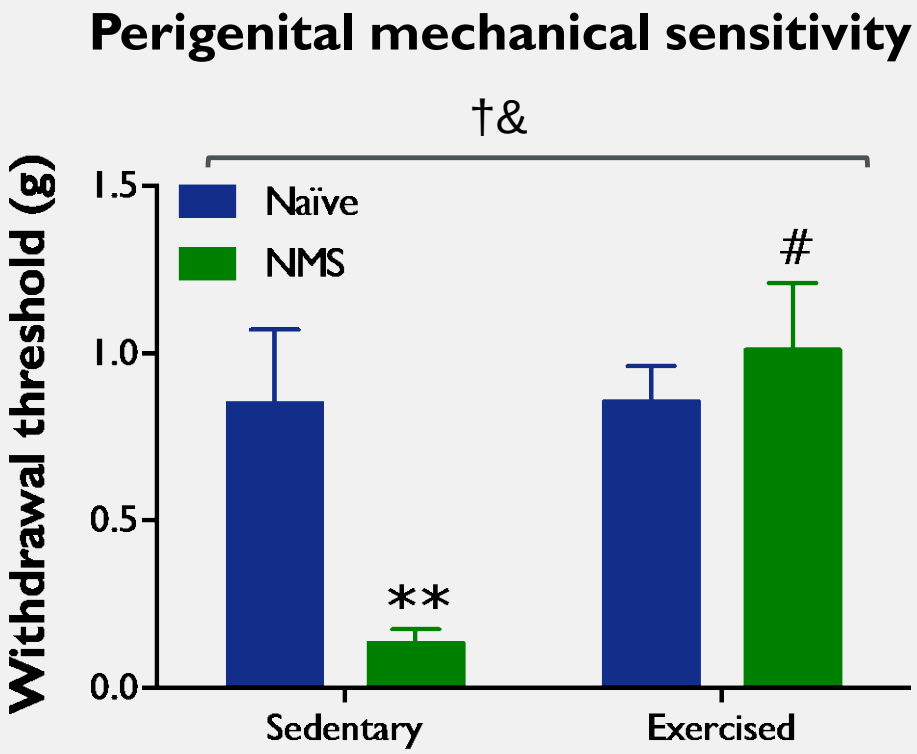
Exercise increased void size only in naïve mice.

Pierce et al., *Neurourol Urodyn,* 2018.

Brackets: NMS (§ $p<0.05$), two-way ANOVA; *, ** $p<0.05$, 0.01 vs. naïve; # $p<0.05$ vs. sedentary; Fisher's LSD posttest.

PELVIC ORGAN SENSITIVITY

Male mice



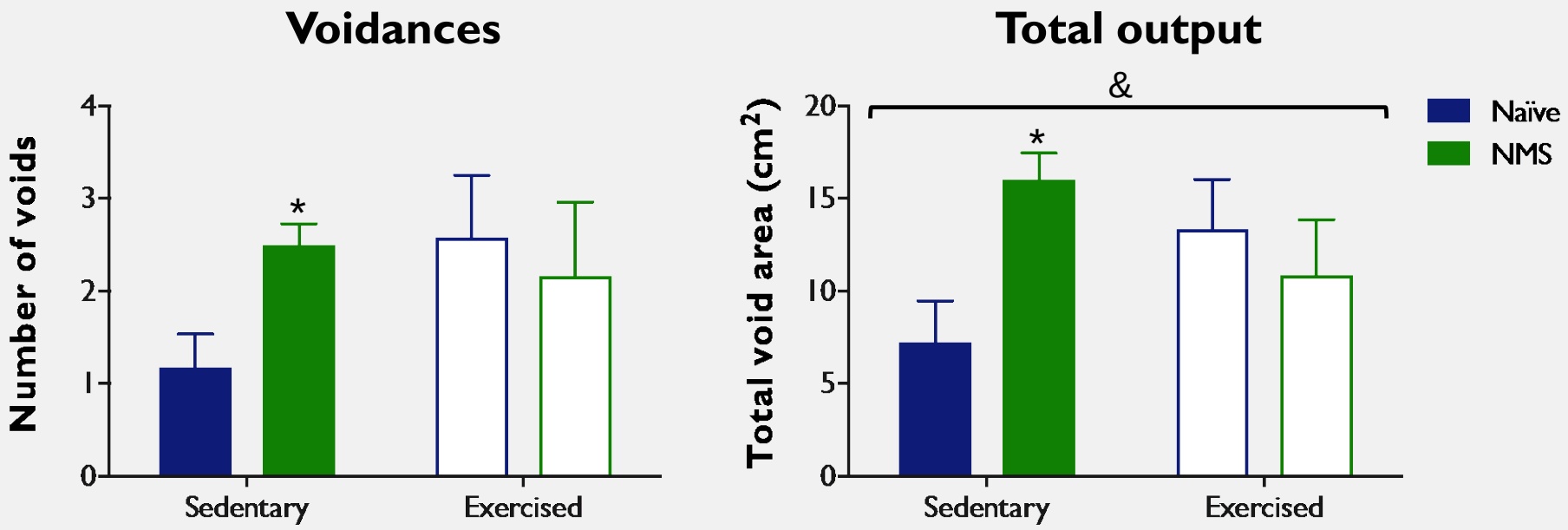
Voluntary exercise prevents perigenital mechanical hypersensitivity in male NMS mice.

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.

