36.22 Using the isotropic fractionator method to assess the effects of domestication on neuronal and non-neuronal cell numbers in the rat *(Rattus norvegicus)*

L. WILLIAMS[1], A. NGWENYA[2], R. STRYJEK[3], K. MODLINSKA[3], W. PISULA[3], S. M. PELLIS[1], A. N. IWANIUK[1];

[1]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Rhodes Univ., Grahamstown, South Africa; [3]Inst. of Psychology, Polish Acad. of Sci., Warsaw, Poland

Introduction

Domestication is the process by which wild organisms are adapted for human use. The lab rat, an important model organism in scientific research, is the result of domestication of the wild Norway rat. Similar to other domesticated animals, lab rats have relatively smaller brains compared to wild rats. Although the "ecological niche" of a domesticated lifestyle produces changes in brain size, little is known about the underlying neuroanatomical changes responsible for differences in brain morphology between domesticates and their wild counterparts. For example, changes in brain size or in the size of brain regions in domesticates could reflect changes in neuron size, connectivity or neuron number. Here, we used the recently developed isotropic fractionator (IF) technique to test for differences in the cellular composition in the brains of female wild Norway rats and two widely used laboratory strains: Long-Evans and Sprague-Dawley. The IF is a non-stereological means of estimating total number of cells, neurons, and non-neuronal cells in the entire brain, or any dissectible region. This technique is faster compared to traditional stereological methods and provides accurate estimate of neuron numbers, particularly for brain regions that are highly heterogeneous (e.g., neocortex) or have exceptionally high neuronal density (e.g., cerebellar granule cells).

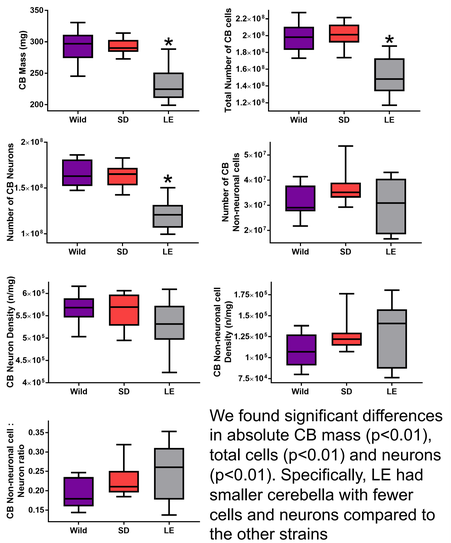
Methods

We examined 36 female rats (71-82 days of age): wild rats (n = 12), Long-Evans (n = 12) and Sprague-Dawley (n = 12). Wild rats were obtained from a wild-breeding colony at the Polish Academy of Science (Warsaw, Poland), whereas the lab rat strains came from commercial sources. All animals were trans-cardinally perfused with saline followed by 4% buffered paraformaldehyde. The brains were dissected into four regions: olfactory bulbs, cortex, cerebellum and the remaining tissue ("rest of brain").

Fixed-tissue was mechanically grinded into a homogenous mixture of suspended nuclei and samples were incubated with the DNA-specific fluorescent label DAPI. To estimate total cells, aliquots were loaded into a hemacytometer and counted under the 40X lens of a Zeiss Axioskop 2 MOT fluorescent microscope. To estimate neuron numbers samples were immunoreacted with the neuronal nuclear antigen (NeuN) and visualized with the 40X lens of a Zeiss Axiocam Imager MRm microscope. At least 500 DAPI-labelled nuclei were examined for NeuN labelling. To estimate neuron numbers, the proportion of NeuN labelled cells was multiplied by total cells. The number of non-neuronal cells was calculated by subtracting the number of neurons from total cell numbers.

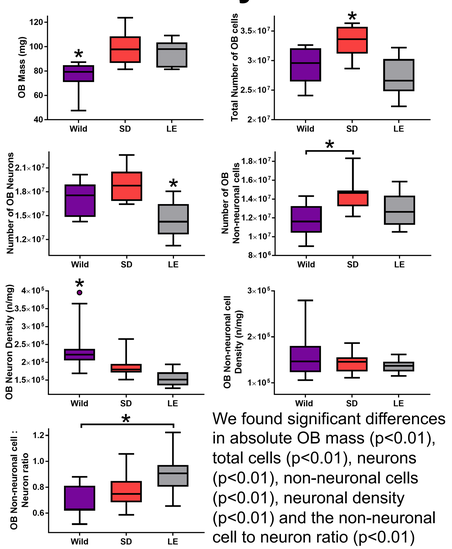
Statistical analysis was performed using analysis of variance (ANOVAs) to test for differences among strains. Absolute measurements were analyzed with 1-way ANOVAs and 1-way ANCOVAs were used to test for differences in relative measurements using brain mass as a covariate. Any significant effects were then further tested with Tukey HSD post-hoc tests.

