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Analyzing Predicted Open Chromatin Regions in the Brain across Homeothermic and Non-Homeothermic Species

Abstract

Understanding whether a trait can exhibit evidence of convergent evolution is crucial for uncovering the adaptive process that drives similar phenotypes across distant species. The trait analyzed in this project is homeothermy. Homeotherm is the ability for a species to regulate its own body temperature at a constant level regardless of its environment. Homeothermy is a discrete trait that has evidence of convergent evolution by body size adaptations. Some mammal species like humans (*homo sapiens*) regulate their body temperature well, whereas other mammals, like the house mouse (*mus musculus*) don't do it as well and rely on their environment to cool and for heat.

Maintaining bodily temperature has been shown to be a function of the brain. Two common cell types that help mediate this temperature regulation are astrocytes and VIP neurons. Therefore, by looking for the presence of a correlation between open chromatin regions from these cell types and the homeotherm trait, this study analyzed nearby relevant genes: two human genes, TSKU and HSPB3, and two mouse genes, Spef1 and TEAD1. These genes tended to have higher open chromatin regions across multiple cortical cell types on the Allen Brain Map for human and mouse cells, indicating evidence of a broader mechanism in these gene's effect on homeothermy. Additionally, myo-inositol transport, a biological process related to transport of the sugar inositol, was an enriched gene ontology term in significant upregulated peak regions in VIP neurons, which may suggest a pathway that allows homeothermic species to regulate their body temperature. For the field moving forward, a better sense of how species are able to effectively regulate their body temperature in a stable fashion can be obtained through analyzing expression levels from various cortical cell types. This could even lend itself to genetic engineering approaches in which species are modified to become stronger homeotherms, and thus become well suited to varying environments.

Introduction

A. Background of Homeotherm

The trait studied is homeothermy. If an animal is homeothermic, it should have "maintenance of a constant internal body temperature" (Scholander). As a clear example, modern humans are homeothermic because they maintain a constant internal body temperature. Conversely, reptiles are not homeothermic. Their body temperature depends on "the temperature of their environment." On the other hand, a species can be classified as poikilothermic, which means their body temperature is dependent on their ambient temperature. Although there are also

species that are heterothermic, which act in a sort of medium between these two traits where they can both self-regulate their body temperature and adapt to their environment, this analysis only labeled species binarily. Species were considered homeotherms or non-homeotherms, which is the category that includes heterotherms.

Evidence of convergent evolution for this trait is seen especially when comparing the body size information of certain species over history. This stems from unrelated species evolving similar body sizes due to requiring a balance between storing heat and releasing heat. It is generally seen that larger body sizes help reduce heat loss due to a smaller surface area-to-volume ratio. This suggests that there was a selective pressure for larger species to develop the homeotherm trait which may not be due to the ancestry of the species.

Homeothermy is related to the brain because “our internal body temperature is regulated by a part of our brain called the hypothalamus” (“In Brief: How Is Body Temperature Regulated and What Is Fever?”). This means that homeothermy is vitally regulated by the brain and crucial to the survival of a species. For example, when internal body temperature is raised too high in humans, the hypothalamus will help the individual sweat to cool skin or release heat. If the body temperature is too low, the hypothalamus tries to create and maintain heat. Therefore analyzing homeothermy in open chromatin regions of cell types found in the cortex, another related brain region, would be useful.

B. Background of Species

As part of this investigation project, an analysis was done on a set of 163 species from the Zoonomia dataset annotated with the homeotherm trait. Among the 163 species, 37 species were annotated as “Non-Homeotherms” and 126 species were annotated as “Homeotherms.” In Figure 1, a phylogenetic tree of these species with their annotation can be visualized.

To further analyze why a species was labeled a “Non-Homeotherm”, it is important to consider the definition of what criteria scientists use. In ‘The Mouse: An “Average” Homeotherm’, Gordon argues that merely possessing the capability to regulate body temperature should not be the sole criteria for classifying a species as homeothermic. For example, while the mean difference in core temperature between a mouse and an elephant over several days is typically within 2.7°C , small enough to meet the homeothermic criteria, the variability in core temperature within a 24-hour period clearly differentiates these species. A mouse’s core temperature can fluctuate by as much as $2\text{-}4^{\circ}\text{C}$ within a single hour. Therefore, the trait homeotherm also considers the size of the species. Small mammals, such as rodents and bats, whose higher surface-area-to-volume ratio causes them to lose heat more rapidly to the environment, results in their bodies having greater temperature variability compared to larger mammals. On the other hand, larger species similar to elephants, with larger body size and a lower surface-area-to-volume ratio allow them to better retain heat and maintain a more stable body temperature classifying them as homeotherms. In this project, both homeotherms and non-homeotherms were analyzed to see patterns in open chromatin regions that lead to the species being classified as such.

