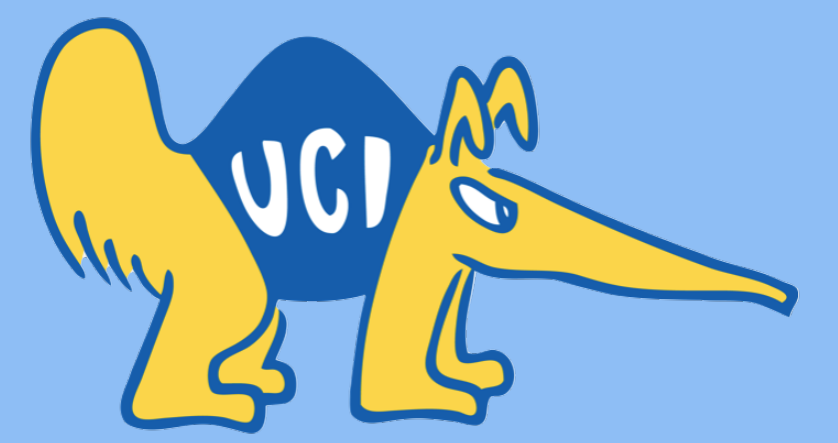


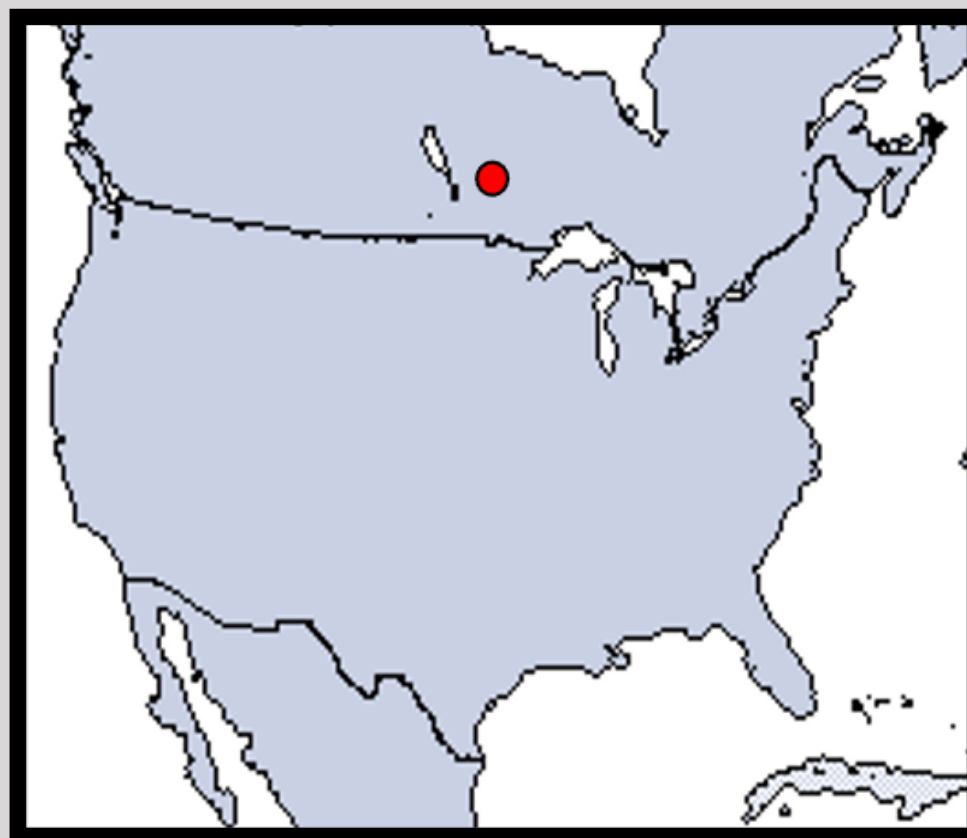


Why Did the Snake Cross the Road?



