Long-term GSHR-mediated morphological alteration of hypothalamic nuclei for induced Anorexia Nervosa and Obesity in *Wistar* Rats

Nino Koci

Tufts University — Graduate School of Arts and Sciences — Biology

Introduction and Hypothesis

Ghrelin is a 28-amino-acid-long peptic hormone synthesized in the fundus region of the stomach. It is often considered the appetite hormone, as ghrelin levels are highest before a meal. Additional responses to lack of energy in the body will also trigger ghrelin secretion. Eventually, ghrelin levels will decrease after eating.

In the hypothalamus, ghrelin will bind to the GHS-Receptor (GHSR), a G-receptor, in the ventromedial hypothalamus nucleus VHN, where it will initiate a calcium cascade that will signal the hypothalamic arcuate nucleus (ARC) by promoting the production of NPY/AgRP and inhibiting POMC, as POMC codes for anorexigenic peptides CRH and α-MSH. NPY/AgRP travel to the paraventricular nucleus (PVN), where they inhibit the release of CRH and stimulate the production of orexin in the lateral hypothalamus (LHA), which will stimulate the appetite response.

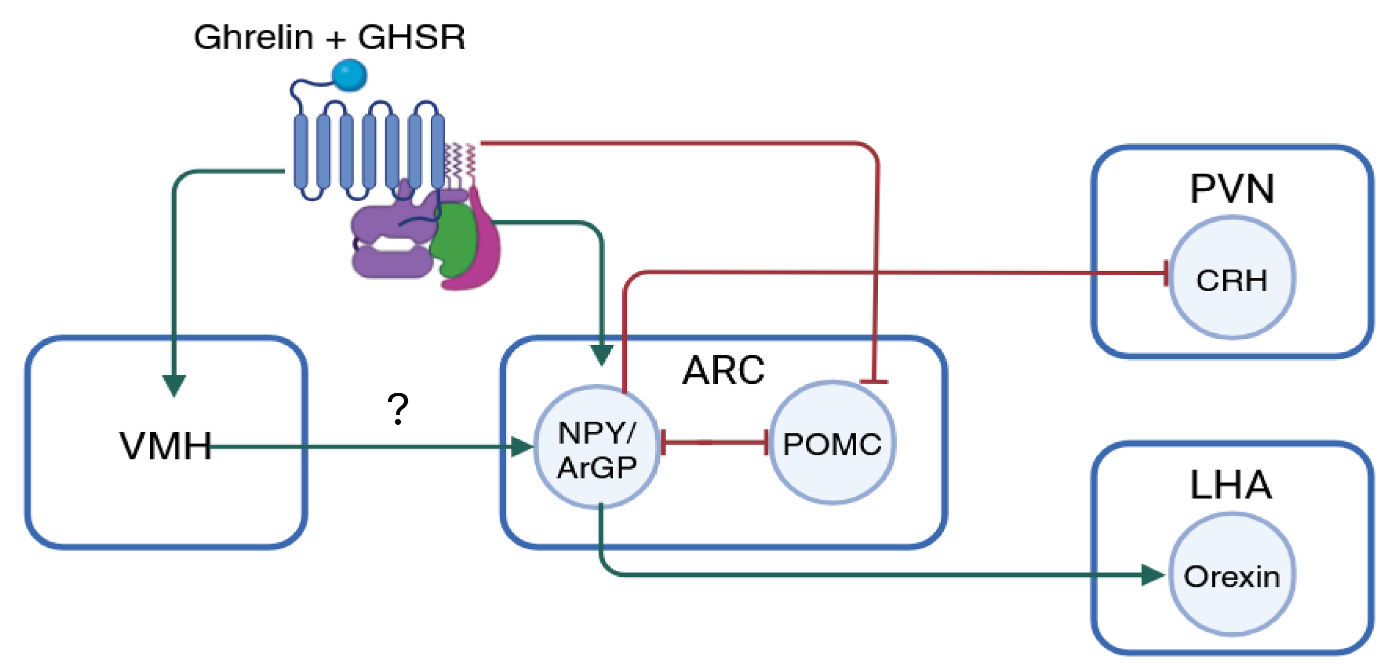

Figure.1: Graphical representation of the Ghrelin Orectic Pathway. Green denotes promotion and red inhibition. '?' denotes that we do not know the intermediary.