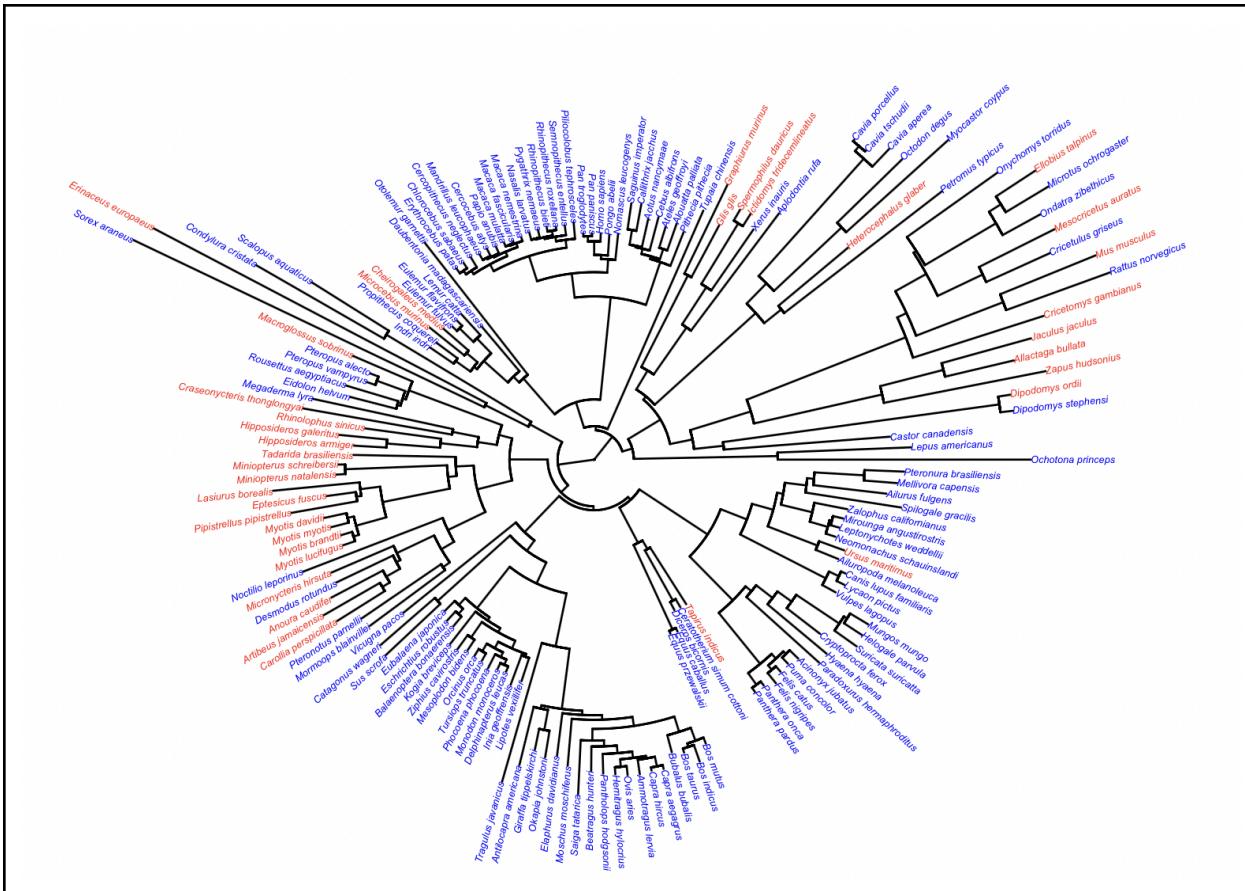


Figure 1: Phylogenetic Tree labeled based on Homeothermic properties

In the phylogenetic tree, all species analyzed in this project are displayed in a fan template showing their ancestral relationship and annotation of the homeothermic trait. Note that the trait homeotherm (species in blue) show evidence of convergent evolution since the trait emerges independently across unrelated lineages. This means this trait was developed as a result of similar selective pressures rather than shared ancestry.

C. Background on Cell Types

As previously mentioned, the ability for a species to exhibit the homeothermic trait is a direct function of the hypothalamus in the species' brain. Given cortical cell data for this investigation, two cell types of interest were astrocytes and VIP neurons. Astrocytes, a subtype of glial cells, are found throughout the central nervous system. Astrocytes play a lot of roles, but are most commonly involved in “metabolic, structural, homeostatic, and neuroprotective tasks” (Wei & Morrison). They do not conduct electrical signals, but help the neural cells that do conduct electrical signals. They look similar to stars, in what is known as “radially-arranged foot processes” (Wei & Morrison).

Astrocytes seem to have a strong association with homeothermy since this cell type is involved in homeostasis by helping to control the blood-brain barrier through secreted factors that aid in the formation of tight junctions and the association between cells in the brain-blood

barrier (Manaserh et al). More directly, however, it was found in a study that mice lacking insulin receptors in their astrocytes had lower energy expenditure and lower fasting body temperature. In the cold, “they were able to mount a thermogenic response” (Manaserh et al). This indicates that although insulin played a key role, the absence of them from astrocytes affected their body temperatures. As a result, one can assume that astrocytes play a role in body temperature regulation across more species than just mice.

VIPs or Vasoactive Intestinal Peptide neurons are a small set of neurons found in the mammalian cortex that display the peptide. These neurons, found in the cortex and the suprachiasmatic nucleus (SCN) of the brain, play an important role in modulating cortisol activity of an organism (Zou & Hires). By inhibiting or disinhibiting other neurons, VIPs are crucial in fine-tuning activity in the neural network. One of the primary roles of VIPs in the SCN is to maintain circadian rhythms. They do this by being responsible for responding to light in the retinas, are involved in stimulating glycogenolysis to regulate metabolism and are continuously active at night to regulate ‘siesta’ sleep (Todd et al.). It has been shown that an organism’s body temperature follows its circadian rhythm pattern (Yu and Li). This is because during times of rest, body temperature decreases. While doing activity, body temperature rises. This is common amongst most endothermic homeotherm organisms. Thus by coordinating circadian signals, VIP neurons can help ensure that the thermoregulation aligns with the body’s daily active cycle which is necessary for survival of homeothermic organisms (Szadai et al.). Unfortunately the data accessible for this study had cortical VIP neurons rather than hypothalamic VIP neurons. However, it is possible that both cell types have similar expression and since cortical neurons do receive sensory information on temperature through the spinal cord, patterns observed in the cortical VIP neurons could translate to patterns observed with hypothalamic VIP neurons.

