The Potential Impact of Environmental Complexity on Cerebrovasculature in Wild and Laboratory *R. norvegicus*

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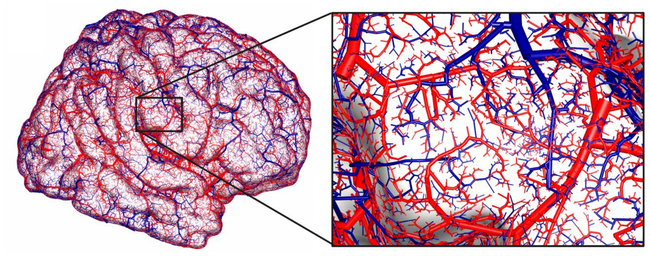
Lab and Wild-Trapped Urban Rats

- Rodents such as *Rattus norvegicus* represent the dominant mammalian model used in approximately 95% of biomedical research (Grimm, 2021), but very little is known about their wild counterparts.
- Recently, our lab reported increased neural cell counts and higher stress hormone levels in wild-trapped rats from Richmond, VA, compared to weight-matched Long-Evans laboratory rats (Jacob et al., 2022).
- While many laboratory enrichment (Bennett et al., 1964) and negative unexpected event (Dudley & Papini, 1995) experiments have been conducted, how unexpected events affect rodents is largely unexplored. Also of interest is how anticipation can potentially increase optimism as enrichment has been shown to do (Brydges et al., 2011).

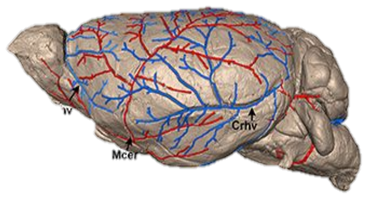
Human and Rodent Cerebrovasculature

- Vascularization is the primary means by which the brain's metabolic needs are satisfied.
  - Human brain has approximately 400 miles of vasculature (Begley & Brightman, 2003).
  - Capillary and endothelial cell density not associated with neuronal density, but rather blood flow and glucose use at rest (Ventura-Antunes et al., 2022).
- Previous investigations of rats raised in enriched environments suggest that environmental complexity is positively associated with cortical microvascular density (MVD) (Sirevaag et al., 1988).



Human Cerebrovasculature

Adapted from li et al., 2020; https://doi.org/10.1371/journal.pcbi.1007943



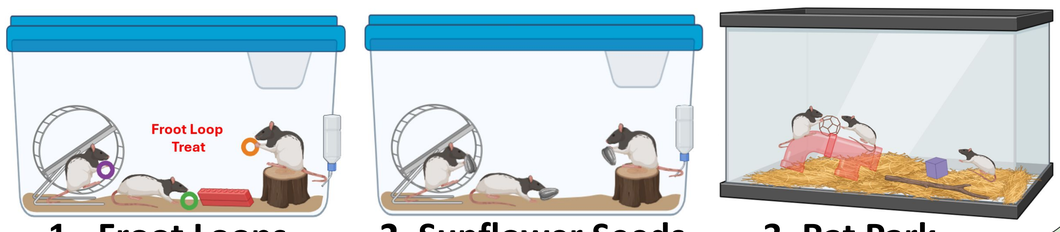
Rodent Cerebrovasculature

Adapted from Xiong et al., 2017; https://doi.org/10.3389/fnana.2017.00128

- **Part 1 Hypothesis:**
  - Considering the enhanced complexity of their environment, wild rats will exhibit higher MVD scores than their laboratory counterparts.
- **Part 2 Hypothesis:**
  - Considering the enhanced complexity of their environment, and enrichment's previous association with optimism, rats anticipating positive events will have higher MVD than solely enriched animals, who will have higher MVD than controls.

Method

- **Part 1:**
  - Fresh brains were post-fixed and sectioned at 40 µm through the anterior cingulate and dorsal hippocampus areas and processed for immunoreactivity of a mineralocorticoid receptor antibody found to demarcate blood vessels.
  - MVD was assessed in 4x images of wild-caught and laboratory-bred female rats (*Rattus norvegicus*; n=5 each group) in targeted brain areas involved in emotional and cognitive functions (i.e., hippocampus and anterior cingulate).
    - Anterior cingulate chosen for its role in higher-order functions like decision-making and social behavior (Apps et al., 2016), and dentate gyrus of the hippocampus for memory encoding and retrieval (Hainmueller & Bartos, 2020).
- **Part 2:**
  - Fresh brains were post-fixed and sectioned at 40 µm through the dorsal hippocampus areas and processed for immunoreactivity of Collagen-IV, a marker for the basal lamina of blood vessels.
  - MVD was assessed in 20x images of the dentate gyri of rats deprived of positive experiences (DEPR), one group with positive experiences that were all provided at once in the morning (CTRL), and another with positive experiences that would randomly be encountered throughout the day (UPER).
    - Positive experiences were:

