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# Polystyrene Nanoplastics Initiate the HPA Axis in Hypothalamic Nerve Cells

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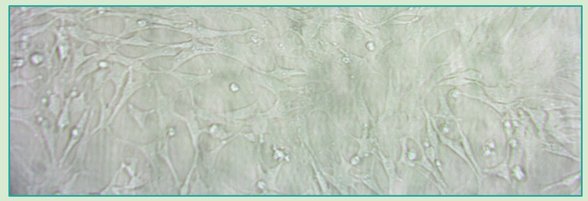

Figure 1: mHypoA-55 cells (Cedarlane).

## Background

The hypothalamic-pituitary-adrenal (HPA) axis is a major stress response system in vertebrate animals. In response to a stressor, the hypothalamus produces corticotropin-releasing hormone (CRH). CRH signals the pituitary gland to produce adrenocorticotropic hormone (ACTH), which signals the adrenal glands to produce cortisol. The circulation of cortisol alters blood sugar, blood pressure, and the immune system in order to adapt to the perceived threat (Fig. 2).[1,2] Due to its effects on metabolism and the immune system, chronic stress and prolonged activation of the HPA axis can lead to insulin resistance, increased susceptibility to infection, and cancer.[4,5] CRH gene transcription is activated by binding of phosphorylated cAMP response element binding protein (pCREB) to the CRH gene.[6]

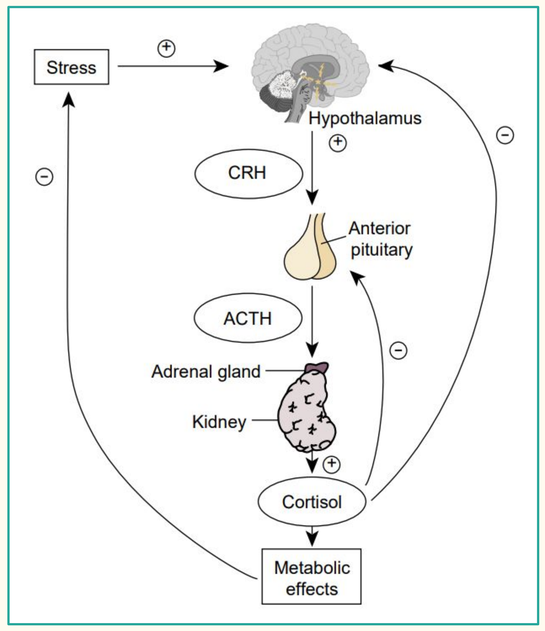


Figure 2: The HPA axis.[3]

Recent decades have seen the rise of a new potential stressor: nanoplastics (NPs). Plastics pervade today's society as a versatile but disposable material, thereby resulting in large amounts of waste.[7] This waste breaks down into small particles that can enter the body through ingestion, inhalation, and dermal absorption. Leslie and colleagues (2022)[8] found a mean concentration of 1.6 µg plastic particles/mL in human blood, indicating that plastic is capable of crossing epithelial and endothelial layers to enter the bloodstream and then circulate to other regions, such as the brain.

Research has shown that polystyrene (PS) NPs can be taken up by neural tissues following exposure by ingestion and inhalation and affect cell health, but literature addressing effects on the hypothalamus is scarce.[9-13] Investigations into the effects of NPs on CRH is nonexistent, despite the importance of the HPA axis in the stress response of humans and other vertebrates.

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## Results

### ELISA Assay: CRH

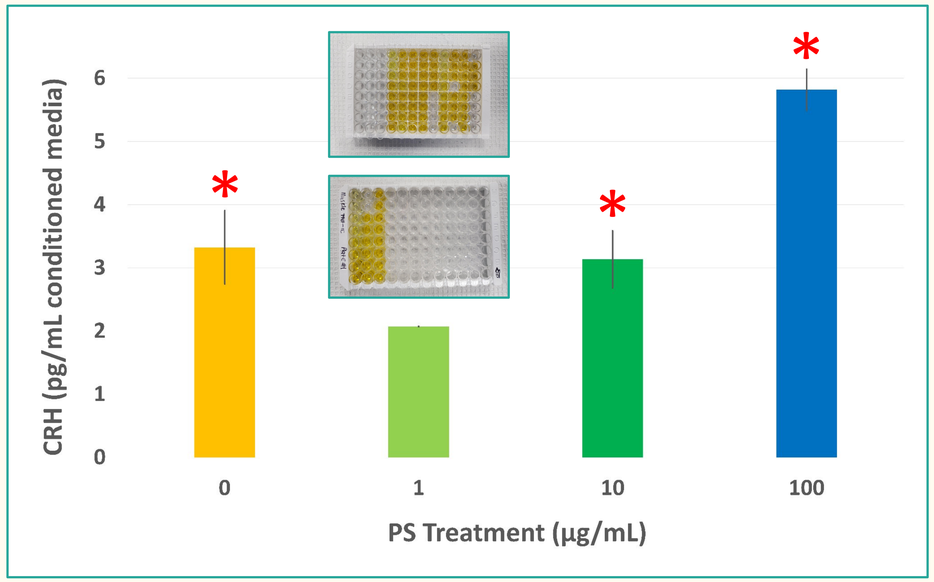


Figure 3: Concentration of CRH in conditioned media by plastic treatment. Concentration of CRH is significantly different between the 0 and 100 µg/mL treatment ($p < 0.05$) and between the 10 and 100 µg/mL treatment ($p < 0.01$).