PELVIC ORGAN FUNCTION

Male mice

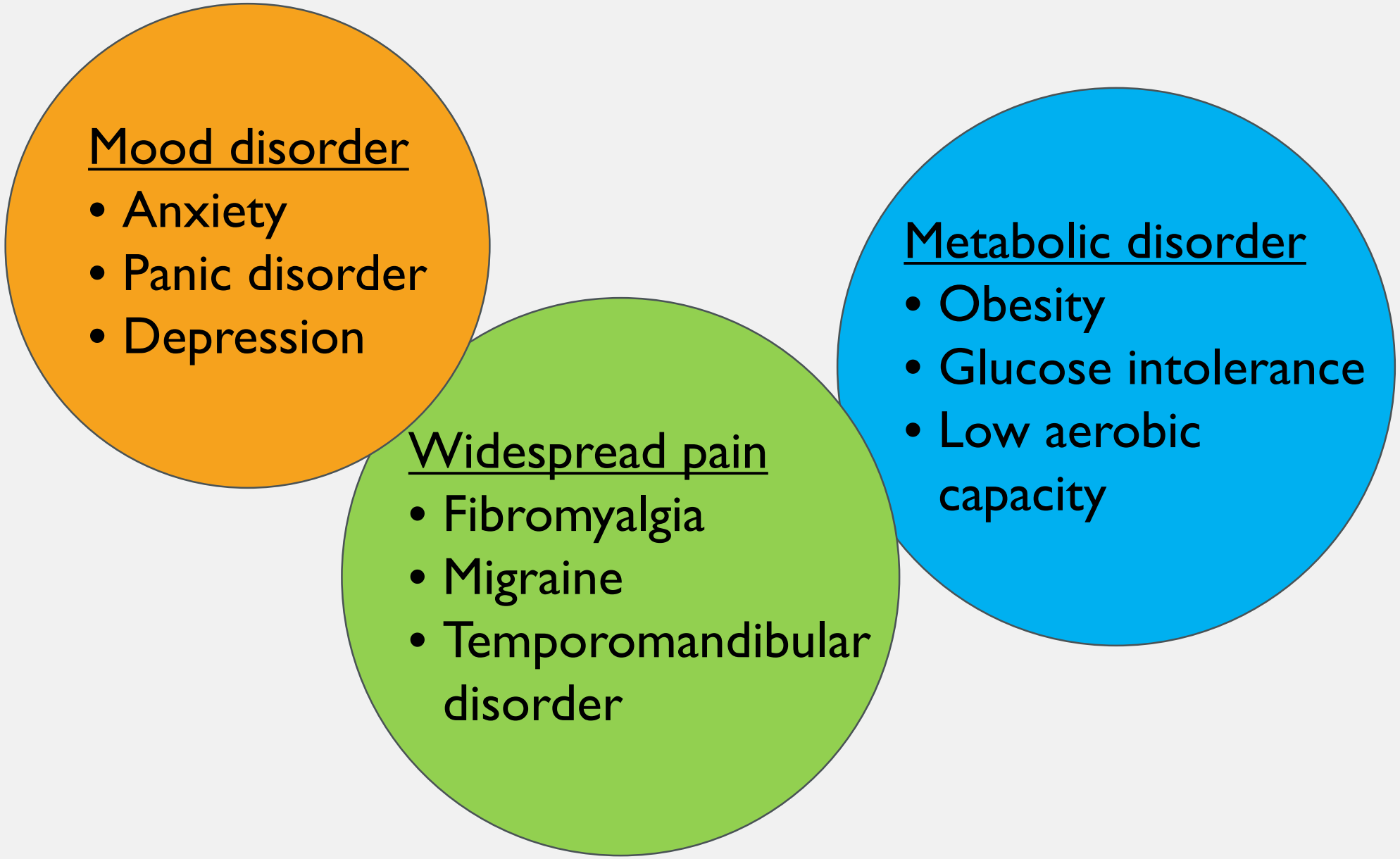


Void frequency and total output was increased at 8 weeks in sedentary NMS male mice.

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



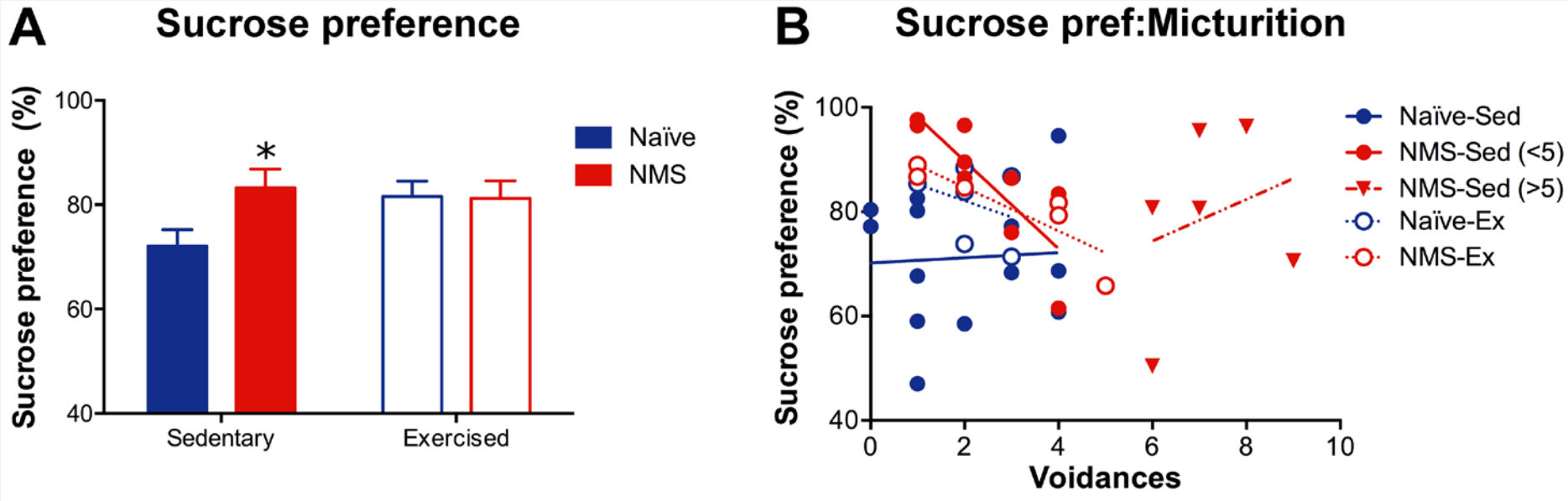
MOOD DISORDER

Sucrose preference test

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

ANHEDONIA

Female mice



Sedentary NMS females have higher sucrose preference at 8 weeks of age than naïve.

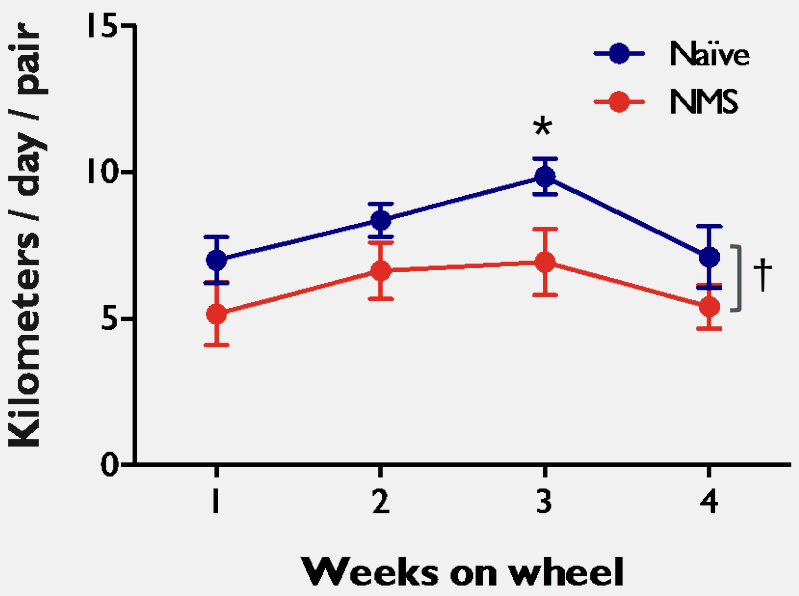
Decreased sucrose preference correlates with increased micturition frequency (within a "normal" range).

Pierce et al., *Neurourol Urodyn,* 2018.
Two-way ANOVA; * $p<0.05$ vs. naïve; Fisher's LSD posttest.
NMS-Sed (<5): $r^2=-0.8190$, $p=0.0069$; NMS-Ex: $r^2=-0.8715$, $p=0.0237$; Pearson's correlation.