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The red dot depicts the location of these snakes, in Manitoba Canada.

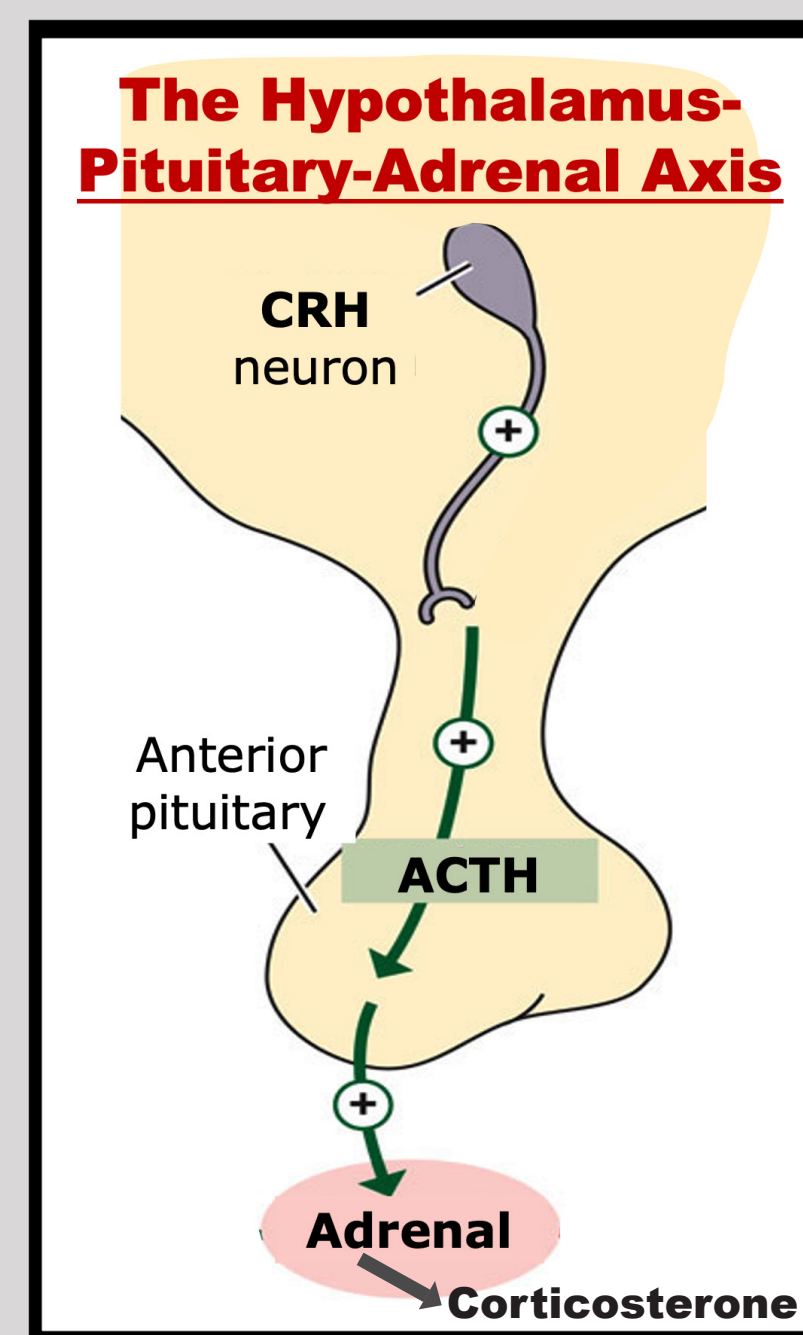


Figure 1. Corticosterone (the stress hormone in snakes) is created within the HPA axis. Corticotropin-releasing-hormone (CRH) triggers the cascade that results in the production of adrenocorticotropic hormone (ACTH). ACTH then causes the production of corticosterone.