Results

The initial step in analyzing the association between the trait and open chromatin regions was to perform an association test. ATAC-seq peaks from two cell types, astrocytes and VIP neurons, were sampled. Since humans are classified as homeotherms and mice as non-homeotherms, this study sampled 10,000 annotated peaks from both species in hopes to identify potential differences in the trait association with the chromatin region. Using these 10,000 peaks and the phylogenetic tree from Figure 1, a phylogenetic linear model, phylolm, was fit to model the relationship between homeotherm and the accessibility of the open chromatin region while considering the evolutionary relationship between the species.

As seen in Figure 2, the distribution of p-values and adjusted correlation values for each peak after performing phylolm are plotted for each cell type and each species’ background set of peaks. Looking at the distributions, a Wilcoxon rank-sum test was performed to analyze the differences in the distributions. For the same cell type, the distribution of p-values and adjusted correlation was statistically significant; while for the same species, the distribution of p-values and adjusted correlation was not statistically significantly different. This suggests that the effect

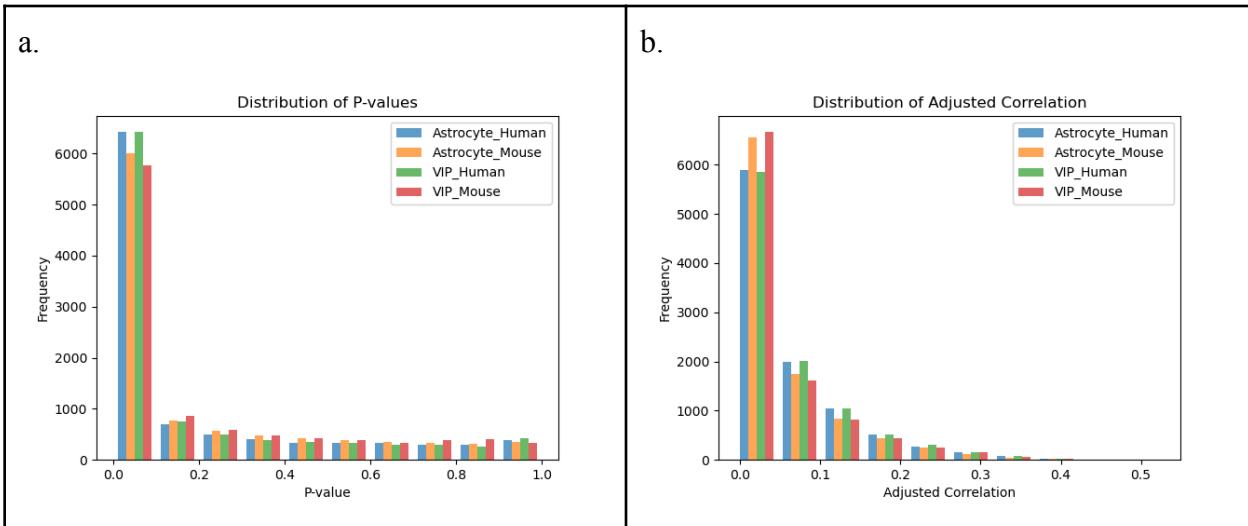


Figure 2: Distribution of p-values and adjusted correlation across astrocytes and VIP neurons in humans and mice. A Wilcoxon rank-sum test was performed to compare the distributions. Comparing the distribution of Astrocyte Human and Astrocyte Mice had an effect size of 0.061 ($p\text{-value} < 0.001$). Comparing the distribution of VIP Neuron Human and VIP Neuron Mice had an effect size of -0.004 ($p\text{-value} < 0.001$). Comparing the distribution of Human Astrocyte and Human VIP Neurons had an effect size of 0.016. Comparing the distribution of Mice Astrocyte and Mice VIP Neurons had an effect size of 0.079 ($p\text{-value} < 0.05$). Note that the effect size of the p-value distribution is inversely equal to the effect size of the adjusted correlation distribution using the normalization method for a rank-sum test.

of homeotherm may be species specific rather than cell type specific. In addition, Figure 2a

	Positively Associated Regions	Negatively Associated Regions
Human Astrocytes	5996	0
Human VIP	5980	0
Mouse Astrocytes	5385	0
Mouse VIP	5124	0

Table 1: Number of Positively and Negatively Associated Chromatin Regions After Performing Multiple Hypothesis Testing. A Benjamini-Hochberg procedure was applied to the full set of peaks to correct for false discovery rate.

shows that a lot of peaks across all 20000 peaks (10000 Human and 10000 Mice) had a p-value close to 0, indicating a lot of possibly statistically significant peaks, regardless of cell type and species the peak originates from. After performing multiple hypothesis p-value correction in both species across both cell types, the number of positively associated and negatively associated regions were identified as seen in Table 1. The number of positively associated regions has decreased from the initial p-value distribution. Even after correction, there were 0 negatively associated regions which was acceptable as sometimes the data lends itself to being statistically underpowered.