Obesity and anorexia nervosa(AN) are believed to correlate with potential disorders in these regions. Obesity is associated with lower ghrelin concentration in plasma, whereas AN is associated with elevated ghrelin levels. Studies have indicated that ghrelin and the GHSR have a role in the plasticity of the orectic regions of the hypothalamus. Based on the work from Cabral et al., GHS-Rs anorexia nervosa altered the dendritic fibers that connect the ARC to the PVN. Alzaid et al. explored the morphological effect of anorexia and obesity in young-adult hypothalamic regions, indicating that obesity causes an overall increase in the volume of the hypothalamus, whereas in AN, the opposite occurs. However, it is still necessary to discover how these morphological changes can be compared in a controlled population that expresses these ailments. We need to observe a cohort that is initially maintained under similar conditions. Then altered by dietary changes to create both obese and anorexic groups, while also maintaining a control group that does not deviate from the original population.

Hypothesis: We hypothesize that GHSRs increase the volume of VMH and ARC in obese rats and shrink it in anorexic rats. PVN and LHA do not change in volume since they rely on activation from the ARC.

Summary

In this poster, we hypothesized that different eating habits would induce morphological changes in the orectic regions of the hypothalamus due to varying ghrelin concentrations in obesity and anorexia nervosa. To test this hypothesis, we developed a method that induces these changes with control in three *Wistar* rat populations that lived under similar conditions but varied diets. We measured the weight and blood plasma ghrelin levels weekly. After 12 weeks, we sacrificed the rats and performed Nissl staining on coronal brain segments to observe the changes in three key orectic nuclei: VMH, ARC, and PVN. We found that obese rats have lower ghrelin and increased hypothalamic VMH and ARC volumes compared to the control. The opposite is true for rats with AN. PVN volume does not change in all three conditions. Sexual dimorphism was present and accounted for.

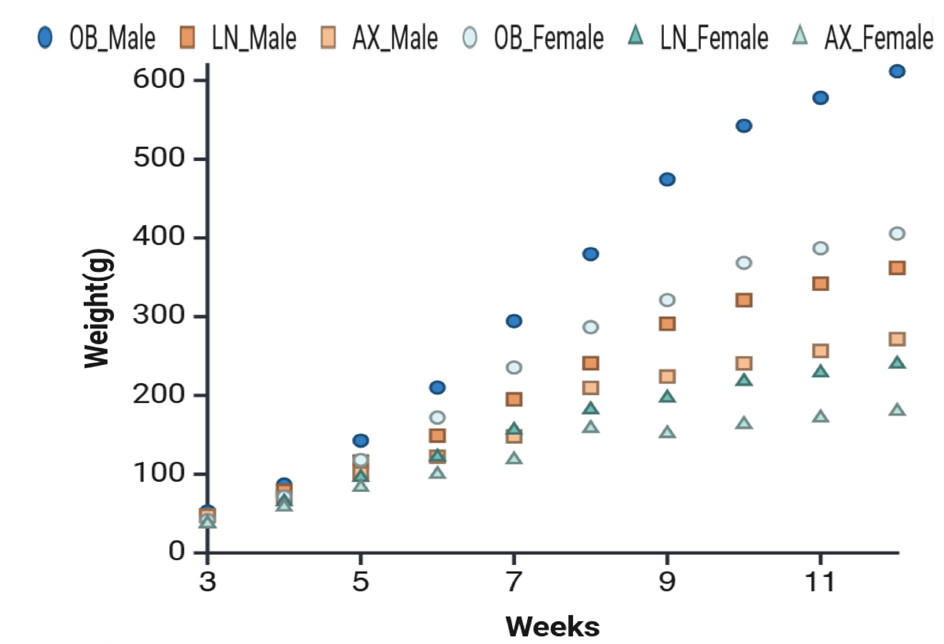

Figure.3: Weight change in male and female rats starting from 3 weeks until 12 weeks.