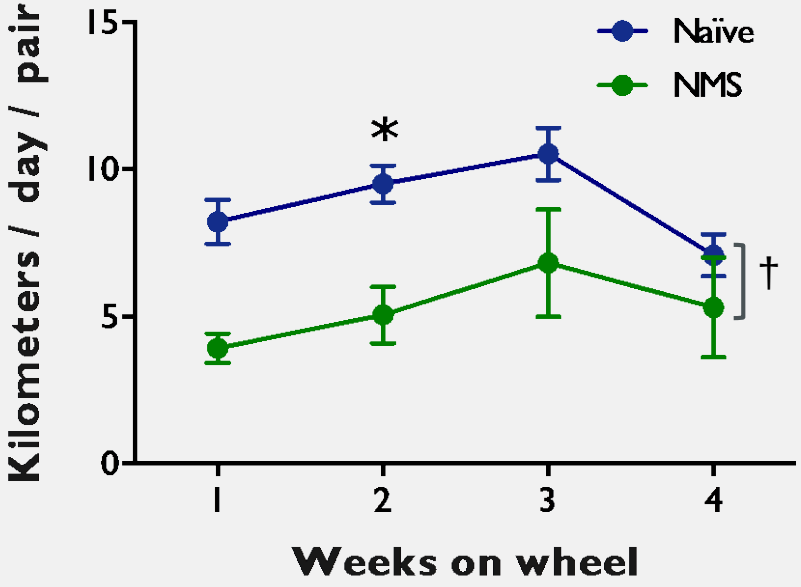
ANHEDONIA

Running distance

Female mice



Male mice



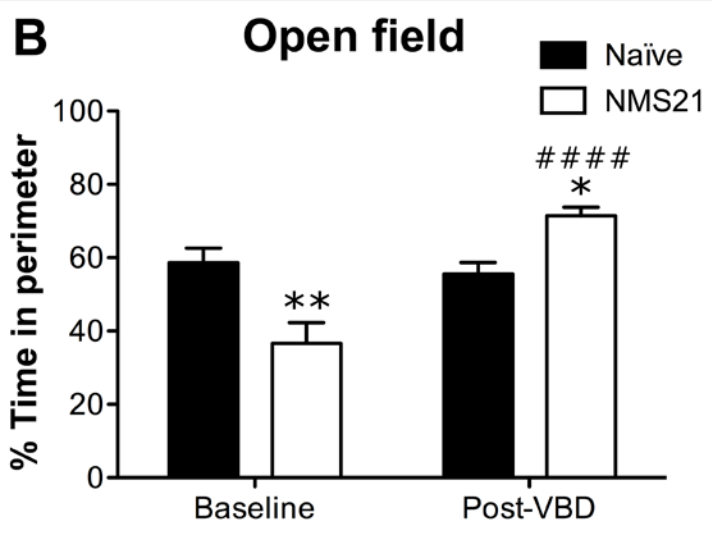
Both female and male NMS mice run a shorter distance than naïve mice.

Anhedonia or low aerobic capacity?

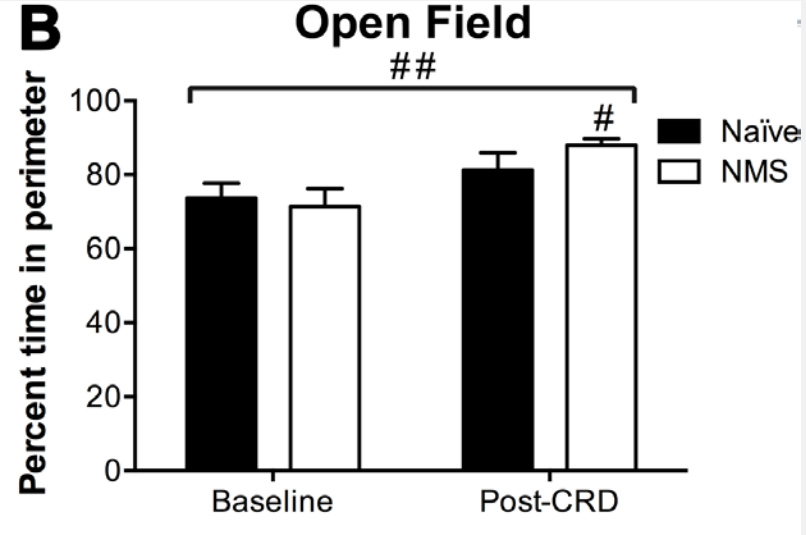
Pierce et al., *Neurourol Urodyn,* 2018; Fuentes et al., manuscript in preparation.
Brackets: time († $p < 0.01$), two-way RM ANOVA; *$p < 0.05$ vs. naïve, Bonferroni posttest.

ANXIETY

Female mice



Male mice



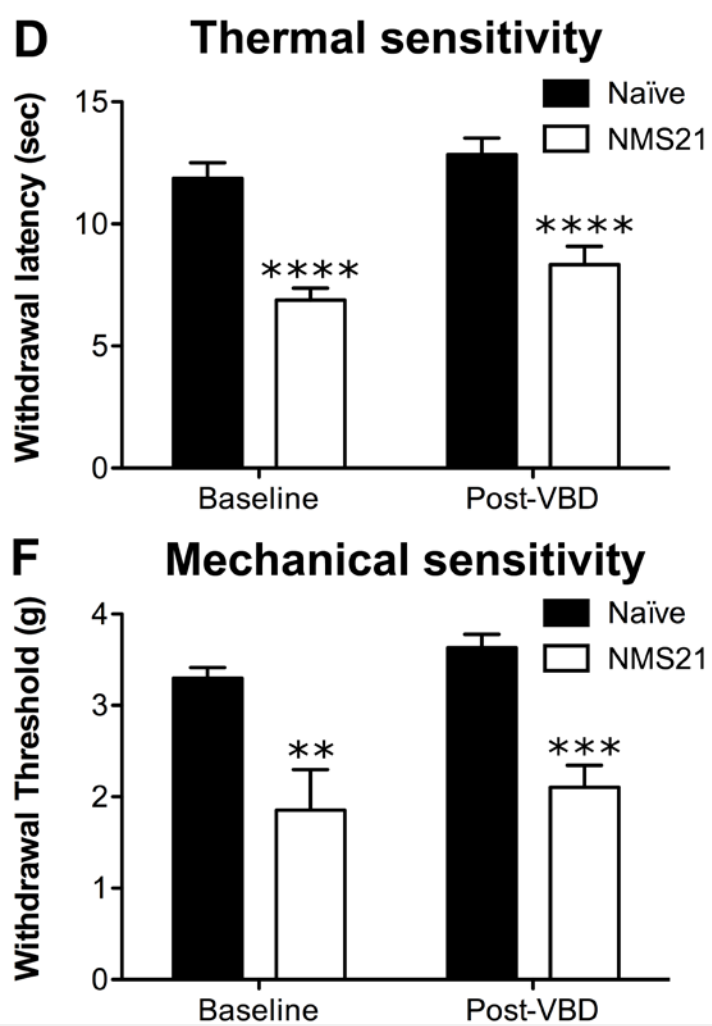
Sedentary NMS females display significant anxiety prior to VBD.

Pelvic organ distension decreases anxiety-like behavior in NMS male and female mice.

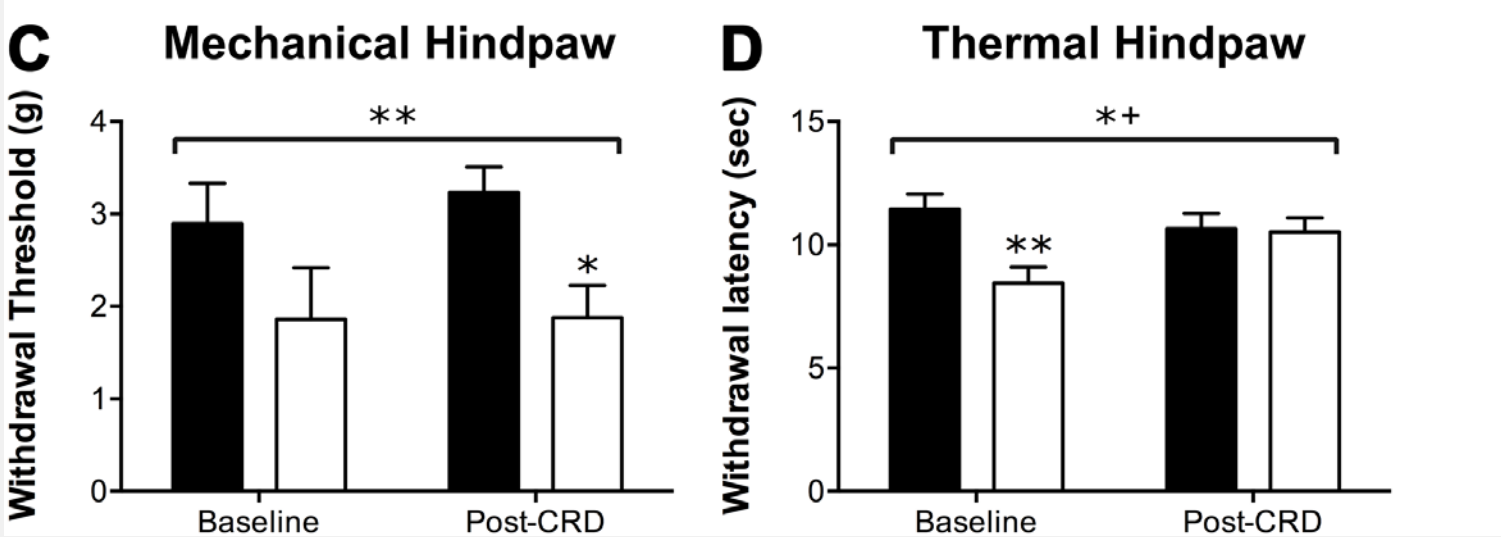
Pierce et al., *Neurosci,* 2014; Fuentes et al., *IBRO Rep*, 2016.
Brackets: CRD (## $p<0.01$), two-way ANOVA;
*, ** $p<0.05$, 0.01 vs. naïve; #, #### $p<0.05$, 0.0001 vs. baseline; Bonferroni's posttest.