Introduction

- Red-sided garter snakes (*Thamnophis sirtalis parietalis*) come out of hibernation and enter mating season. We refer to the location of their mating season as the “den”.
- Once mating season ends the snakes migrate from the den to their feeding grounds. The “road” is the best place to find and capture the migrating snakes.
- Corticotropin-releasing-hormone (CRH) initiates the production of corticosterone (Figure 1), which is a type of glucocorticoid and known as a stress hormone in snakes.
- Glucocorticoids are known to reach their highest levels at den sites (Figure 2; Cease et al., 2007). This is interesting because stress hormones are known for suppressing reproductive capabilities.
- We collected snakes and measured levels of CRH at the den and at the road.
- We predicted that after mating CRH levels would decline, triggering the snakes’ migration. Therefore, we expected to find lower CRH levels in snakes collected while migrating (at the road).

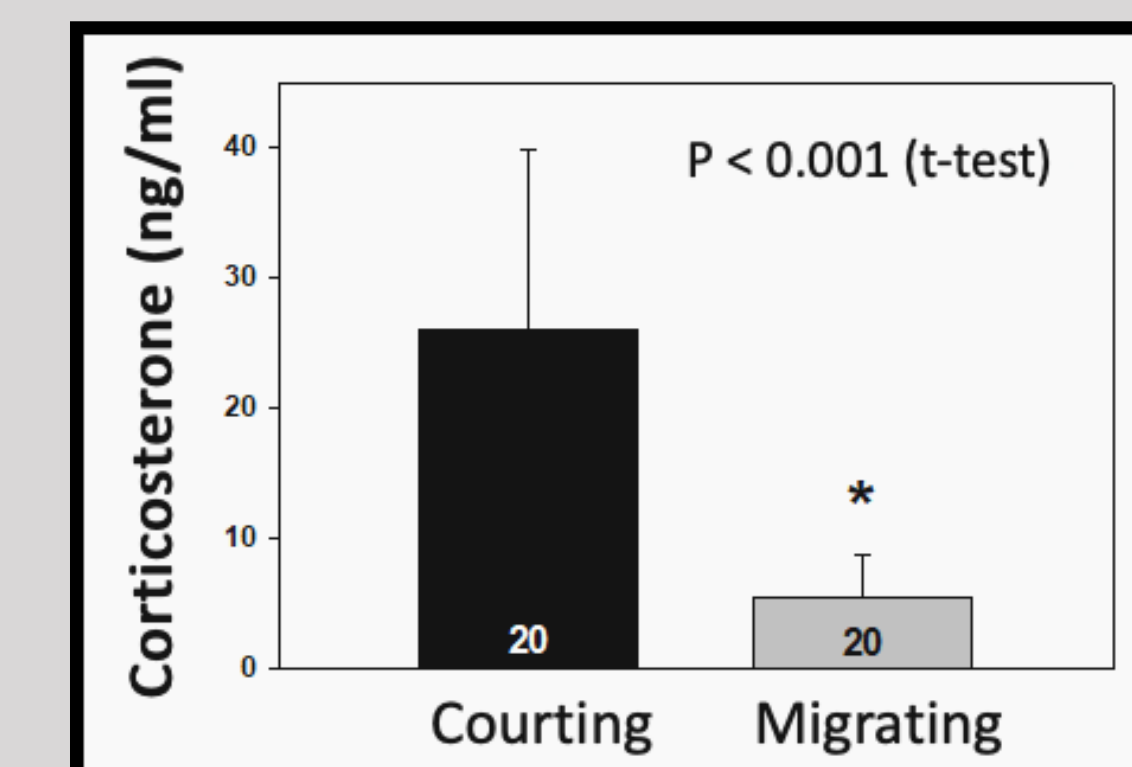
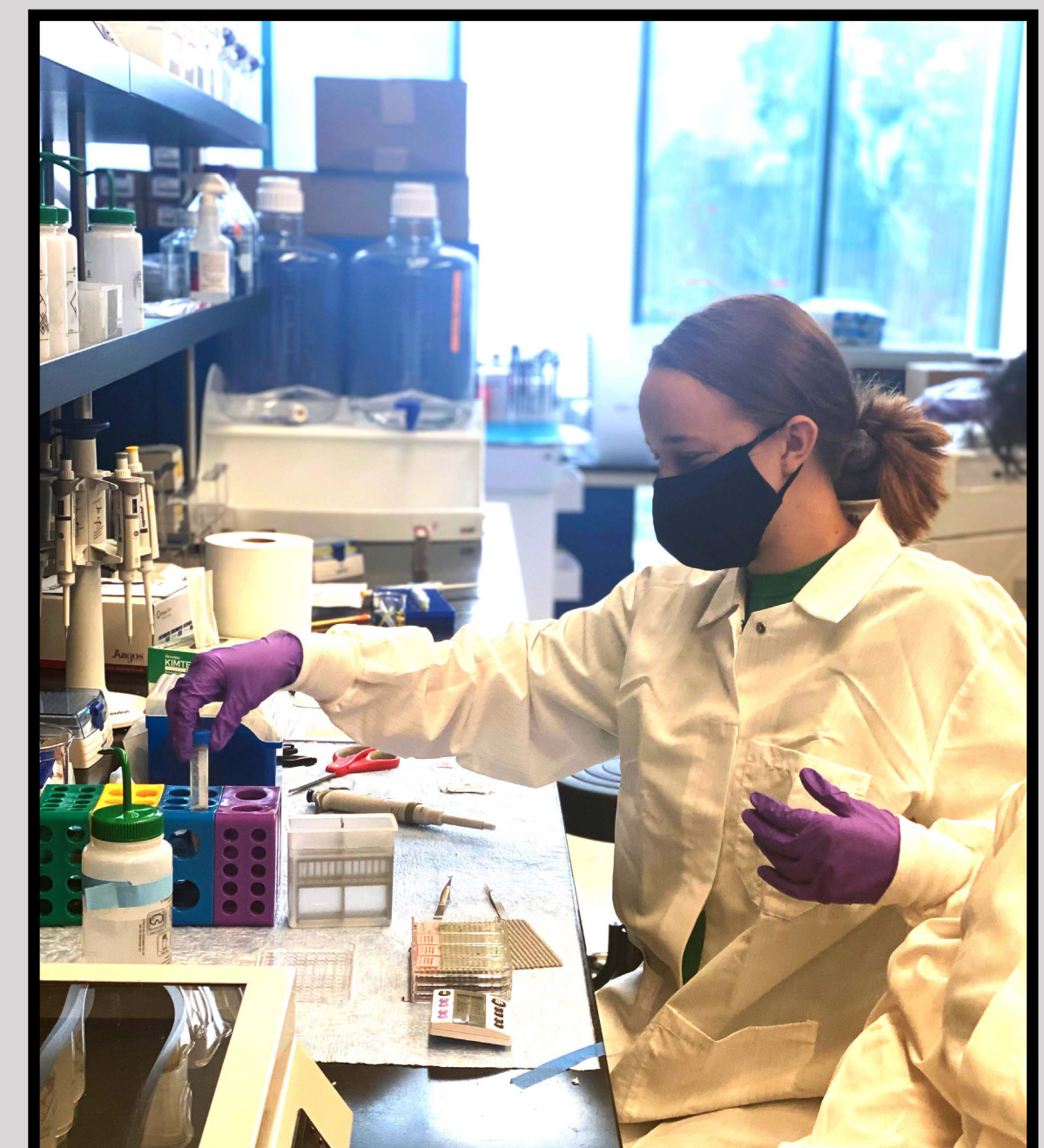


Figure 2. Baseline corticosterone is significantly lower in migrating male snakes (Cease et al., 2007).