<p>a. Test: Positively Associated Human Astrocyte</p> <p>Background: Whole Genome</p>	<p>b. Test: Negatively Associated Human Astrocyte</p> <p>Background: Whole Genome</p>	<p>c. Test: Positively Associated Human Astrocyte</p> <p>Background: All Astrocyte Peaks</p>	<p>d. Test: Negatively Associated Human Astrocyte Background: All Astrocyte Peaks</p>
<p>e. Test: Positively Associated Human VIP Neuron</p> <p>Background: Whole Genome</p>	<p>f. Test: Negatively Associated Human VIP Neuron</p> <p>Background: Whole Genome</p>	<p>g. Test: Positively Associated Human VIP Neuron</p> <p>Background: All VIP Peaks</p>	<p>h. Test: Negatively Associated Human VIP Neuron</p> <p>Background: All VIP Peaks</p>
<p>i. Test: Positively Associated Mice Astrocyte</p> <p>Background: Whole Genome</p>	<p>j. Test: Negatively Associated Mice Astrocyte</p> <p>Background: Whole Genome</p>	<p>k. Test: Positively Associated Mice Astrocyte</p> <p>Background: All Astrocyte Peak</p>	<p>l. Test: Negatively Associated Mice Astrocyte</p> <p>Background: All Astrocyte Peaks</p>
<p>m. Test: Positively Associated Mice VIP Neuron</p> <p>Background: Whole Genome</p>	<p>n. Test: Negatively Associated Mice VIP Neuron</p> <p>Background: Whole Genome</p>	<p>o. Test: Positively Associated Mice VIP Neuron</p> <p>Background: All VIP Peaks</p>	<p>p. Test: Negatively Associated Mice VIP Neuron</p> <p>Background: All VIP Peak</p>

Figure 3: GREAT analysis for the genes associated with the top 200 Peaks. Each of the outputs from performing GREAT analysis on the set of peaks and the corresponding background is displayed above. Note that most of the inputs showed no significant results except Figure 5f, 5g, 5j and 5k.

To analyze these regions, it may be interesting to look at a set of peaks to see if they correspond to a pathway. Therefore the top 200 significantly positively and negatively associated regions were used to perform gene ontology analysis with GREAT. This will hopefully identify if there is a pathway enrichment present in homeotherm species which encourages them to display the trait. In Figure 3, all of the permutations of background and peaks are presented. From the analysis in Figure 3, most of the peaks have no enriched pathway shown. However, the few enriched pathways that were identified were plotted in Figure 4. Even after looking at these pathways, it is clear that most of the pathways fall in the category of cellular components. The only enriched biological process was from the positively associated VIP neuron human peak (Figure 3g) called the myo-inositol_transport pathway. Inositol is a key molecule in many metabolic pathways by ensuring that inositol, a sugar, is available to maintain body temperature. It is also essential for osmotic balance in the mammalian brain, which fluctuates due to temperature fluctuations. This is very interesting because it shows that significant positive VIP peaks may have a cumulative effect that results in the expression of a relevant biological pathway.

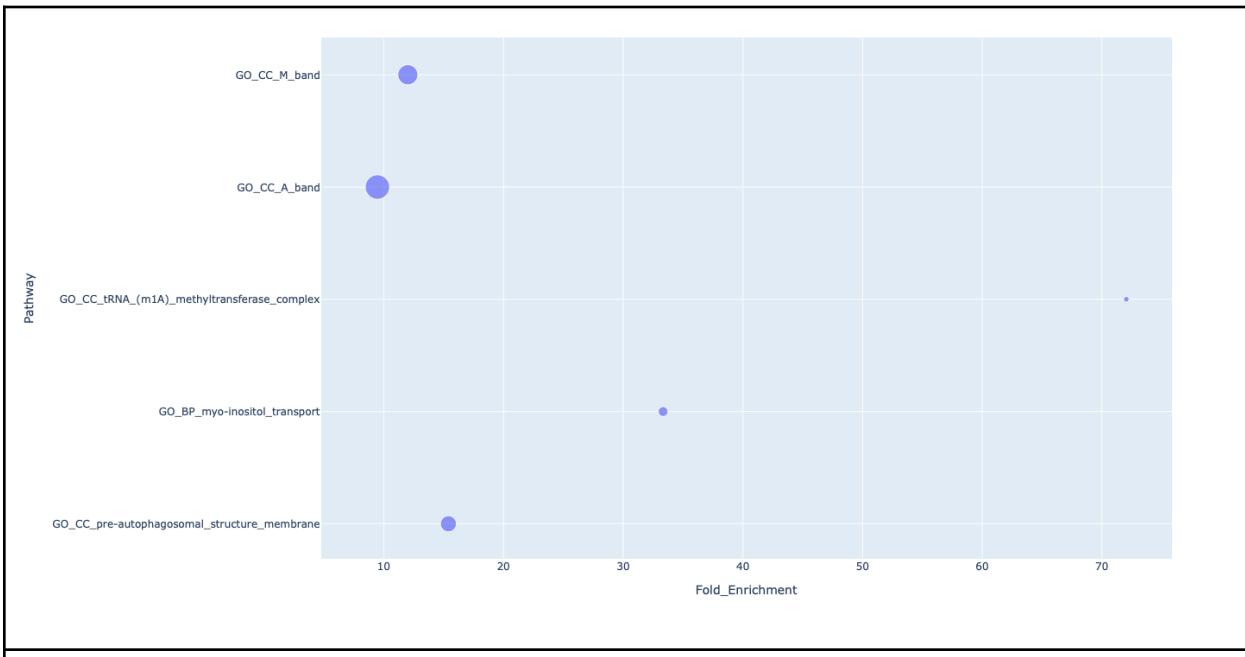
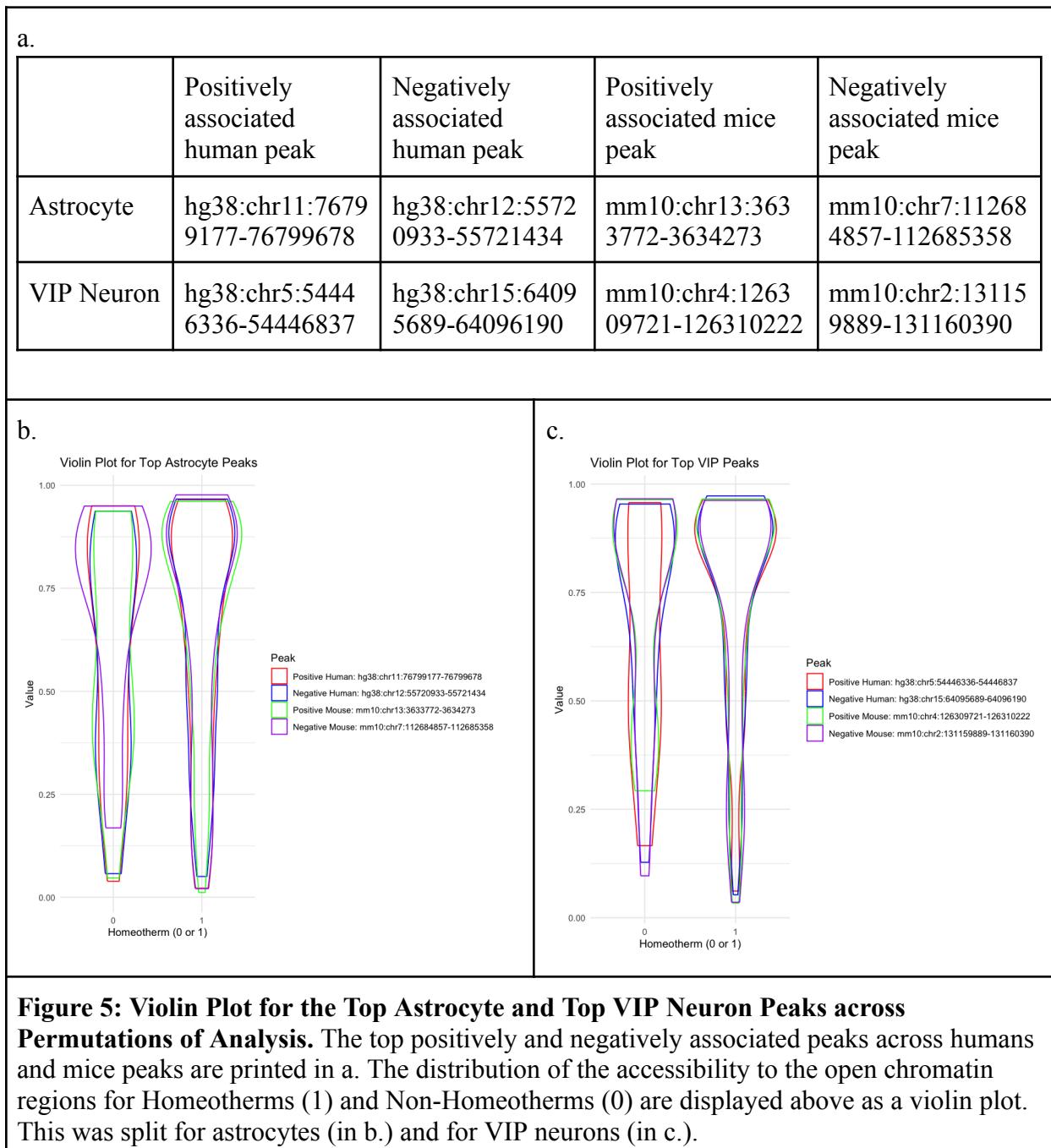