Cerebellum



We found significant differences in absolute CB mass ($p<0.01$), total cells ($p<0.01$) and neurons ($p<0.01$). Specifically, LE had smaller cerebella with fewer cells and neurons compared to the other strains

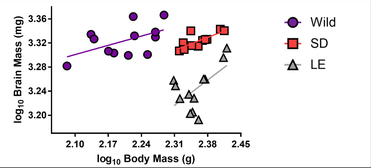
Olfactory Bulbs

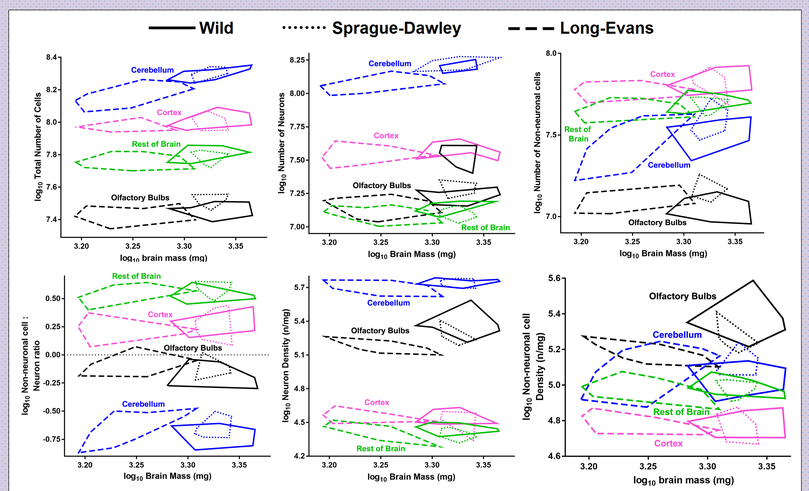


We found significant differences in absolute OB mass ($p<0.01$), total cells ($p<0.01$), neurons ($p<0.01$), non-neuronal cells ($p<0.01$), neuronal density ($p<0.01$) and the non-neuronal cell to neuron ratio ($p<0.01$)

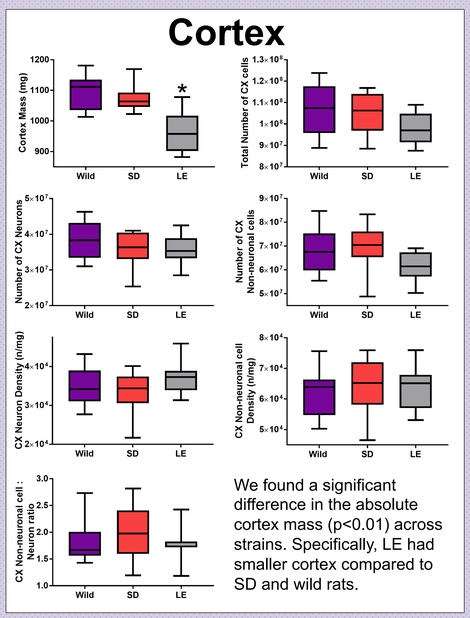
Brain & Body Mass

Brain mass varies significantly by both strain ($p<0.01$) and body mass ($p=0.01$), and no interaction effects were detected ($p=0.85$). Specifically, LE had smaller brains and bodies compared to wild rats and SD.



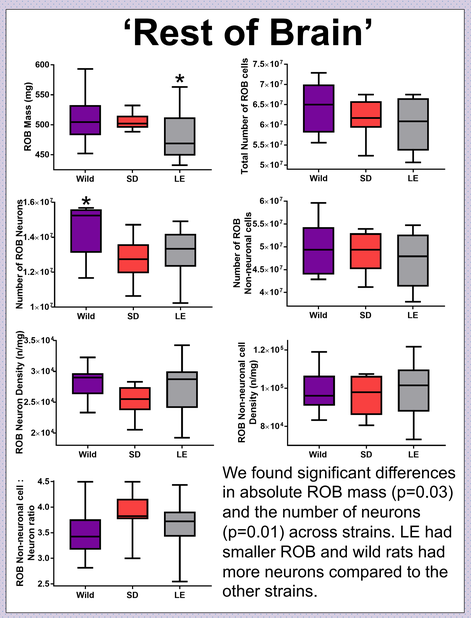


Cortex



We found a significant difference in the absolute cortex mass ($p<0.01$) across strains. Specifically, LE had smaller cortex compared to SD and wild rats.

'Rest of Brain'



We found significant differences in absolute ROB mass ($p=0.03$) and the number of neurons ($p=0.01$) across strains. LE had smaller ROB and wild rats had more neurons compared to the other strains.

Cerebellum

- LE had relatively fewer neurons, more non-neuronal cells and a greater non-neuronal cell density compared to wild rats
- LE had relatively more non-neuronal cells per CB neuron than SD and wild

Cortex

- No significant effects of strain on the cellular composition of the cortex

Olfactory Bulbs

- LE had relatively larger OBs with lower neuron densities, more non-neuronal cells and more non-neuronal cells per neuron compared to wild
- LE also had relatively fewer OB neurons than SD

Rest of Brain

- LE had relatively smaller ROB than the other two strains
- LE also had relatively smaller ROB neuron densities compared to wild

Conclusions and Future Directions

- The cellular composition of domestic strains differs from wild rats
- The effects of domestication varies across strains and brain regions
- This knowledge will help improve out understanding of how domestication and strain effects brain morphology
- Are there strain-specific effects of environmental enrichment on cerebellar morphology?

Acknowledgements

We thank our colleagues at the Polish Academy of Science for providing our wild rat specimen and NSERC for supporting our research.