General Methods

- Snakes were collected at the “den” site (during mating) (n= 14) and the “road” (while migrating) (n=11) in Manitoba Canada (Figure 3).
- The snakes utilized in this data were euthanized, their brain tissues were fixed, and immunohistochemistry was completed in order to stain CRH.
- CRH containing cells located in the paraventricular nucleus of the hypothalamus were counted (Figure 4). CRH containing cells were identified in the cortex, but not counted.
- Statistical analysis was completed. Significance was determined using a two-tailed t-test.

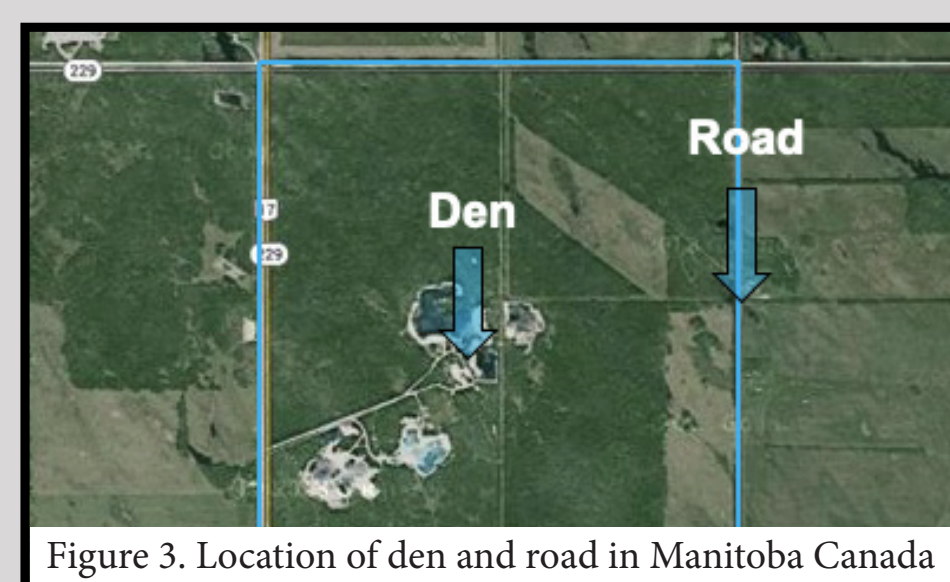


Figure 3. Location of den and road in Manitoba Canada

Counting Corticotropin-Releasing Hormone Cells

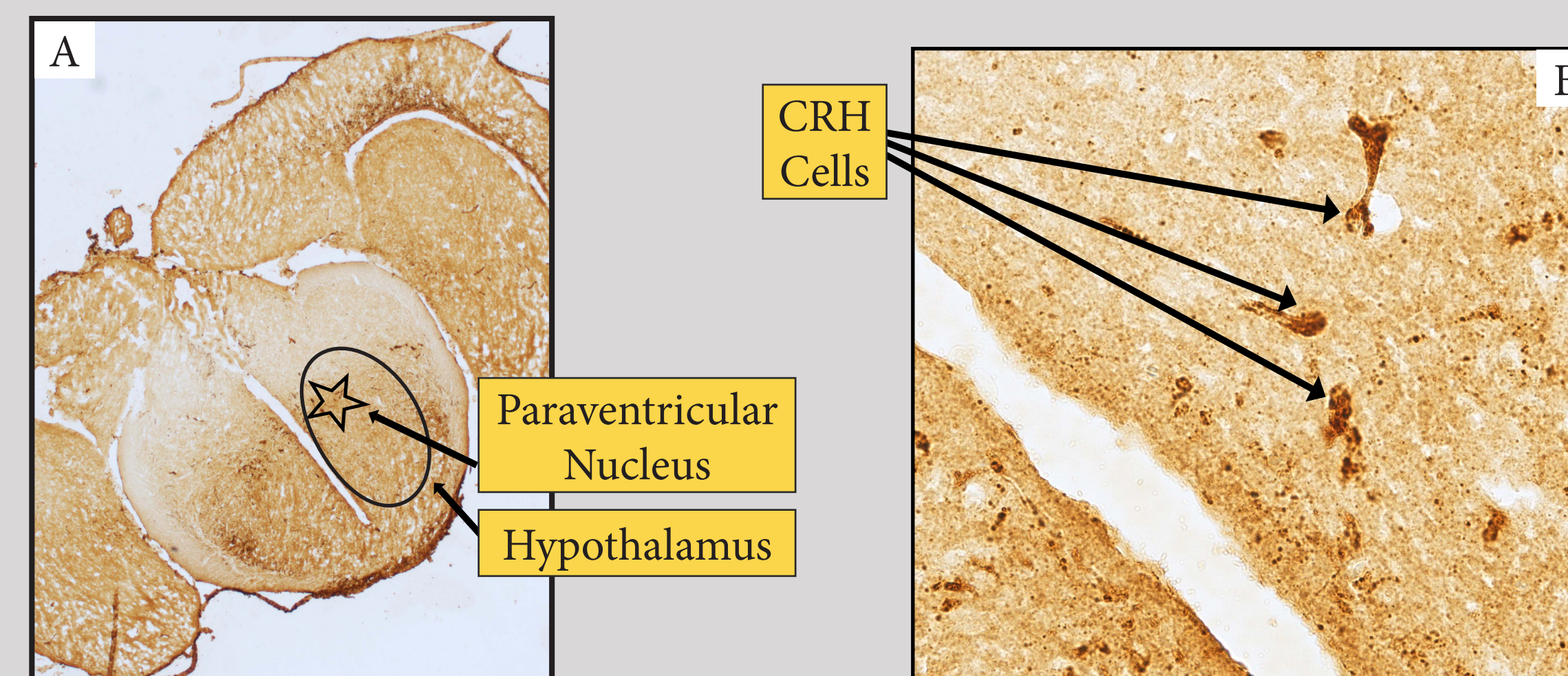