Figure 4: Pathways enriched from the GREAT analysis in Figure 3. The enriched pathways from Figure 3, were plotted on this dot plot with the pathway name and the x-axis representing the fold enrichment of the pathway in homeothermic species compared to non-homeothermic species. The dot size corresponds to the number of genes that are mapped to that pathway in order for them to be considered enriched.

Therefore, to further analyze the effect of the homeotherm trait, it may be important to look at just the top positively associated peak and the top negatively associated peak for all cell types and both background species were analyzed in a violin plot as seen in Figure 5. From the violin plots, the main takeaway is that the distribution of data across astrocyte top peaks and VIP neuron top peaks, regardless of species, is very similar. There does not seem to be any peak that

significantly stands out in terms of predicted open chromatin distribution, especially towards the ends of the violins plots. Generally, there is more predicted open chromatin than not.



Within each peak, genes can be identified that may be associated with the homeotherm trait. Therefore, each peak in Figure 5 was passed into the UCSC genome browser to identify nearby genes. As seen in Figure 6a, the gene near the positively associated human astrocyte peak was TSKU. TSKU has been shown to be involved in bone maturation, especially regulation of bone elongation and bone mass (Yano, et al). This can play a role in upregulating homeothermic

ability because an increase in bone mass leads to an increase in overall body size; this can potentially improve the homeothermic ability of animals. Near the negatively associated human astrocyte peak in Figure 6b was the gene RDH5. RDH5 is thought to be one of the “main dehydrogenases in the retinal pigment epithelium”, which is a layer of cells that plays a role in the health of the retina and photoreceptor cells (Sajovic, et al). Although this is a negatively associated gene, it does not seem to be related to a species’ homeothermic nature. For the top positive VIP peak, genome browsing led to finding the HSPB3 gene as seen in Figure 4c. HSPB3 is a heat shock protein that helps cells adapt to environmental and internal stresses by aiding in protein folding, unfolding, and translocation. It also promotes myogenesis, the “process of developing and forming muscle tissue” (Tiago, et al). Given that HSPB3 is a heat shock protein, this is relevant to homeothermy. Animals need to maintain their body temperature in response to stressors, and HSPB3 can aid in this process. A gene nearby the negatively associated VIP neuron peak in Figure 6d was SNX1. SNX1 is involved in the “tubulation of early endosomes toward ER sites” (Da Graça and Morel). Since endosomes are generally used in membrane trafficking pathways for recycling and degrading proteins, this did not seem to have a relation to homeothermy.