### ELISA Assay: pCREB

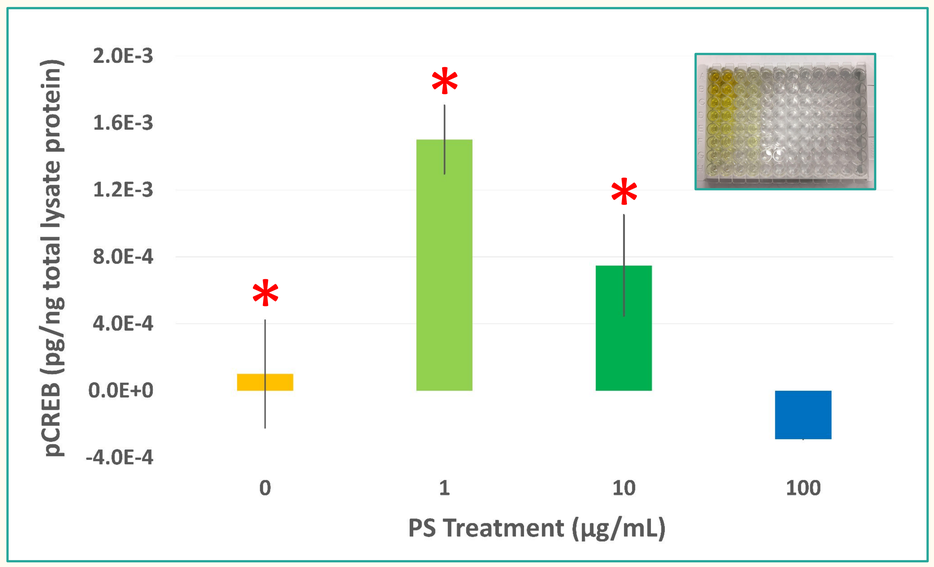


Figure 4: Concentration of pCREB in cell lysates by plastic treatment. Concentration of pCREB is significantly different between the 0, 1, and 10 µg/mL treatments ($p < 0.01$).

## Methods

- Cell culture
  - mHypoA-55 cell line: Immortalized adult mouse hypothalamus cells (Cedarlane)
  - DMEM (10% FBS, 1% Penicillin-Streptomycin, 1% NEAA)
- Plastic beads
  - Carboxylated 0.03 µm PS beads (Sigma Aldrich)
- Experimental design
  - Seed 75,000 cells/well in 12-well plates, settle for 30 hr
  - Treat with DMEM at 0, 1, 10, or 100 µg/mL PS for 40 hr
  - Analyze treated cell pellets and conditioned media
- Uptake assay
  - Cells stained with DAPI
  - Green fluorescent beads
  - View on fluorescent microscope
- Protein extraction
  - Minute™ Total Protein Extraction Kit (Invent Biotechnologies)
- ELISA assay
  - CRH: Human CRH ELISA Kit (Innovative Research)
  - pCREB: CREB [pS133] phosphoELISA™ ELISA Kit (Invitrogen)

## Question/Hypothesis

**Question: Could exposure of hypothalamic nerve cells to polystyrene nanoplastics initiate the HPA axis?**

**Hypothesis: Exposure of hypothalamic nerve cells to polystyrene nanoplastics initiates the HPA axis by increasing levels of CRH and pCREB.**

### Uptake Assay

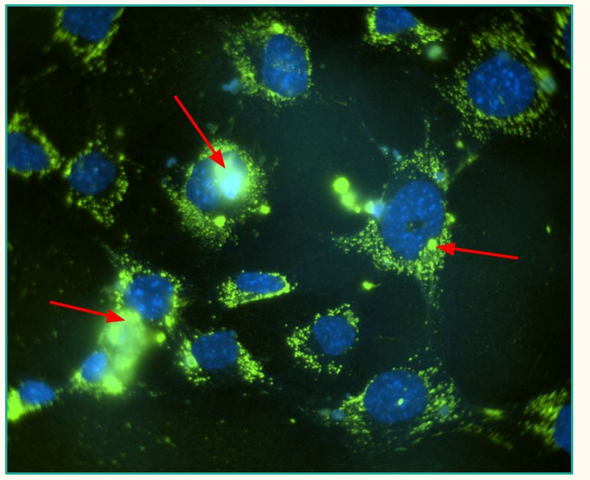

Figure 5: Uptake of 0.03 µm green fluorescent beads (red arrows) by mHypoA-55 cells. Cell nuclei are stained with DAPI (blue).

## Conclusions

- PS NP exposure results in phosphorylation of CREB, activation of the CRH gene, and release of CRH
- pCREB signal diminishes as the CRH gene is activated
- PS NPs stimulate the HPA axis as a stressor and have potential to cause chronic stress

## Acknowledgments

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