Figure 4. images of snake brain tissue. (A) shows parts of the cortex as well as the paraventricular nucleus and the hypothalamus. The hypothalamus (encircled) is responsible for many things. Such as releasing hormones, maintaining homeostasis, and regulating sex, emotions, and hunger. The paraventricular nucleus (starred) regulates stress and reproduction. (B) depicts three CRH cells in the paraventricular nucleus.

Results

- Snakes at the den and the road exhibited CRH fibers (Figure 5) and cells.
- We found no statistical difference in the number of CRH cells between den and road groups (p=0.44) (Figure 6).

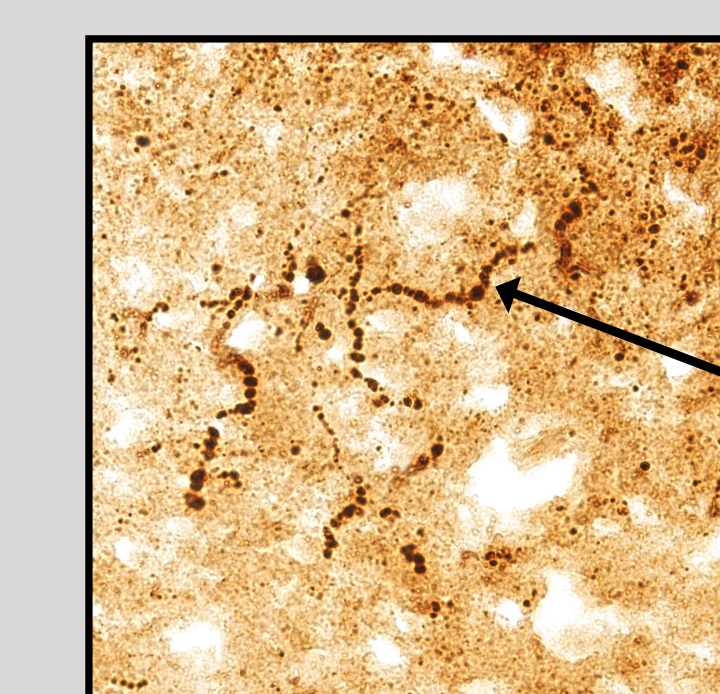


Figure 5. CRH fibers in the paraventricular nucleus of the hypothalamus.