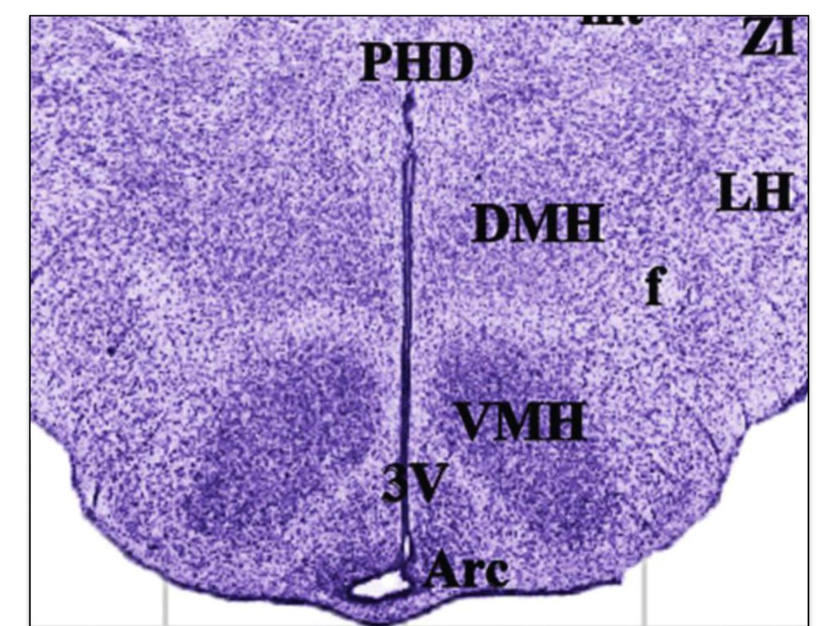

Figure.4: Nissl-stained rat hypothalamus with the nuclei of interest labeled[6].