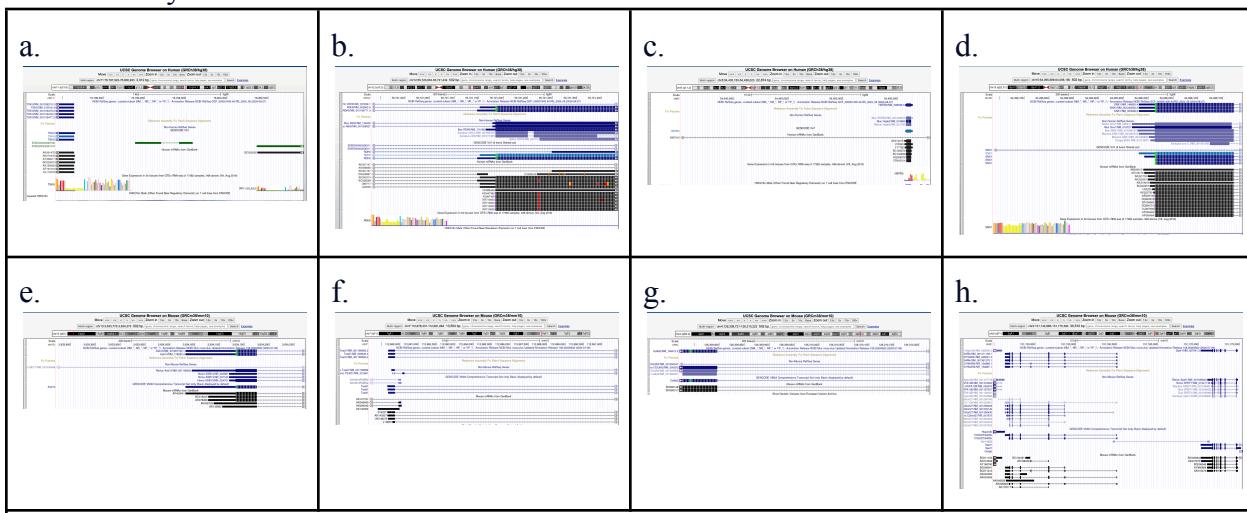
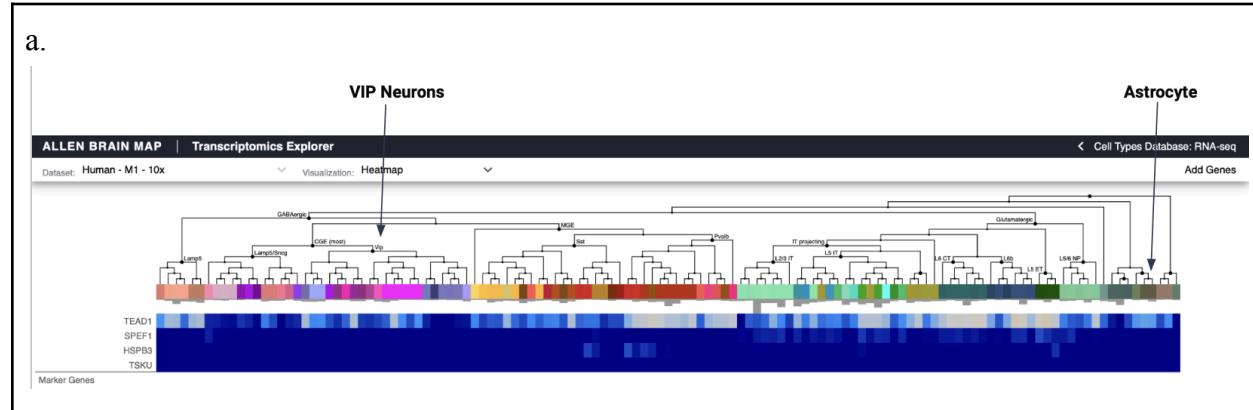


Figure 6: UCSC genome browsing of the top cell peaks to find nearby genes. Nearby mRNA coding genes were identified by zooming out up to 12x magnitude.

The gene nearby the positive astrocyte peak in mice was Asb13 as seen in Figure 6e. In experiments overexpressing Asb13, it was found that Asb13 “suppressed lung metastasis” (Fan, et al). Even though this was an upregulated peak, it does not have a direct connection to homeothermy. The gene near the negatively associated astrocyte mice peak genomic region was the TEAD1 gene as seen in Figure 6f. TEAD1 plays a key role in regulating the oxidative phosphorylation system in mitochondria (Liu, et al). Since this helps regulate the cellular energy of the species, an association to homeothermy can be hypothesized. A species needs enough cellular energy to effectively maintain their internal homeostasis. For the positively associated VIP mice peak, the Col8a2 gene was a nearby gene as seen in Figure 6g. Col8a2 does not relate to homeothermy because it regulates the fate of corneal endothelial cells (Hwang, et al). There is

a lack of clear connection between corneal endothelial cell fate and maintaining body temperature. Lastly, *Spef1* was the gene nearby the negatively associated VIP mice peak as seen in Figure 6h. This has a relationship with homeothermy because *Spef1* promotes microtubule bundling and stabilizes microtubules against depolymerization in response to cold shock (Tapia and Hecht). Maintaining microtubules to adverse stresses is important in homeothermic actions. Since this was near the negatively associated peak this may be a gene that is down-regulated in non-homeothermic species which may correlate with why they are non-homeotherms.

