Exploratory analysis of the neuropil space in the somatomotor, visual and cingulate cortex of the extinct Tasmanian tiger (*Thylacine cynocephlus*)

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Abstract

The Tasmanian tiger (*Thylacine cynocephalus*) was a carnivorous marsupial which was driven to extinction at the start of the 20th Century. While historical records and genetic data have provided us with some insight into thylacine behavior and relatedness, we still know very little about their comparative neurobiology. Through the recent availability of a high-resolution image dataset acquired from hematoxylin-stained sections of a single thylacine brain, we undertook an exploratory analysis of the cortical microcircuitry in the somatomotor, visual and cingulate cortex. The aim of this study was two-fold, 1) to evaluate if there are regional differences in microcircuitry between cortical areas and to access the quality of the image data for follow-up comparisons with the extant Tasmanian devil (*Sarcophilus harrisii*). Using a design based stereological sampling and image analysis approach, we quantified the neuropil fraction and average cell size in the cortical regions of the Thylacine brain. Our preliminary findings are interpreted within the context of published data on the neuropil space across species and recommendations are made for the use of this image dataset in subsequent histological comparisons.

Introduction

The thylacine (*Thylacinus cynocephalus*) was the largest modern day carnivorous marsupial and prior to extinction was found on the Island of Tasmanian in Australia. It is believed that anthropogenic pressure as well as competition from other species as well as climatic challenges contributed towards the extinction of this species (1, 2). The last recorded thylacine was known to have died in the Hobart Zoo in 1936 (3). Very little is known about Thylacine biology, especially neurobiology but H &E-stained sections were recently made available for study from a century old brain specimen. Knowledge on the biology of this species will not only inform our understanding what may have led to their extinction, their potential behavioral repertoire but also provide unique insight into the effect of convergence on brain morphology.

Figure 1: An image of the last Thylacine which died in captivity in 1936.

Methods

Image Acquisition

Digital images of the Thylacine brain were obtained from the online repository https://www.Brainmaps.org. Using the published anatomical map of the Thylacine brain as a reference (see Haines et al., 2023), we sampled the Visual (V) Cingulate (Cg); Motor (M) and Somatosensory cortex (SS). We used Preview on the Macbook to screen capture each region of interest at 20X magnification with at least three sections per region included in sampling. Resulting screen captures ranged in size from 1,200 X 800 pixels to 1,440 X 970 pixels. Images were then imported into ImageJ where a customized macro was developed to sample and crop the images into consistent frame sizes for batch sampling. Image frames were monitored to ensure that all frames fell inside the boundaries of the region of interest. Final image stacks consisted of ~60 images per section were region of interest.

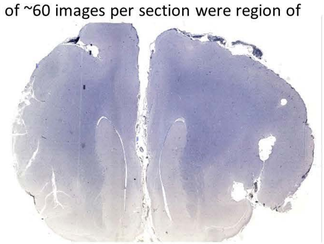

Figure 2: A representative screenshot of the frontal lobe in coronal section as it appeared in

Methods

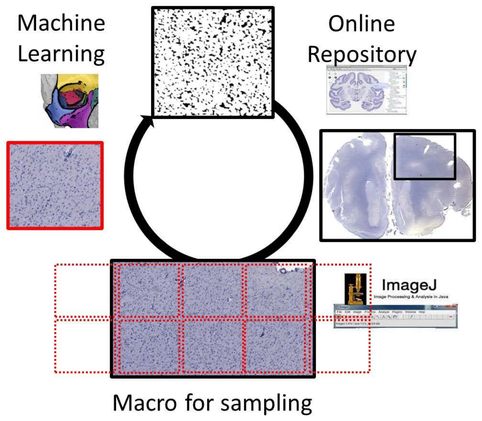

Figure 3: Workflow summary as screenshots from OrbitImage Analysis for the application of machine learning to a histology dataset. a- Define classes ; b- Classify and Train dataset; c- ROI testing and accuracy inspections or Batch processing; d- Object segmentation for quantification of number of objects or size parameters

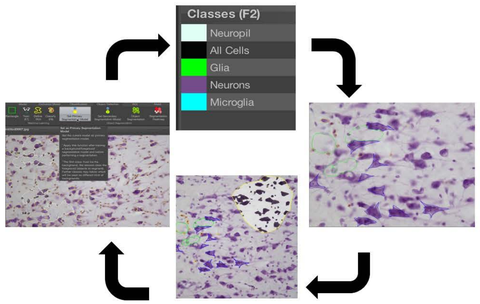

Figure 4: Workflow summary for the application of machine learning to a histology dataset in OrbitImage Analysis . a- Define classes ; b- Classify and Train dataset; c- ROI testing and accuracy inspections or Batch processing; d- Object segmentation for quantification of number of objects or size parameters

Image analysis using machine learning

Once images were captured, each image series was imported into Orbit Image Analysis for automated segmentation of the neuropil space using machine learning (Stritt, Stalder, & Vezzali, 2020). Using the Model toolset in OrbitImage Analysis, we defined our classes of tissue (i.e., Foreground = cells; background = Neuropil). Once the classes were defined, we used the drawing tools under the Classification tab to manually label our classes of interest in a sample of images drawn from our dataset.