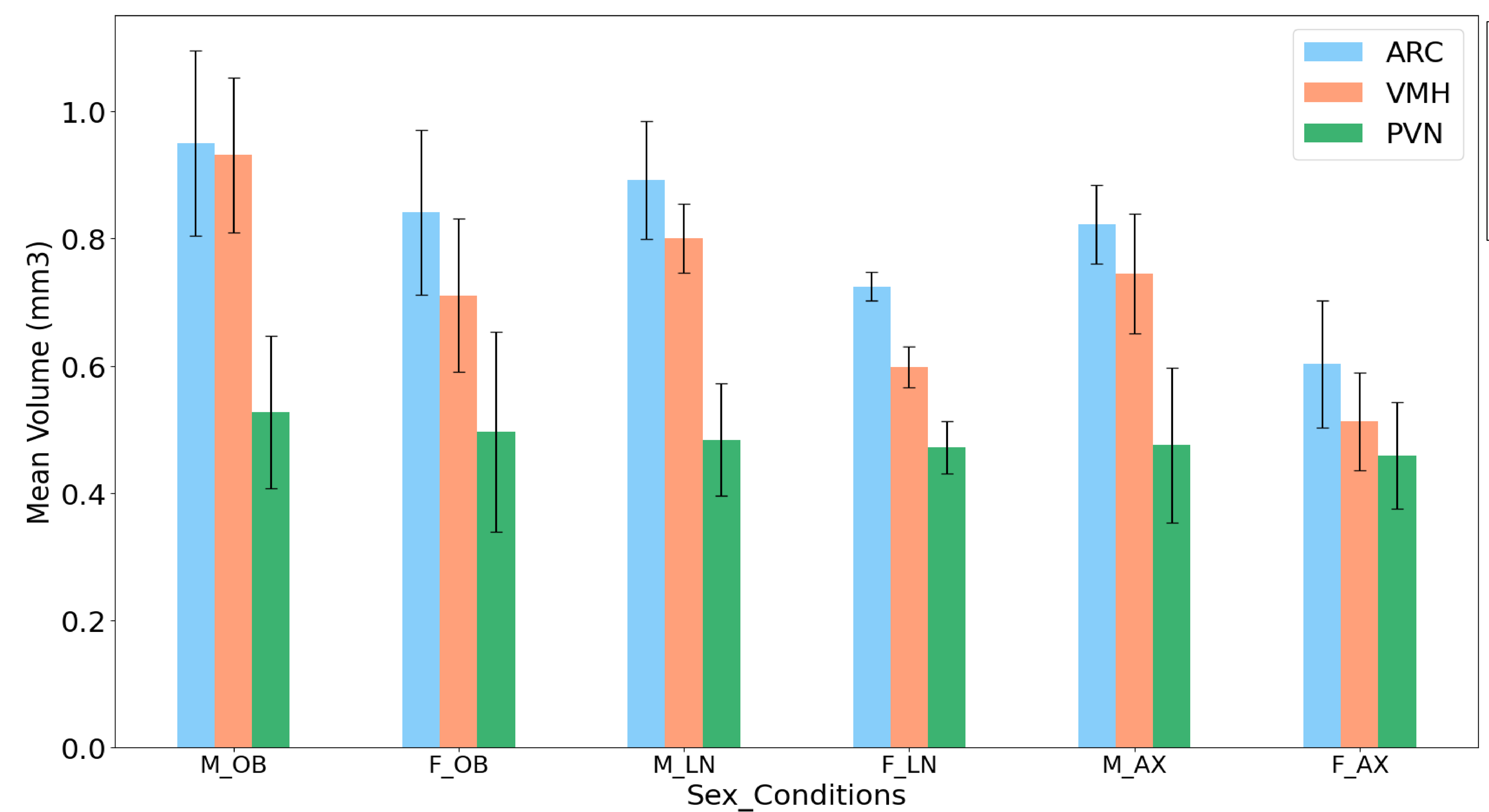

Figure.5: Rat hypothalamic nuclei volume in mm3.

