Effects of Housing Environment on Thermal and Mechanical Nociceptive Responses in Mice

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Introduction

Pain as defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Millions of people worldwide are affected by pain, both acute and chronic, every day. Currently, it is estimated that pain management costs $560-$635 billion annually, making it one of the most pervasive issues of public health (American Academy of Pain Medicine). Multiple areas of the nervous system are involved in the experience of pain. This reflexive response is also known as nociception.

During nociception, intense thermal, mechanical, and/or chemical stimuli are detected by a subpopulation of peripheral nerve fibers or nociceptors (Basbaum et al., 2009). Nociceptors possess a peripheral axon that receives nociceptive stimulation from skin, bones and viscera; and a central axon that synapses onto neurons located in the dorsal horn of the spinal cord (Figure 1). The cell bodies of nociceptors reside in the dorsal root ganglia, which are located in the intervertebral spaces lateral to the spinal cord (Basbaum et al., 2009). There are two classes of nociceptors, the first contains myelinated fibers that are either medium diameter and transmit acute, or "fast" pain, or large diameter which transmit light touch. The second class of nociceptors are unmyelinated C fibers that transmit slow pain. C fibers are able to detect both thermal and mechanical stimuli, making them polymodal, or able respond to multiple types of stimuli (Basbaum et al., 2009).

Enriched environment and nociception

Most of the research regarding the effects of environment on pain focuses on supraspinal mechanism, but very little is known about the effects of environment on nociception. Furthermore, it is unclear how a deprived, or "impoverished" environment alters nociception. In this investigation I will determine whether manipulating the housing environment of mice affects their thermal and mechanical nociceptive responses.

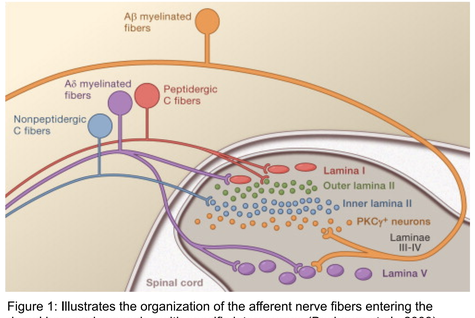

Figure 1: Illustrates the organization of the afferent nerve fibers entering the dorsal horn and synapsing with specific interneurons (Basbaum et al., 2009)

Hypotheses

Mice raised in the Enriched Environment will have longer withdrawal latency times than the mice raised in a Standard Environment in both the mechanical and thermal nociceptive tests.

Methodology

Question 1: Does enriched environment reduce basal thermal nociception in mice?

Mice were divided into two groups, enriched and standard. The enriched environments contained multiple mice in a large cage with running wheels, logs, tunnels, igloos, and hay with water and food *ad libitum.* Mice in the standard environment were housed individually, in standard lab cages with water and food *ad libitum,* but no objects. Mating's were set up in each environment, and the females remained in their environment through pregnancy. Their pups were raised in the same environments throughout testing. Testing was performed on post-weaning aged (P21) male and female pups.

Question 2: Does environment influence mechanical nociception in mice?

To answer this question, mice will be divided into the two different groups as described above. After P21 mechanical nociceptive thresholds were measured using Von Frey filaments and the Up Down Method. Here, the hind paw of a mouse was stimulated with calibrated filaments with different weights. I started with the lowest weight filament and once a response was detected I followed the Up Down Method to obtain the pain threshold with the lowest number of trials.

Question 3: Does environment influence thermal nociception in mice?

To answer this question, mice will be divided into the two different groups as described above. After P21 thermal nociceptive thresholds were measured using the Hargreaves test. In this method the hind paw of a mouse was stimulated with a beam of light. Withdrawal latency was measured using the timing mechanism on the apparatus.

Comparison of Enriched Environments between humans and mice

Figure 2: Example of an enriched environment for human children

Figure 3: Enriched environment used during the experiment

Assays to test for nociception

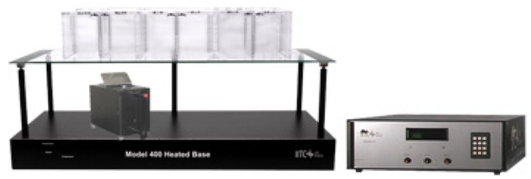

Figure 4: Shows the set up for the Hargreaves testing apparatus. One mouse is placed in each testing chamber, allowing up to twelve mice to be tested at once.