WIDESPREAD HYPERSENSITIVITY

Female mice



Male mice



Female NMS mice display thermal and mechanical hypersensitivity.

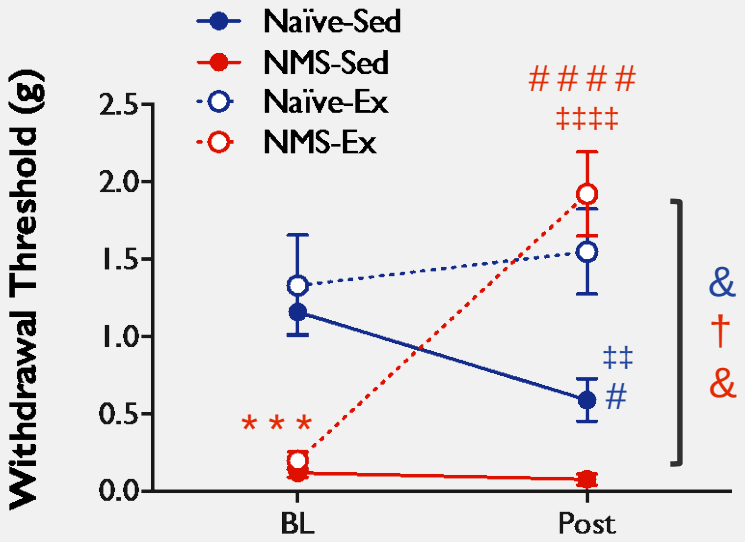
Male NMS mice display stress-dependent thermal and mechanical hypersensitivity.

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.

WIDESPREAD HYPERSENSITIVITY

Female mice

Forepaw mechanical sensitivity



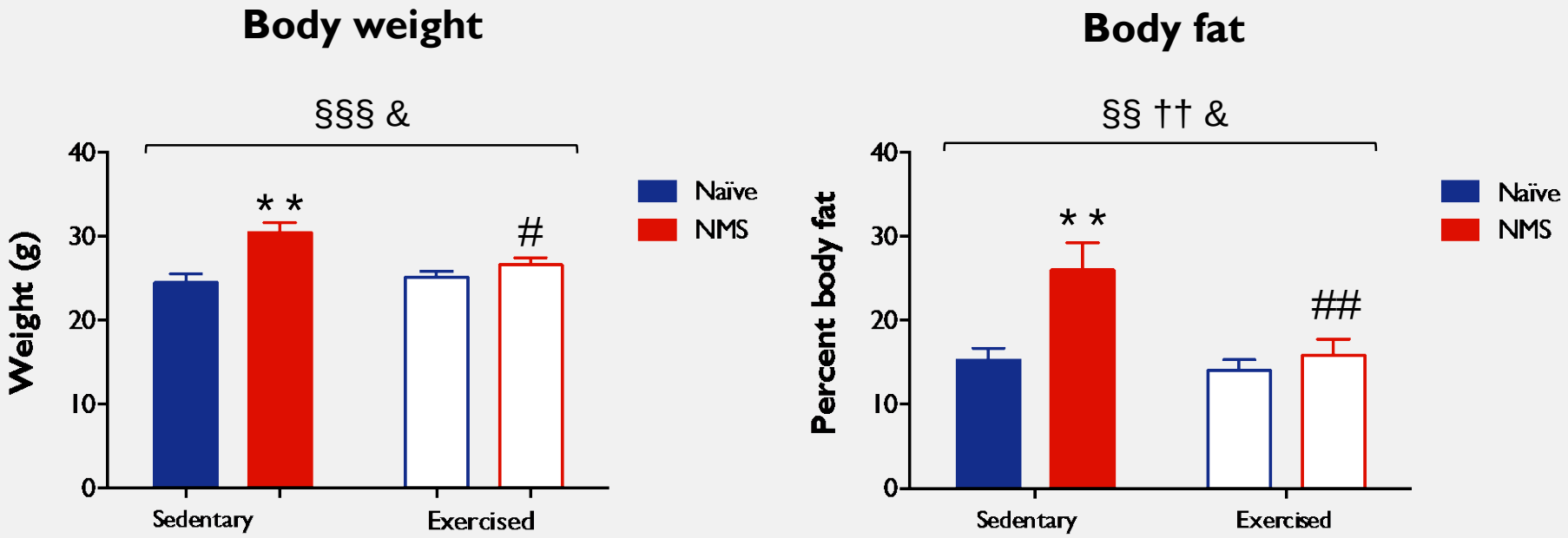
Exercise normalizes forepaw mechanical sensitivity in female NMS mice.

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.

METABOLIC DISORDER

Female mice – 10 months

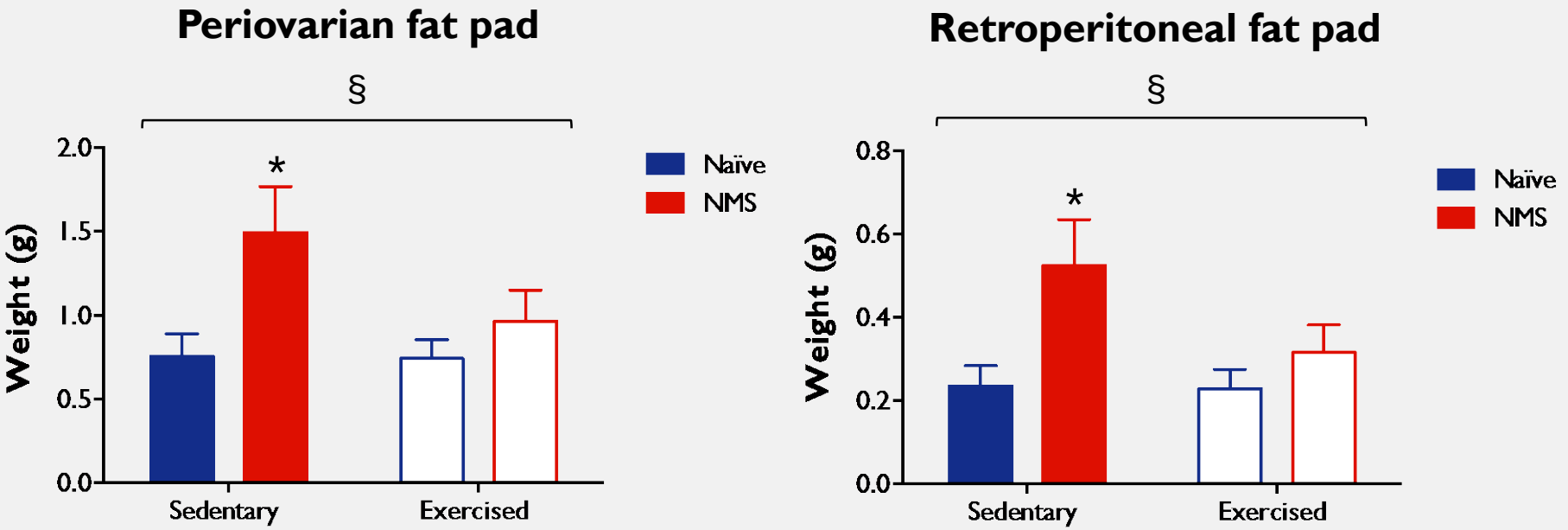


Female sedentary NMS mice are significantly heavier and have more body fat than naïve or exercised NMS mice.