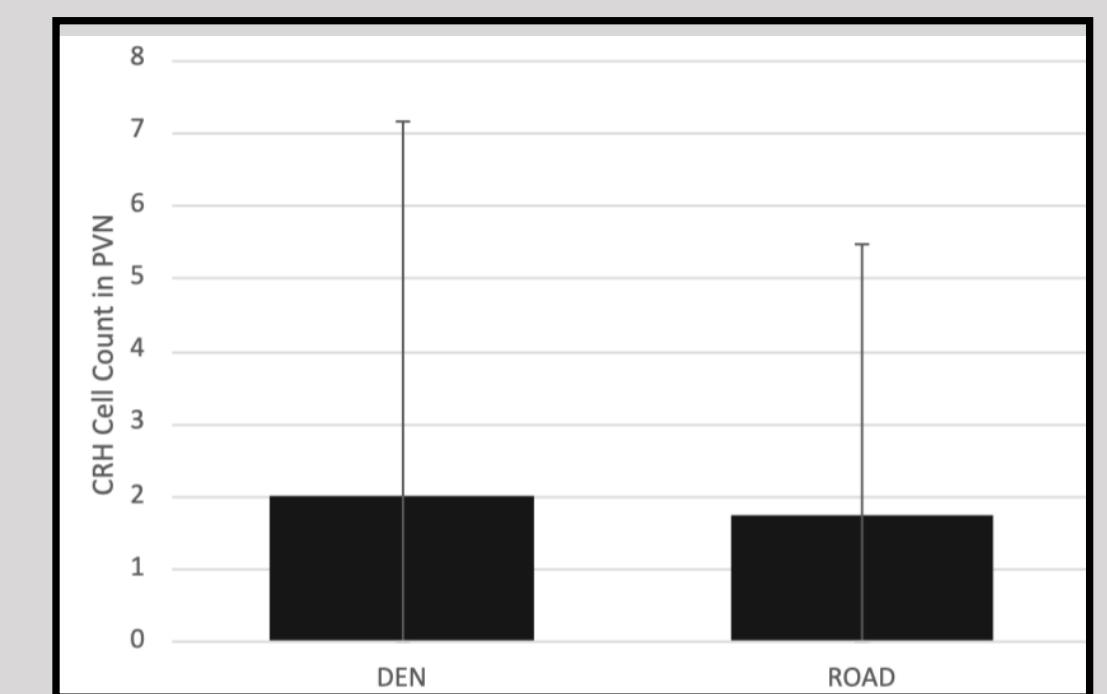


Figure 6. the average number of CRH cells in a single brain section portraying the PVN of a snake at the den or the road. Error bars represent standard deviation.

Results, continued. CRH Found in Cortex (Hippocampus)

- Although we didn’t quantify their density CRH cells were found in the cortex (encircled) (Figure 7).
- CRH is known as a hormone, finding cells containing CRH in the cortex shows its function as a neuromodulator.