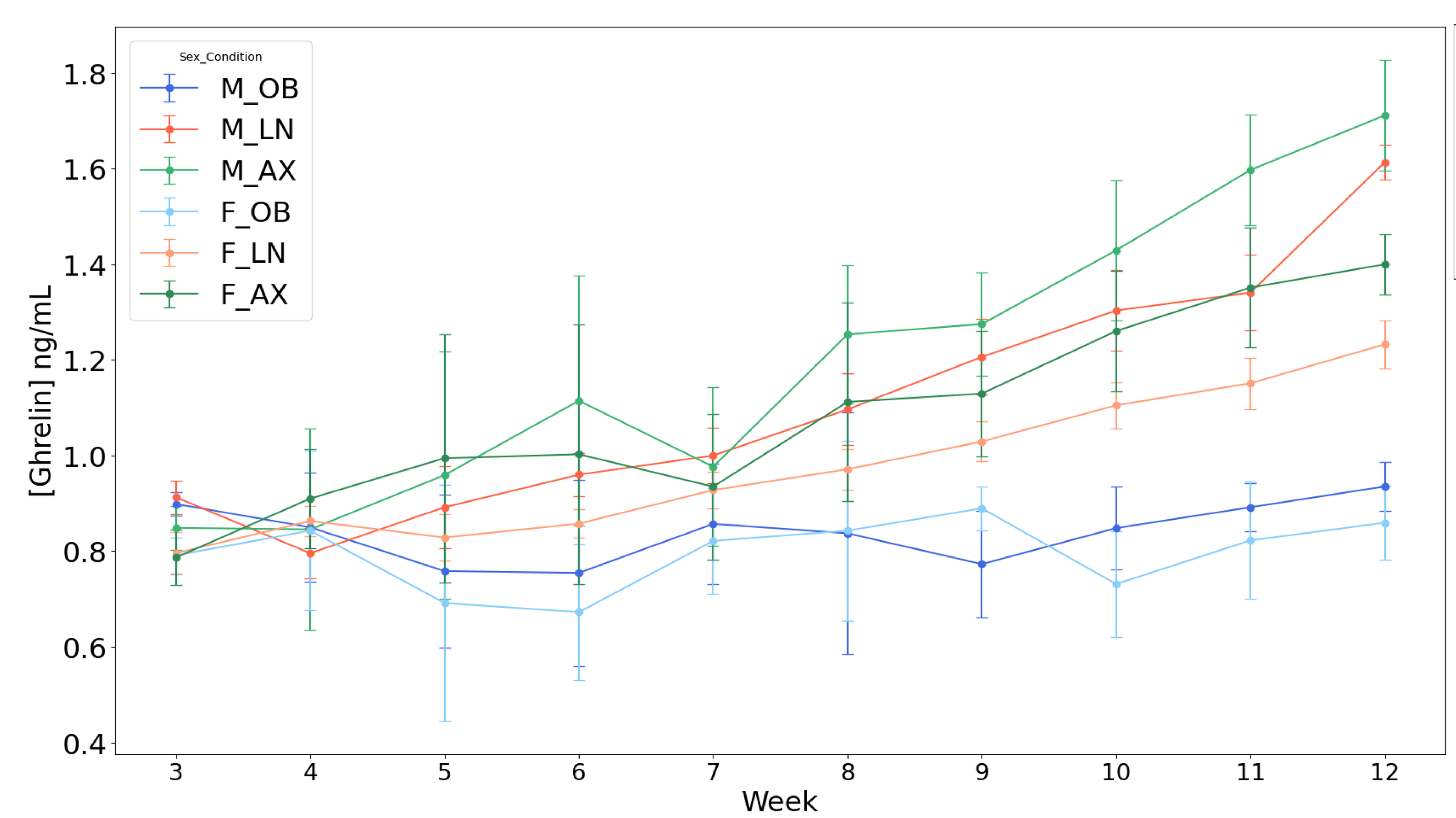

Figure.6: Ghrelin concentration measured in the rats over 12 weeks.

Methodology

For this experiment, we will consider the ghrelin-induced change in morphology in the hypothalamus of *Wistar* rats:

- The study will last 12 weeks
- These rats will be divided into three groups
- Each group will have n=20 rats,
  - Male n=10 male and female n=10,
- We will keep the diet steady until 21 days postnatal, as the rats achieve 90-95% brain maturity at this time

Each rat group will be fed a specific diet that will induce the three desired conditions:

- A high-fat diet that will induce obesity in the rats (OB)
- A balanced diet that will be used to maintain a lean control group (LN)
- A low-calorie diet that will induce an anorexic-like diet (AX)

Weekly measurements:

- Blood plasma will be extracted, and total ghrelin concentration in the bloodstream will be measured through a commercial ELISA kit
- Weight will be calculated weekly as well

After 12 weeks:

- The rats will be euthanized, and their brains will be extracted and coronally segmented
- Cresyl violet (CV) will be used to stain all the nuclei in the rat brain
  - CV does so by binding to a protein called Nissl in the ER
- CV staining will determine the differences in the ghrelin-affected areas in the hypothalamus by microscopic analysis of the coronal segments