Eller-Smith, manuscript in preparation.
Brackets: NMS (§§, §§§ $p<0.01, 0.001$), exercise (†† $p<0.01$), NMS x exercise (& $p<0.05$), two-way ANOVA;
** $p<0.01$ vs. naïve; #, ## $p<0.05, 0.01$ vs. sedentary; Bonferroni's posttest.

METABOLIC DISORDER

Female mice – 10 months

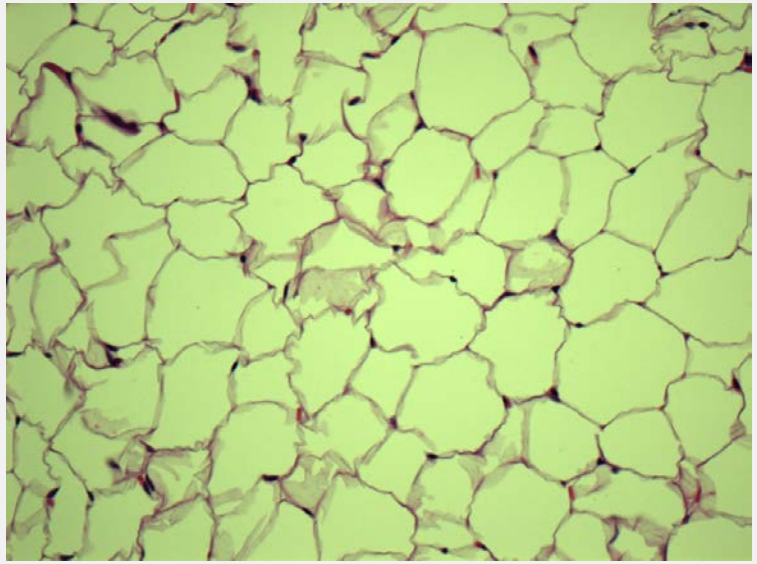


Female sedentary NMS mice have heavier fat pads than naïve or exercised NMS mice.

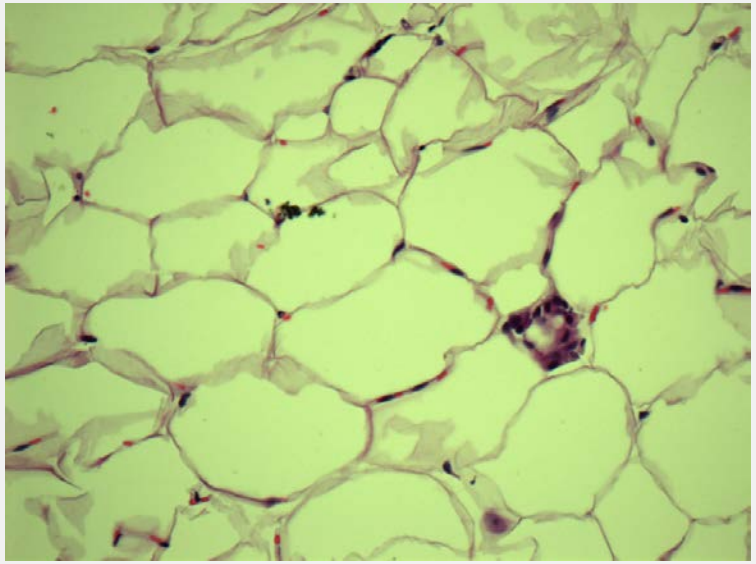
Eller-Smith, manuscript in preparation.

Brackets: NMS (§§, §§§ $p<0.01, 0.001$), exercise (†† $p<0.01$), NMS x exercise (& $p<0.05$), two-way ANOVA;
** $p<0.01$ vs. naïve; #, ## $p<0.05, 0.01$ vs. sedentary; Bonferroni's posttest.