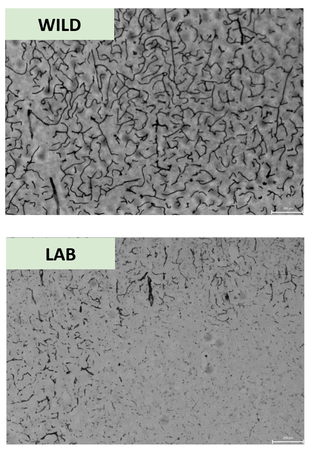


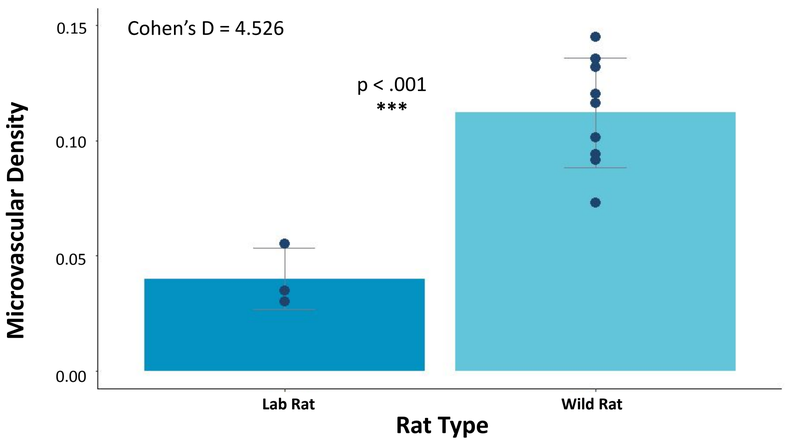
1. Froot Loops
2. Sunflower Seeds
3. Rat Park

Results

I. Anterior Cingulate Vasculature

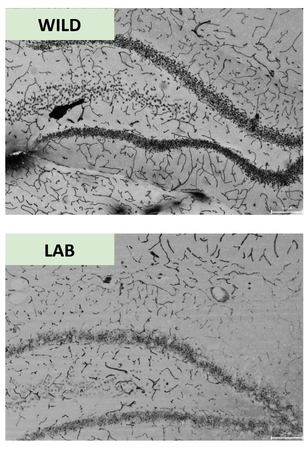
MVD Marker MR

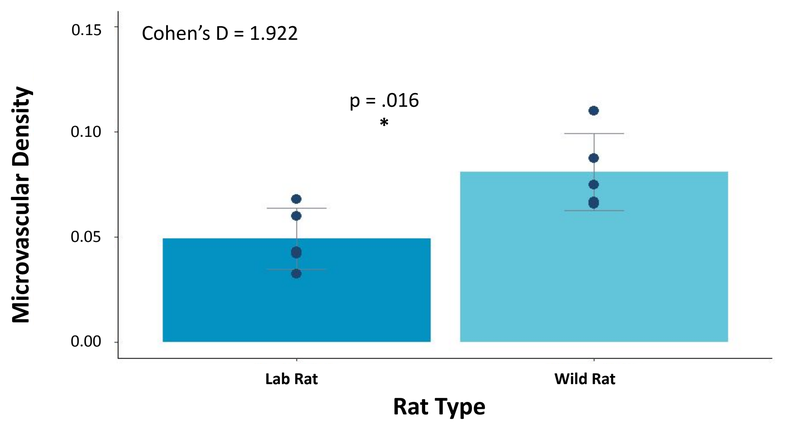




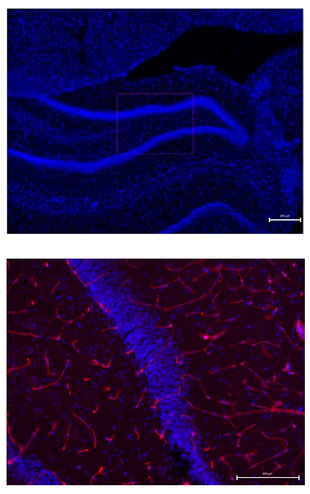
II. Dentate Gyrus Vasculature

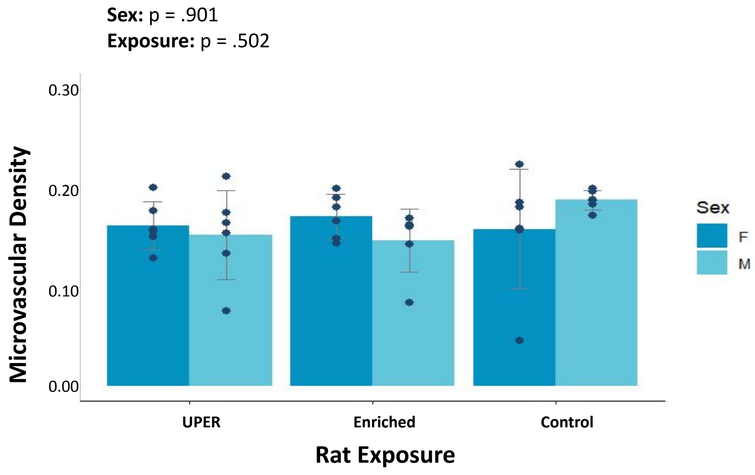
MVD Marker MR





Collagen-IV





Conclusions

I. Increased Levels of MR-ir Tissue in Wild Rats

- Wild rats had approximately 60% higher visible vasculature scores than the lab rats in the dentate gyrus of the hippocampus and approximately 200% higher scores than the lab rats in the anterior cingulate cortex.
- Association of MR activation with increased blood pressure might reflect wild rats' need to activate fight or flight response (ter Heegde et al., 2014).

II. Similar Vasculature Amounts Between Enriched Rats and Controls

- Vasculature percentages between DEPR, CTRL, and UPER rats were similar.
  - While increased blood flow in the DG is associated with exploration in an enriched environment (Shen et al., 2019), these similar vasculature amounts imply that short positive experiences, even when prolonged by anticipation, don't significantly change existing vasculature.

Future Directions

- Nestin stain to examine angiogenesis in UPER, DEPR, and CTRL rats.
- Experimentation with other complex environments, including comparing virtual reality and physical enriched environments.
  - Investigate existing and emerging vasculature for potential clinical translation to brain injuries.