None of these genes seem to directly cause the activation or inhibition of the homeotherm trait. However, some of the genes seem to be somewhat correlated with the homeotherm trait. TEAD1, *Spef1*, HSPB3 and TSKU seem to all be related to pathways that may be adjacently related to homeotherms and thus should be analyzed further across other cell types. This would allow for a better understanding of how other cortical cell types upregulate or downregulate these genes and cause a mixed effect relationship in these gene's association with homeothermy. As seen in Figure 7a, the expression of these genes were analyzed across other cell types. TEAD1, which was found from the negatively associated astrocyte peak in mice, has a lot of increased expression in inhibitory L5-6 parvalbumin cells, excitatory 5-6 cells, and Inhibitory L3-5 VIP neurons. There is also some increased expression in astrocyte L1-6 cells. Since this was from a negatively associated peak, the expectation is that increased expression in these cells, specifically astrocytes, is downregulating homeothermic ability, as mice are not homeotherms. *Spef1*, the negatively associated VIP neuron peak in mice, had no expression in the VIP neurons, but rather a very small amount of expression in excitatory L2 and excitatory L5 cells. HSPB3, the positively associated VIP peak for humans, had also a small amount of expression in inhibitory L5-6 parvalbumin neurons. TSKU, the positively associated astrocyte peak in humans, had no expression anywhere. The results for all genes other than TEAD1 were surprising because the hypothesis was that there would be localized increased expression in these specific cortical cell type regions.



b.

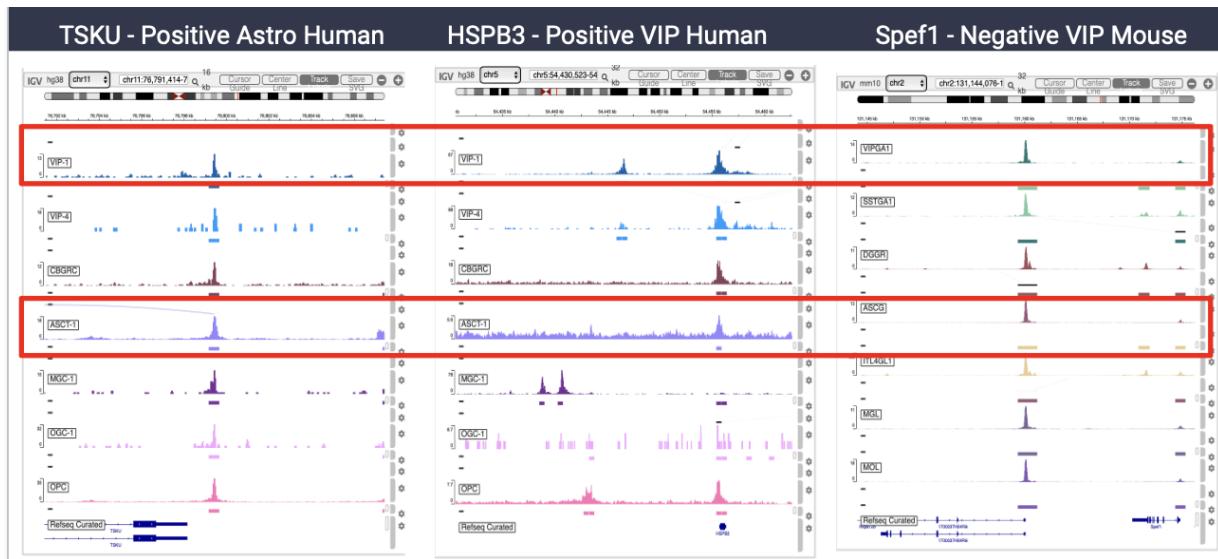


Figure 7: Cell Type Specificity of Relevant Genes and Visualized Open Chromatin. To visualize cell type specificity in the cortex, the Allen Brain Map Transcriptomics Explorer was used. The heatmap visualization allowed for a clear depiction of the gene's expression levels across the cortical cell types. Using the Catlas Human and Mouse brain map tracks, the open chromatin across cortical cell types were examined. Factors that affected the cortical cell types displayed were chosen due to initial cell types displayed, types used in this investigation, and increased public information regarding cell type.

Beyond the specific gene expression, it is possible that the open chromatin differs across cortical cell types from associated open chromatin regions. A similar pattern might suggest there is a broader mechanism occurring, or rather a localized mechanism to the subset of cells explored. Looking at Figure 7b, in both TSKU and HSPB3, there is clear evidence of a broader mechanism since there are large peaks across multiple cortical cell types, including the respective cell type the gene was found from. This could mean that these genes are also highly expressed in other cell types and play a larger role in association with homeothermy. In Spef1, there are very small peaks across the cell types, which hint at what is more likely to be open chromatin localized to VIP neurons.

In conclusion, the goal of this investigation was to examine a trait, homeothermy, whose difference across species may be associated with different cortical cell types. Astrocytes and VIP neurons, two different cortical cell types, were analyzed to see if open chromatin regions in the cell types were correlated with a species presenting the trait. By analyzing the differentially expressed peaks, pathways and genes were identified that may be related to the mechanism of homeothermy. After performing a literature review on these genes and pathways, it doesn't seem that they are directly related to the homeotherm trait but may have some loose associations. Comparing genes identified from VIP neuron data and from astrocyte data, it seems that the genes identified from the significant peaks from VIP neurons are more associated with the

homeotherm trait. This was since HSPB3 which helps adapt to the environment and Spef1 which reacts to cold shock are more likely to lead to a species developing the homeotherm trait. Further analysis showed that these peaks for Spef1 may just be localized to VIP neurons which suggest greater evidence in this cell type developing an association with the homeotherm trait.

Further exploration across more cortical cell types will aid in better understanding the association between cell type and homeothermy. By doing so, the most significant and relevant cell types can be identified. This would create a larger knowledge base of broader mechanistic actions versus cell type specific actions. As a result, genetic engineering approaches could even be developed to strengthen homeothermic ability. If the most significant cortical cell type has a direct relationship to homeothermy, modifications of the gene expression in that cell type can improve a species' ability to maintain a stable internal temperature.