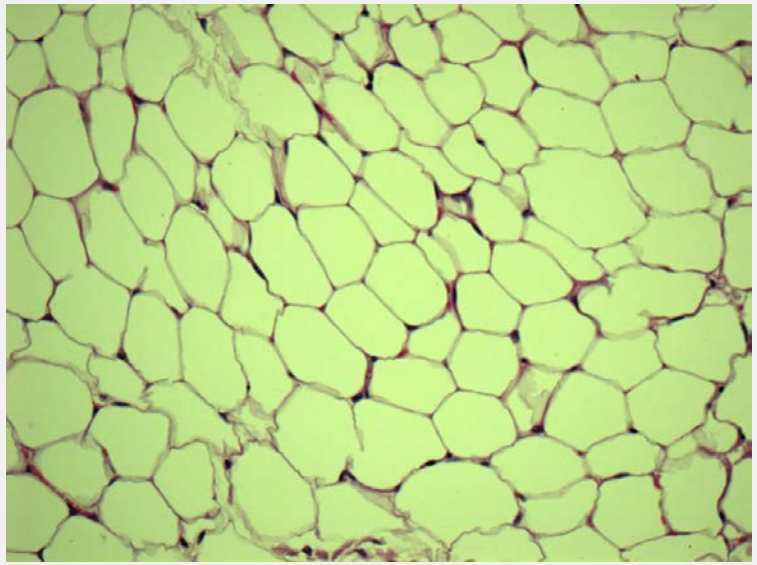
Naïve-Sed



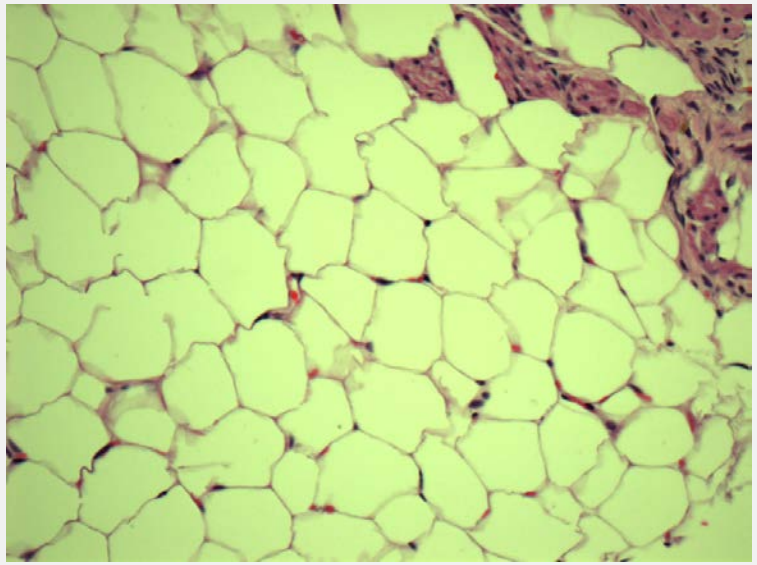
NMS-Sed



Naïve-Ex



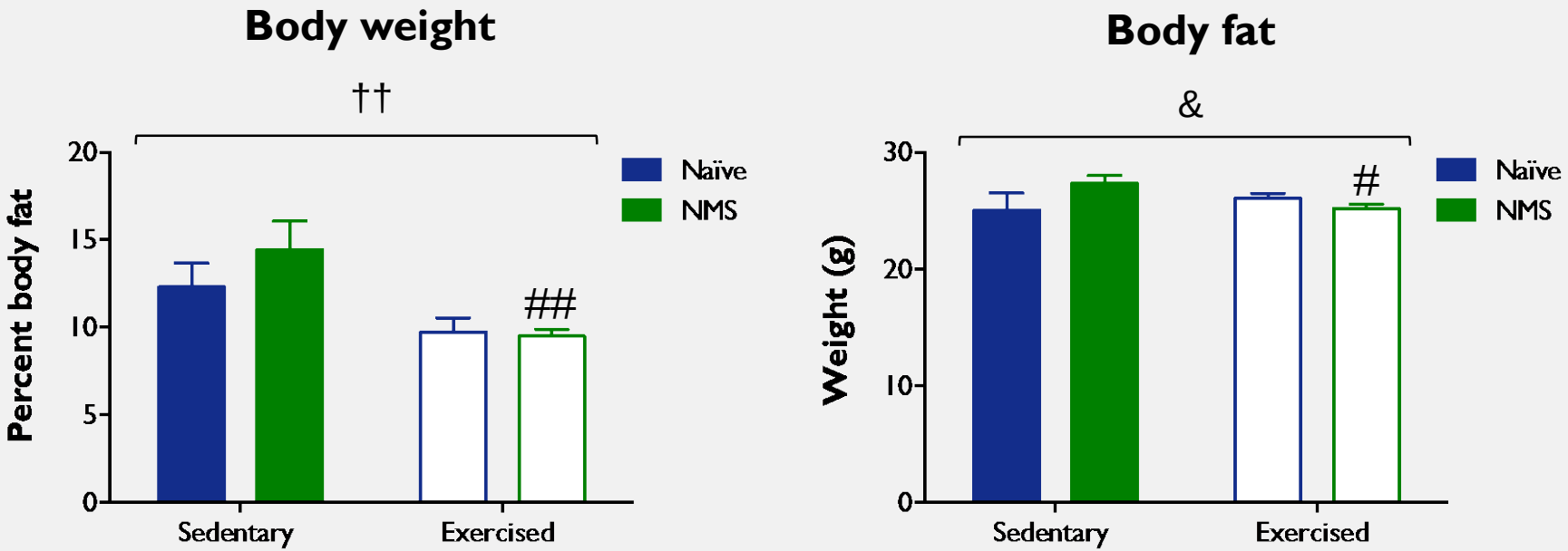
NMS-Ex



20x

METABOLIC DISORDER

Male mice – 3 months

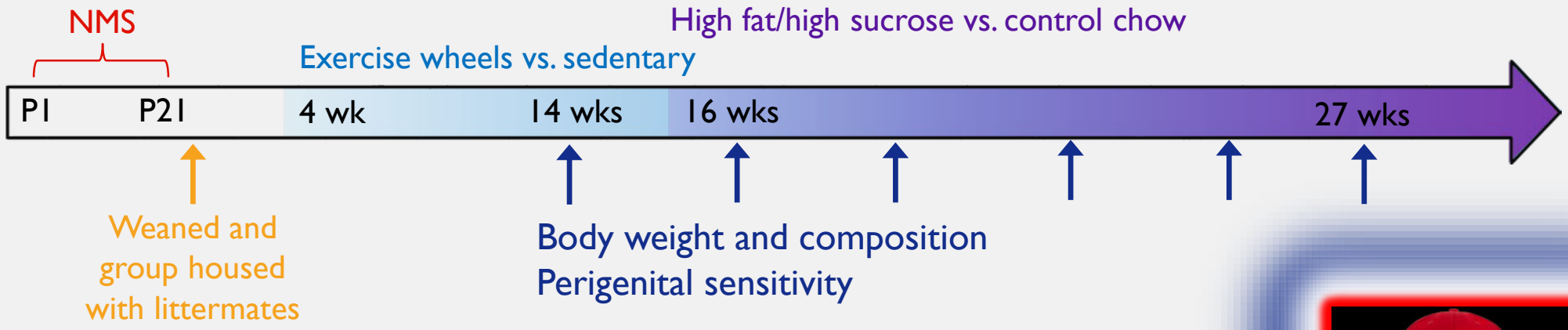


Male sedentary NMS mice trend toward greater body weight and fat, exercise significantly lowers both.

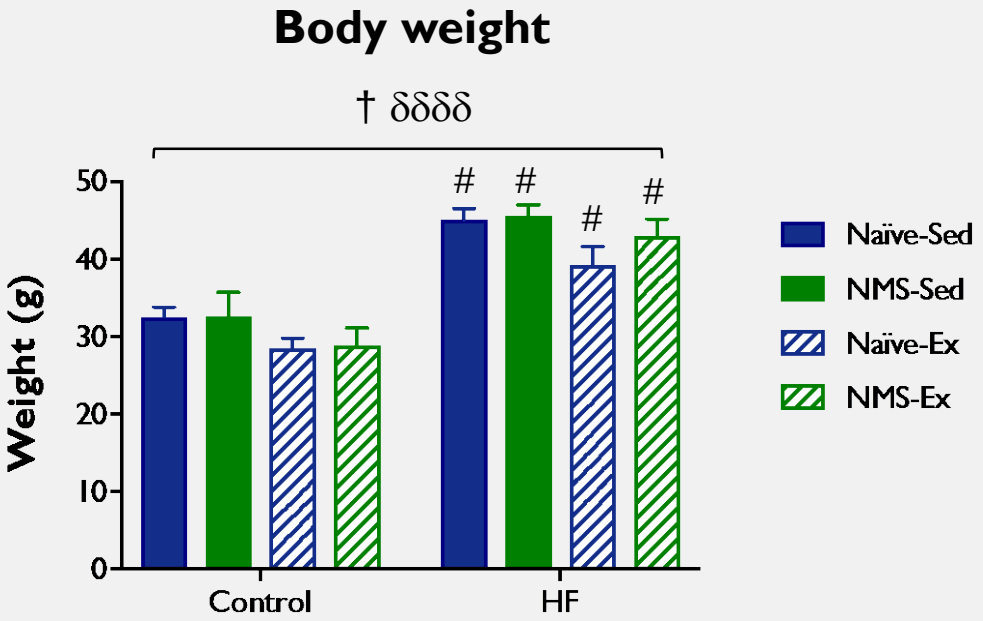
Eller-Smith, manuscript in preparation.
Brackets: exercise (†† $p<0.01$), NMS x exercise (& $p<0.05$), two-way ANOVA;
#, ## $p<0.05, 0.01$ vs. sedentary; Bonferroni's posttest.

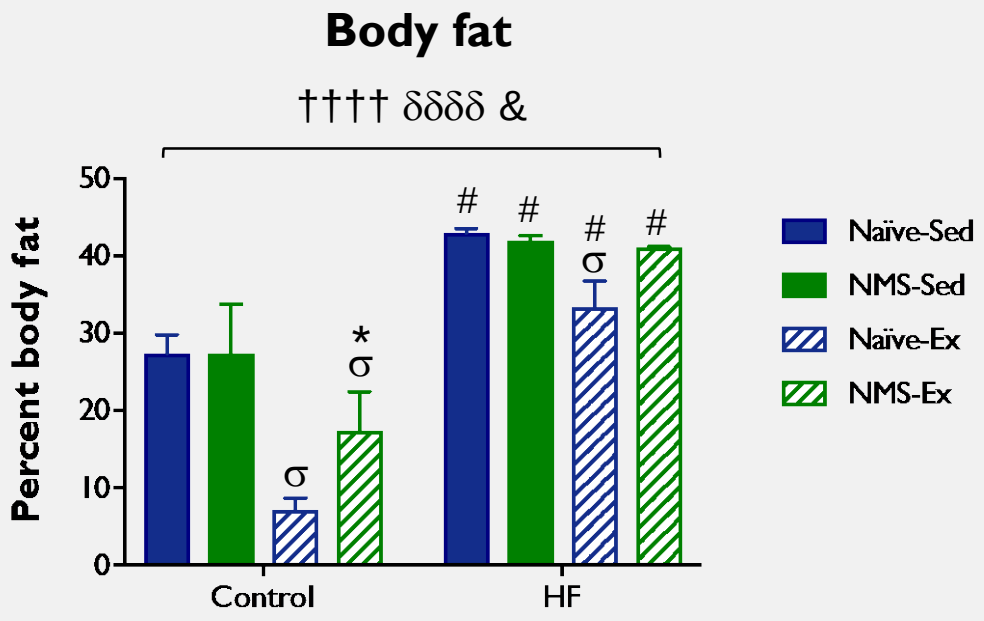
WESTERN DIET

3h/day X 21 days
34°C
50% humidity



BODY COMPOSITION





All mice gained more weight on the Western diet.