Methods

To facilitate accurate training of the dataset, we made sure to outline regions that were close to the borders of each of our classes and to sample across the images to ensure that the training dataset included a range of image intensity values. After the classification system was setup, the model was then built and trained. Average model training took between 10-20 seconds depending on the number of images included in the image stack. After the model was trained, we used the Region of Interest (ROI) tool to define a region within our sample and visually inspected the results to evaluate the accuracy of the classification model. Models were then saved before being applied to the entire image dataset using the Batch processing function.

Results & Discussion

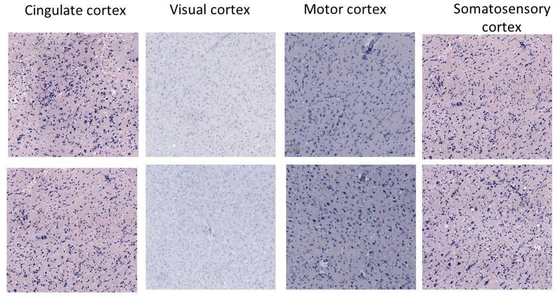

Figure 5: Representative images of the visual, cingulate, motor and somatosensory cortex. Images were screen captured from brainmaps.org before being imported into ImageJ and cropped for processing as an image stack in OrbitImage Analysis using machine learning.

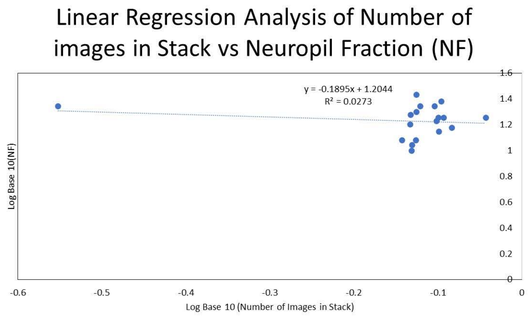

Figure 6: Linear regression analysis (least squares) of the number of images in each stack vs the neuropil fraction (NF) in three regions (somatosensory, motor and cingulate cortex). Note that there is no significant relationship between the the neuropil fraction (i.e., the proportion of neuropil sampled) and the number of sampling sites included in the analysis. This result indicates that sampling has not played a major role in determining the average neuropil fraction estimated for each region.

Conclusion

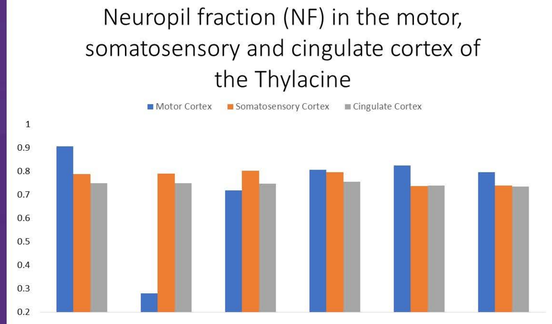

Figure 7: Bargraphs of the neuropil fraction (NF) in the motor, somatosensory and cingulate cortex of the Thylacine brain.

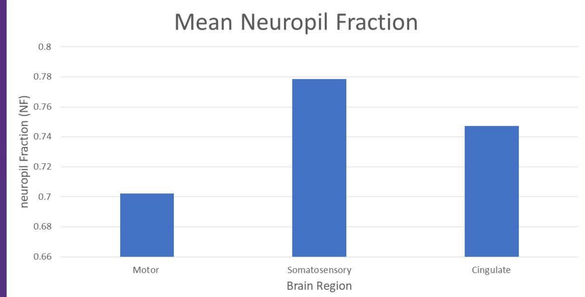

Figure 8: Bargraphs of the mean (calculated as weighted means by number of images in each stack) neuropil fraction (NF) in the motor, somatosensory and cingulate cortex of the Thylacine brain.

Preliminary data indicates that there is cortical variation in neuropil space in the Thylacine brain as has been shown for other mammalian species. Notably we observed the largest neuropil fractions in the somatosensory, followed by cingulate and then motor cortex. These data while speculative suggest that there was greater cortical connectivity in the putative sensory cortex and cingulate cortex compared to the motor cortex. This observation aligns generally with that observed in studies of other mammals (Spocter et al., 2018, 2012, 2015).

References

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Acknowledgments

This work was supported by funding from the Iowa STEM BEST (MAS),. We are also grateful for our community partnership with the Des Moines School District (Central Campus) which has helped to foster interest in STEM fields through supporting high school student involvement in our research.