References

Da Graça, Juliane, and Etienne Morel. "Canonical and non-canonical roles of SNX1 and SNX2 in endosomal membrane dynamics." *Contact*, vol. 6, Jan. 2023, <https://doi.org/10.1177/25152564231217867>.

Fan, Huijuan, et al. "ASB13 inhibits breast cancer metastasis through promoting snai2 degradation and relieving its transcriptional repression of YAP." *Genes & Development*, vol. 34, no. 19–20, 17 Sept. 2020, pp. 1359–1372, <https://doi.org/10.1101/gad.339796.120>.

Gordon, Christopher J. "The Mouse: An 'average' homeotherm." *Journal of Thermal Biology*, vol. 37, no. 4, July 2012, pp. 286–290, <https://doi.org/10.1016/j.jtherbio.2011.06.008>.

"Homoeothermic or Poikilothermic " World Ocean Review." *World Ocean Review*, worldoceanreview.com/en/wor-6/polar-flora-and-fauna/living-in-the-cold/homoeothermic-or-poikilothermic/#:~:text=Birds%20and%20mammals%20have%20a,called%20homoeothermic%20or%20endothermic%20organisms. Accessed 14 Sept. 2024.

Hwang, Jin Sun, et al. "COL8A2 regulates the fate of corneal endothelial cells." *Investigative Ophthalmology & Visual Science*, vol. 61, no. 11, 15 Sept. 2020, p. 26, <https://doi.org/10.1167/iovs.61.11.26>.

"In Brief: How Is Body Temperature Regulated and What Is Fever?" *InformedHealth.Org [Internet]*, U.S. National Library of Medicine, 6 Dec. 2022, www.ncbi.nlm.nih.gov/books/NBK279457/.

Kappel, Th., et al. "Variability in brain ganglioside content and composition of endothermic mammals, heterothermic hibernators and ectothermic fishes." *Neurochemistry International*, vol. 22, no. 6, June 1993, pp. 555–566, [https://doi.org/10.1016/0197-0186\(93\)90030-9](https://doi.org/10.1016/0197-0186(93)90030-9).

Liu, Ruya, et al. "TEAD1 is essential for mitochondrial function in cardiomyocytes." *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 319, no. 1, 1 July 2020, <https://doi.org/10.1152/ajpheart.00732.2019>.

Manaseri IH; Maly E; Jahromi M; Chikkamenahalli L; Park J; Hill J; "Insulin Sensing by Astrocytes Is Critical for Normal Thermogenesis and Body Temperature Regulation." *The Journal of Endocrinology*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/32698146/. Accessed 24 Sept. 2024.

Sajovic, Jana, et al. "The role of Vitamin A in retinal diseases." *International Journal of Molecular Sciences*, vol. 23, no. 3, 18 Jan. 2022, p. 1014, <https://doi.org/10.3390/ijms23031014>.

Scholander, P. F. "Evolution of Climatic Adaptation in Homeotherms." *Evolution*, vol. 9, no. 1, 1955, pp. 15–26. *JSTOR*, <https://doi.org/10.2307/2405354>. Accessed 14 Sept. 2024.

Szadai, Zoltán, et al. “Cortex-Wide Response Mode of VIP-Expressing Inhibitory Neurons by Reward and Punishment.” *eLife*, eLife Sciences Publications, Ltd, 23 Nov. 2022, elifesciences.org/articles/78815.

Tapia, Rocio, and Gail A. Hecht. “SPEF1/clamp binds microtubules and actin-based structures and regulates cell migration and epithelia cell polarity.” *Annals of the New York Academy of Sciences*, vol. 1515, no. 1, 16 June 2022, pp. 97–104, <https://doi.org/10.1111/nyas.14845>.

Tiago, Tatiana, et al. “Small heat-shock protein HSPB3 promotes myogenesis by regulating the lamin B receptor.” *Cell Death & Disease*, vol. 12, no. 5, 6 May 2021, <https://doi.org/10.1038/s41419-021-03737-1>.

Todd, William D., et al. “Suprachiasmatic VIP Neurons Are Required for Normal Circadian Rhythmicity and Comprised of Molecularly Distinct Subpopulations.” *Nature News*, Nature Publishing Group, 2 Sept. 2020, www.nature.com/articles/s41467-020-17197-2#:~:text=Surprisingly%2C%20VIPcre/cre::,DD%CF%84%20=%20free%20running%20period%20length.

Wei, Dereck C. “Histology, Astrocytes.” *StatPearls [Internet]*, U.S. National Library of Medicine, 1 May 2023, www.ncbi.nlm.nih.gov/books/NBK545142/#:~:text=Astrocytes%20are%20a%20subtype%20of,barrier%2C%20and%20promoting%20synapse%20formation.

Xiaozheng Yu, et al. “Comparative Insights into the Integration Mechanism of Neuropeptides to Starvation and Temperature Stress.” *General and Comparative Endocrinology*, Academic Press, 23 Nov. 2021, www.sciencedirect.com/science/article/pii/S0016648021002380.

Yano, Kosei, et al. “The role of Tsukushi (TSK), a small leucine-rich repeat proteoglycan, in bone growth.” *Regenerative Therapy*, vol. 7, Dec. 2017, pp. 98–107, <https://doi.org/10.1016/j.reth.2017.08.001>.

Zoonomia, zoonomiaproject.org/. Accessed 14 Sept. 2024.

Zou, Jing, and Samuel Andrew Hires. “Inhibitory neurons: VIP neurons expect rewards.” *Current Biology*, vol. 33, no. 17, Sept. 2023, <https://doi.org/10.1016/j.cub.2023.07.059>.