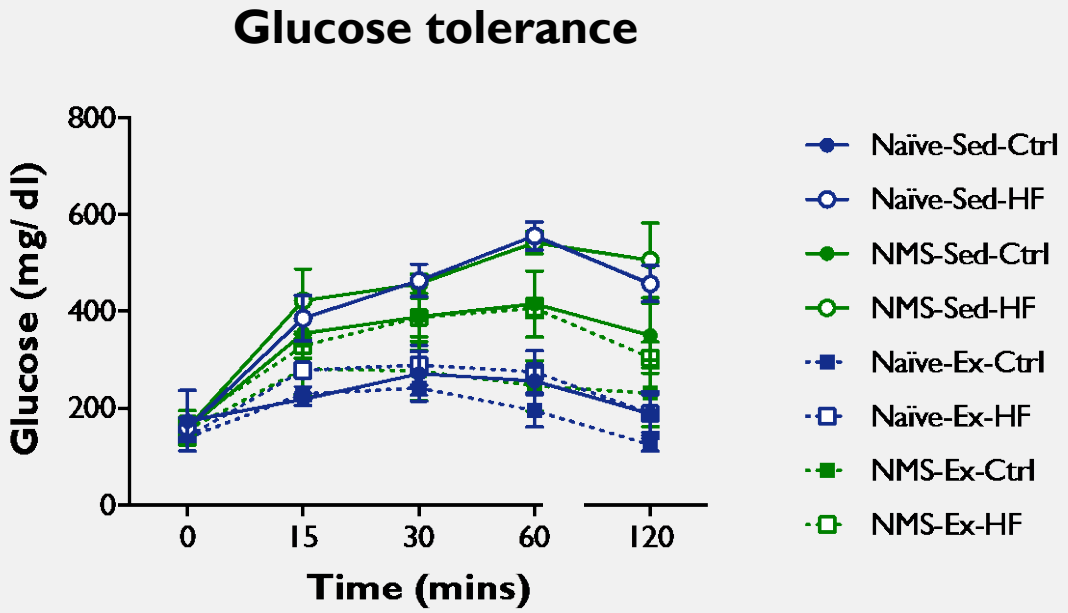
Exercise lowered body fat in control-fed, greater effect on naïve mice.

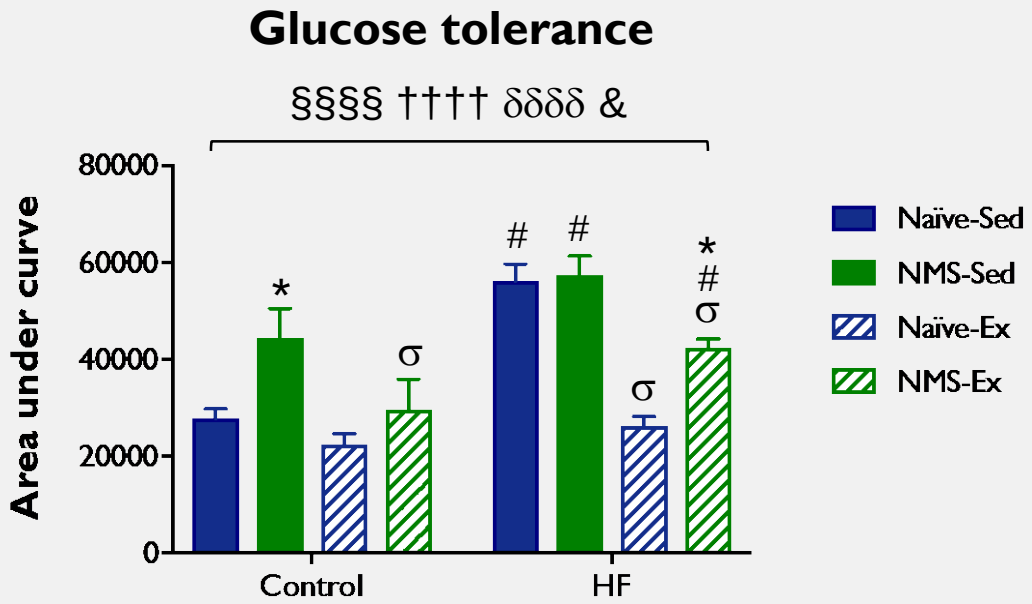
Exercise was not protective of NMS HF-fed mice.

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





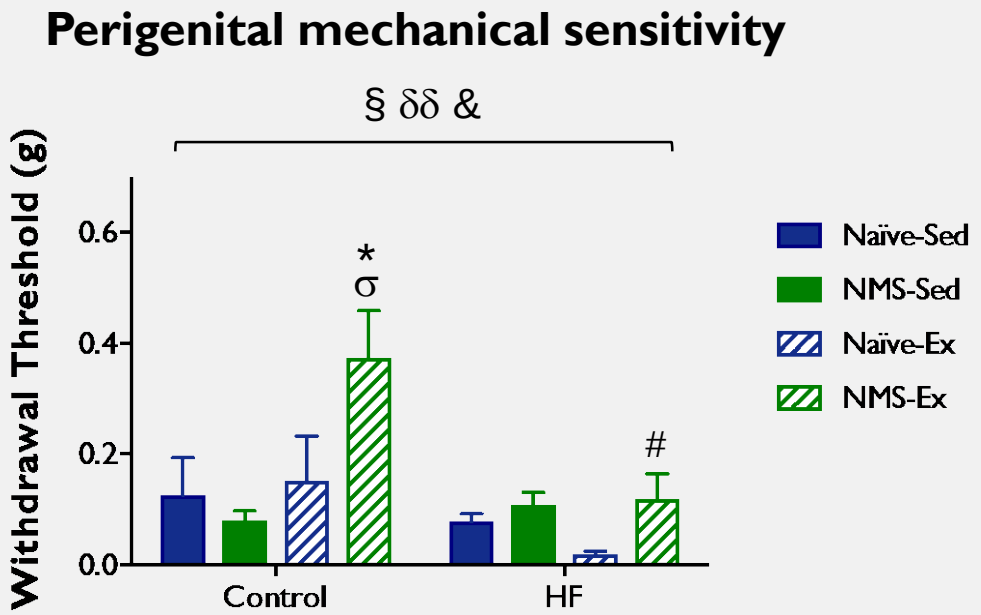
Stratified effect of stress, exercise, and diet on glucose tolerance.

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.