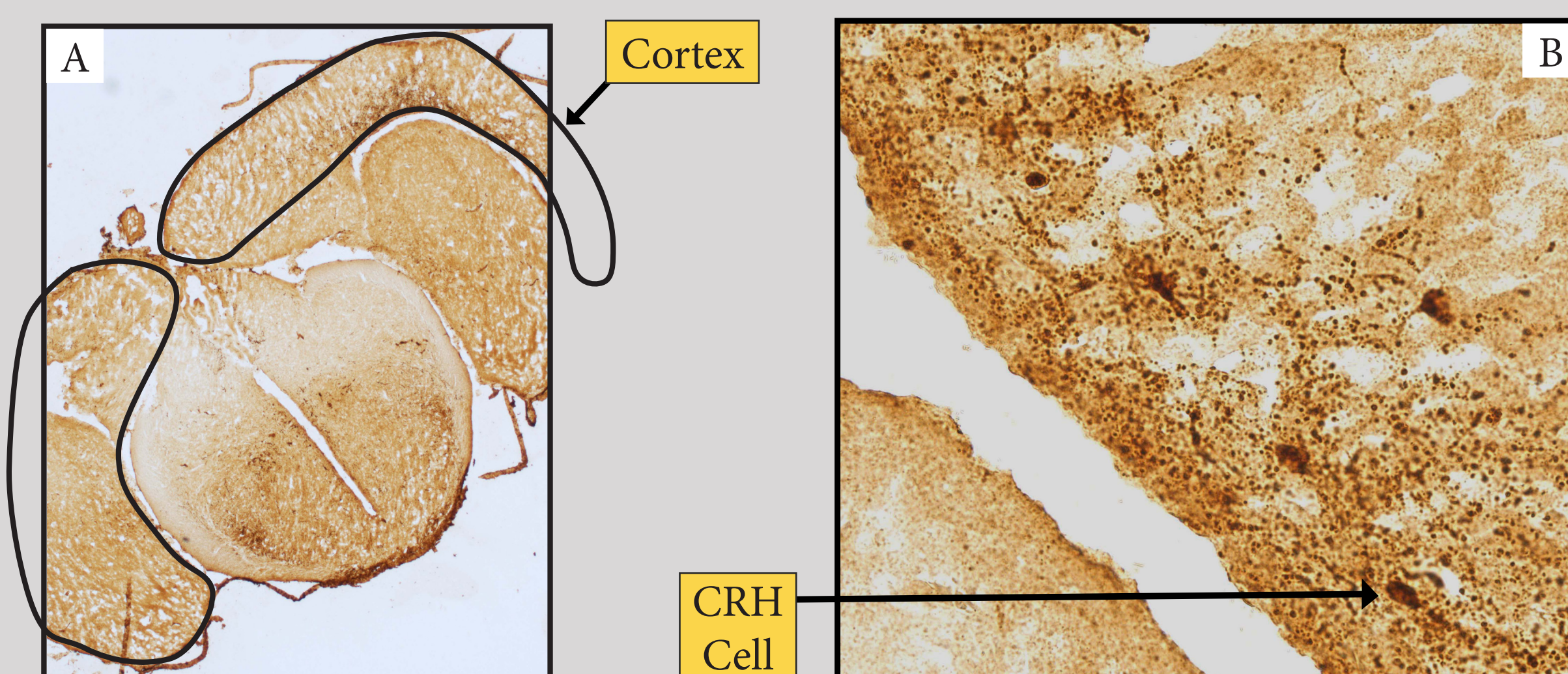


Figure 7. images of snake brain tissue. (A) cross section highlighting the cortex. (B) close up showing four CRH cells in the cortex.

Conclusion

- The snake crossed the road because he was hungry!
- CRH was found in the hypothalamus, but there was no difference in density between den and road populations
- Red-sided garter snakes are known to exhibit lower levels of glucocorticoids in migration than during mating season (Cease et al., 2007). Since we did not find differences in CRH levels between mating and migrating snakes it does not appear the change in glucocorticoid levels is due to change in CRH levels.
- The presence of CRH in the cortex suggests that it is functioning as a neuromodulator.

Future Directions

- What is causing the change in glucocorticoids?
- Is there a difference between CRH cells in the cortex between den and road snake populations?

Acknowledgements

