The painful consequences of stress

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Child abuse and neglect

2012 Adverse Childhood Experiences (ACE) study

- 27.4/1000 children are maltreated.
  - Steadily increased over 5 years.
- 676,569 unique cases in 2011.
  - 27% under the age of 2.

- 64% reported at least one ACE.
- 22% reported three or more.
- Risk of health problems increases with number of ACEs.

NICU admittance

A neonate in the NICU experiences, on average, 16 procedures/day.
  • 10 are considered painful.
  • Number increases for babies born <29 gestational weeks.
  • ~80% performed without specific analgesic intervention.

Prolonged periods of maternal separation.

Early life stress implicated in:
  • Learning disabilities.
  • Mental illness.
  • Functional pain disorders.
    - Migraine
    - Fibromyalgia
    - Chronic pelvic pain

Carbajal et al., 2008, JAMA.
Early life stress paradigm

**Neonatal Maternal Separation:** C57Bl/6 mouse pups born in-house and separated as litters for 3 hours per day from P1-21.

Fuentes et al., 2015, JoVE.
Adult intervention

**Water avoidance stress:** Naïve and NMS mice are singly placed on a 5cm round platform surrounded by water for one hour.

Pierce et al., 2016, Brain Res; Fuentes et al., manuscript submitted.
Adult intervention

**Water avoidance stress:** Naïve and NMS mice are singly placed on a 5cm round platform surrounded by water for one hour.

- Pelvic organ sensitivity
- Bladder dysfunction
- HPA axis regulation

Pierce et al., 2016, Brain Res; Fuentes et al., manuscript submitted.
Neonatal maternal separation

- Pelvic organ sensitivity
- Bladder dysfunction
- HPA axis regulation
Pelvic organ distension

- Electromyographic (EMG) recordings of abdominal muscles.
- Reflexive contraction termed the visceromotor reflex (VMR).
Pelvic organ sensitivity – Female mice

Urogenital organ-specific hypersensitivity in female NMS mice.

Pierce et al., 2014, Neuroscience; Pierce et al., 2016, Brain Res.
Brackets: NMS (§ p < 0.05), two-way RM ANOVA; *, **p < 0.05, 0.01 vs. naïve, Bonferroni posttest.
Pelvic organ sensitivity – Male mice

Fuentes et al., 2015, JoVE; Fuentes et al., manuscript under review.

\( p > 0.05 \), two-way RM ANOVA.
Pelvic organ sensitivity – Male mice

Urogenital organ-specific hypersensitivity in male NMS mice.

Fuentes et al., 2015, JoVE; Fuentes et al., manuscript under review.

*p < 0.05 vs. naïve, Student's t-test.
WAS effect – Female mice

WAS exposure further increases bladder sensitivity in NMS mice.

WAS increased colorectal sensitivity only in naïve mice.
WAS effect – Male mice

WAS significantly increased colorectal sensitivity in NMS mice.

Fuentes et al., manuscript under review.

Brackets indicate effect of WAS (# p < 0.05), two-way RM ANOVA; **p < 0.01 vs. naïve, #, #### p 0.05, 0.001 vs. baseline, Bonferroni posttest.
**WAS effect – Male mice**

WAS did not significantly impact perigenital mechanical sensitivity.

Fuentes et al., manuscript in preparation.

Brackets indicate effect of NMS (§ § $p < 0.01$), two-way RM ANOVA; 
**** $p < 0.05$, 0.01, 0.0001 vs. naïve, Bonferroni posttest.
Neonatal maternal separation

- Pelvic organ sensitivity
- Bladder dysfunction
- HPA axis regulation
Micturition

Measure urine output over one hour to determine how bladder function is impacted by early life and adult stress.
Micturition

Measure urine output over one hour to determine how bladder function is impacted by early life and adult stress.
WAS increases micturition frequency and volume, only in NMS mice.

No impact on fecal output.
Micturition – **Male mice**

NMS increases urine and fecal output in male mice. Normalizes by 8d post-WAS.

Fuentes et al., manuscript in preparation.

Brackets indicate effect of NMS (§, §§ $p < 0.05, 0.01$), two-way RM ANOVA; *, **$p < 0.05, 0.01$ vs. naïve, Bonferroni posttest.
Neonatal maternal separation

- Pelvic organ sensitivity
- Bladder dysfunction
- HPA axis regulation
Response to stress

Stress

CRF

AVP

ACTH

Glucocorticoids

Hypothalamus

Anterior pituitary

Adrenal cortex

Mast cells

Cytokines

Tryptase

Histamine

CRF

Hippocampus

Amygdala

Hippocampal gene expression – Female mice

No significant effects of NMS at baseline.

Transient decrease in regulatory gene expression at 1d in NMS.

- Reduce hippocampal negative regulation of HPA axis.

Pierce et al., 2016, Brain Res.

NMS (§ p < 0.05), WAS (†, †††† p < 0.05, 0.0001), NMSxWAS (& p < 0.05), two-way ANOVA;

*, ** p < 0.05, 0.01 vs. naïve, #, ##, ###, #### p < 0.05, 0.01, 0.001, 0.0001 vs. BL, ‡‡, ‡‡‡, ‡‡‡‡ p < 0.01, 0.001, 0.0001 vs. 1d, Bonferroni posttest.
Hypothalamic gene expression – Female mice

No significant effects of NMS at baseline.

Significant impact of WAS at 8d, especially in NMS.

• Possibly due to hippocampal changes at 1d post-WAS.

Pierce et al., 2016, Brain Res.

WAS (††, †††† p < 0.01, 0.0001), two-way ANOVA;
##, #### p < 0.01, 0.0001 vs. BL, ‡, ‡‡‡ p < 0.05, 0.001 vs. 1d, Bonferroni posttest.
Mast cell degranulation – Female mice

Mast cell degranulation is increased in female NMS bladder. WAS significantly increases degranulation, primarily in naïve.

Pierce et al., 2016, Brain Res. NMS (§ § § p < 0.001), WAS († p < 0.05), two-way ANOVA; *, ***, p < 0.05, 0.001 vs. naïve, # p < 0.05 vs. BL, Bonferroni posttest.
Mast cell degranulation – Female mice

WAS increased protein levels of PAR2 and TRPA1 in both groups.

Pierce et al., 2016, Brain Res. WAS († p < 0.05), two-way ANOVA
Mast cell degranulation – Male mice

Histological evidence of mast cell degranulation is increased in male NMS bladder and prostate.

Fuentes et al., manuscript in preparation.

**p < 0.01, 0.001 vs. naïve; Student’s t-test
Conclusions

• Neonatal maternal separation increases:
  • Urogenital-specific sensitivity in male and female mice.
  • Urinary and fecal output in male mice.
  • Mast cell degranulation in urogenital organs.

• Exposure to water avoidance stress causes:
  • Exacerbation of urinary bladder sensitivity in female NMS mice.
  • Increased colorectal sensitivity in female naïve mice and male NMS mice.
  • Increased urinary output in female NMS mice.
  • Transient decrease in regulatory hippocampal gene expression, followed by hypothalamic changes.
  • Mast cell degranulation and associated protein expression in the bladder.

• Exposure to early life stress induces baseline changes within the urogenital organs, and possible priming within the limbic structures, that result in increased susceptibility to stress exposure later in life.
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