PERIGENITAL SENSITIVITY

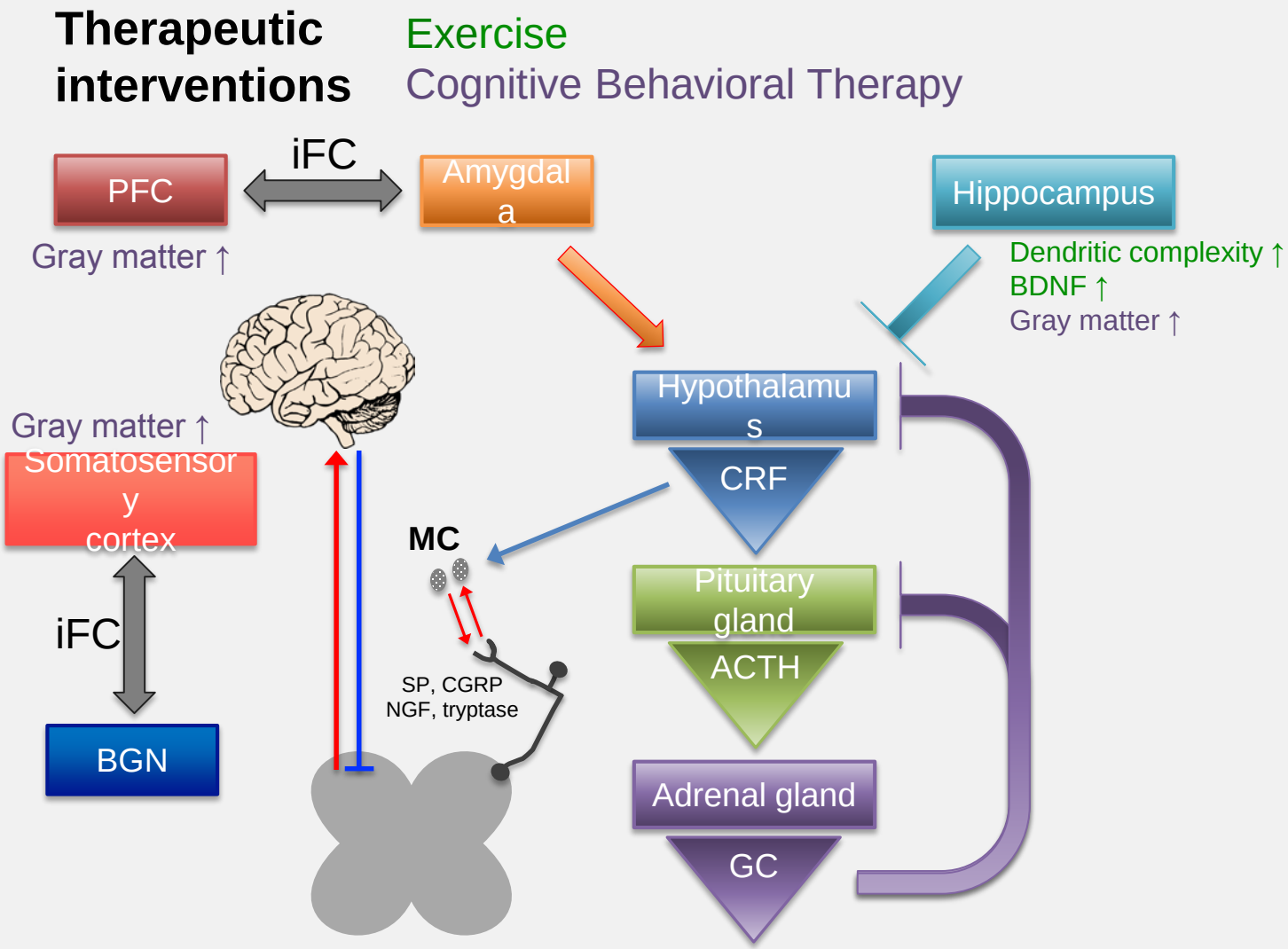


Unlike metabolic measures, exercise was protective ONLY for NMS.

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 INTERVENTION



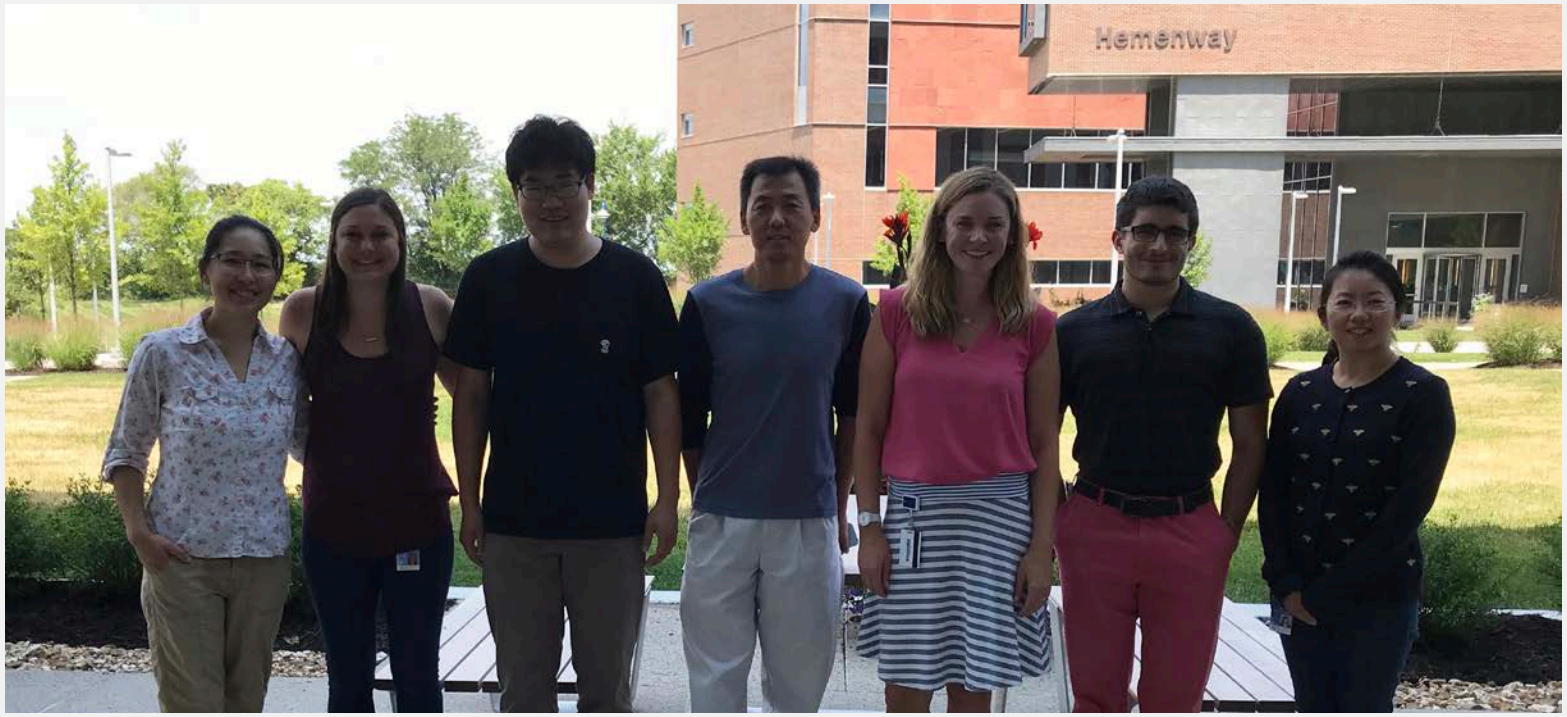
Eller-Smith, Nicol, & Christianson, *Front Cell Neurosci*, 2018

SOAPBOX/CONCLUSIONS

- 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

TEAM JACLAB

Current members:
Isabella Fuentes, PhD – just left for Pitt
Olivia Eller-Smith – PhD candidate
Pau Yen Wu – PhD candidate
Frank Wang – Research Associate
Aaron Brake – Research Assistant
Xiaofang Yang, MD – Postdoctoral scholar
Janelle Ryals – Senior Research Associate



Alumni:
Angela Pierce, PhD – PhD student/Postdoc
Alison Greelief – MS student
Molly Maloney – K-INBRE Summer scholar ('16)
Kellen Wright - K-INBRE Summer scholar ('15)
Rachel Supple – K-INBRE Summer scholar ('14)
Lizzie Di Silvestro – K-INBRE Summer scholar ('13)
Natalie Walker – K-INBRE Summer scholar ('12)

Collaborators
Doug Wright, Ph.D.
- KUMC, Anatomy and Cell Biology

John Thyfault, Ph.D.
Matt Morris, Ph.D.
- KUMC, Physiology

Robert Sorge, Ph.D.
- UAB, Dept. Psychology

Funding sources
R01 DK099611
R01 DK103872
T32 HD057850 (Fuentes, Eller-Smith)
Chancellor's Fellowship (Eller-Smith)
KUMC SOM
COBRE – P20 GM104936
K-INBRE – P20 RR016475
KIDDRC – P30 HD002528

Thank you!