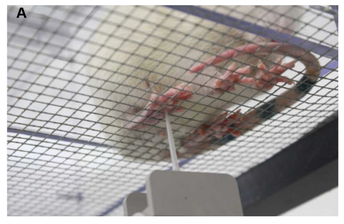

Figure 5 A: Shows the Von Frey test being performed on a mouse

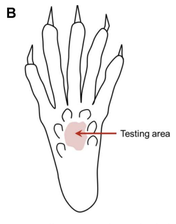

Figure 5 B: Illustrates where the light (Hargreaves) or filament (Von Frey) is applied to the paw during testing

Effect of enriched environment on development of nociception

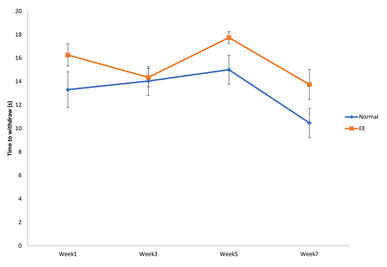

Figure 6: Illustrates the development of thermal nociception over the seven week testing period.

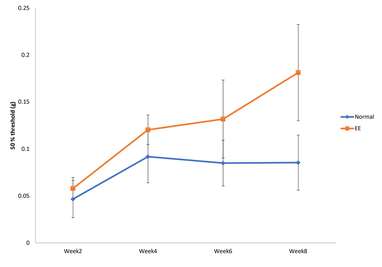

Figure 7: Illustrates the development on mechanical nociception over the seven week testing period.

Enriched environment reduces nociception in mice

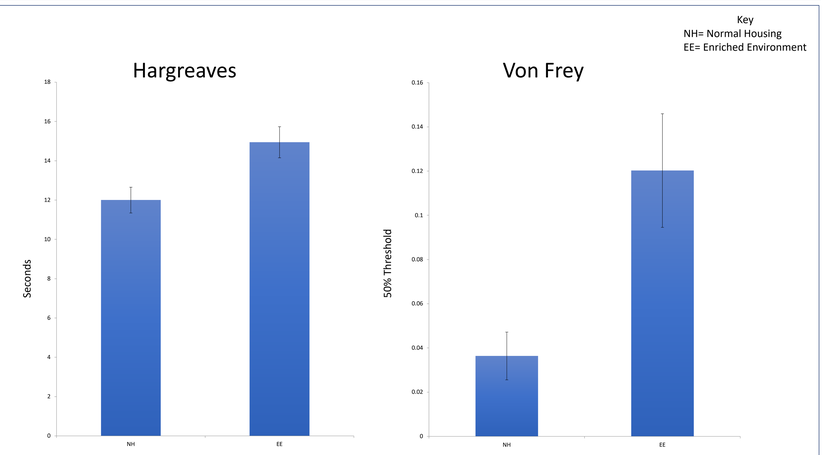

Figure 8: Reports the average withdrawal latency for all males that were tested

Figure 9: Reports the 50% threshold for all males that were tested

Conclusions

Males raised in normal housing showed lower thermal and mechanical thresholds as measured by Hargreaves and Von Frey filaments. Hargreaves: Normal housing = 11.9s ±0.66; Enriched environment = 14.94s ±0.79; P = 0.0118. Von Frey filaments: Normal housing = 0.036g ±0.011; Enriched environment = 0.12g ±0.026; P = 0.0069.

Females were tested at the conclusion of the experiment, but didn't show significant differences in any of the tests that we performed. However, the variability seems to be higher than in the male groups. This may be related to differences in estrous cycle, which was not controlled for due to the need for more animals, more time, and further training.

Future Directions

- Repeat tests with female population controlling for estrous cycle
- Test different aspects of poverty such as food deprivation or insecurity, overpopulation, poor nutrition, isolation
- Test tissue samples collected after this study for DNA methylation
- After further studies with mice, begin human studies

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