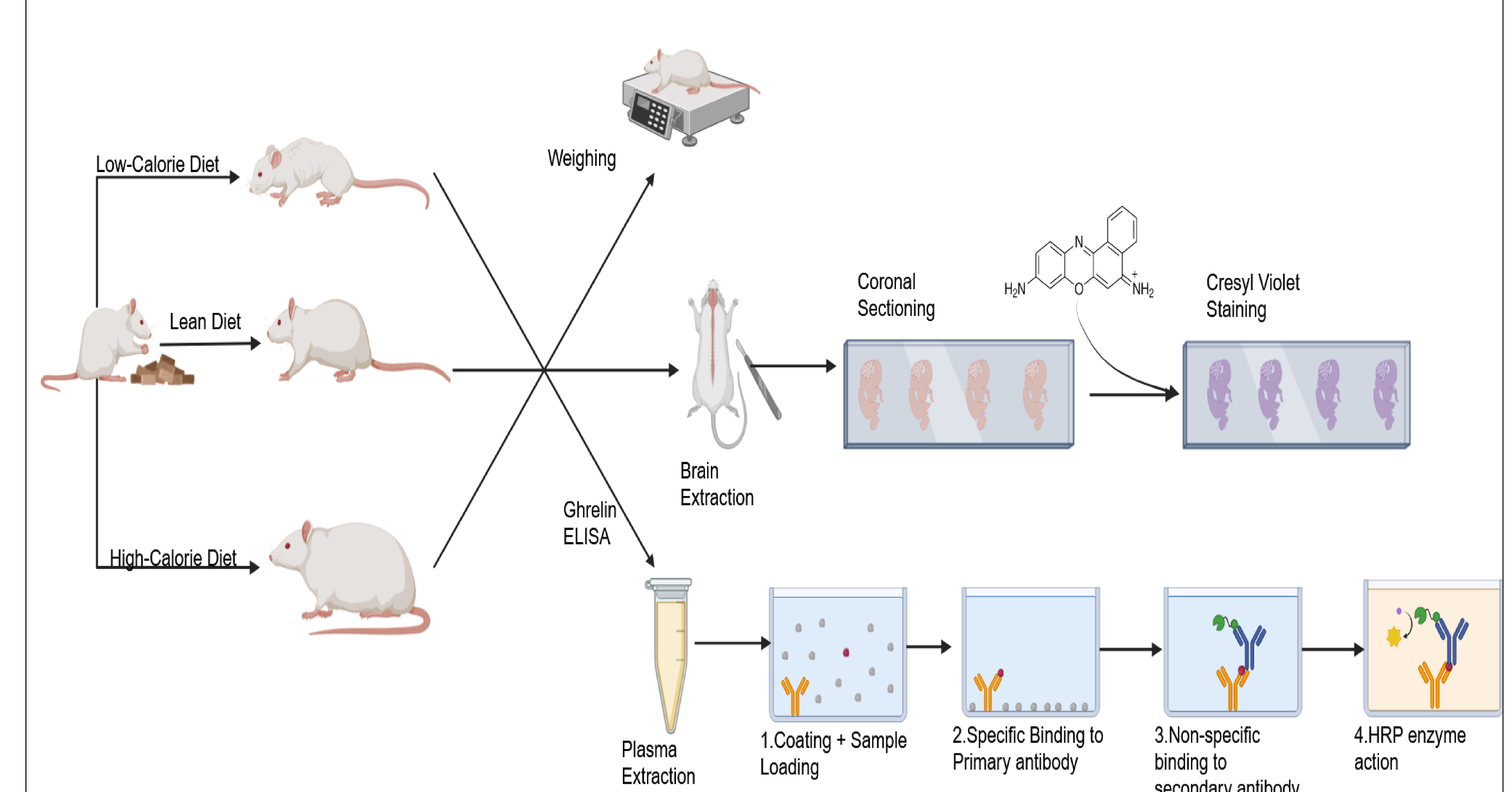

Figure.2: Figurative representation of the methodology.

- We will analyze the VMN, ARC, and PVN
  - The LHA will be noted, but its volume is difficult to calculate and will not be analyzed
- We can use the Nissl stain data to measure the volume of each subject through the equation provided by Matsumoto and Arai[16]:

$$V=\frac{h}{3}(A_1+\sqrt{A_1A_2}+2A_2+\sqrt{A_2A_3}+2A_3+\cdots\cdots$$
$$+2A_{n-1}+\sqrt{A_{n-1}A_n}+A_n)$$

  - V is the nuclear volume, A(n-1) is the area of the (n-1)th section, An is the area of the nth section, and h is the distance between the (n-1)th and nth sections.
- The volume differences between the three groups will be recorded as averages on a bar plot
  - These plots will also account for the sex of the rats, as sexual dimorphism has an impact on the volumes of the VHN and ARC.

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17. I developed Figures 1-3 through Biorender. Figure 2 takes coronal cutting of the brain and Nissl staining from Ref.[2]. Figure 3 uses data from Ref.[16] that I altered myself.
18. Figure 5 used data from Ref.[10,13,14] and Figure.6 uses data from Ref.[3]. Values were randomly generated in python and visualization code was aided by ChatGPT. Code is available on request.