# REWIRING REALITY: HOW VIRTUAL AND PHYSICAL SPACES INFLUENCE *R. NORVEGICUS* NEUROPLASTICITY

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## VR AND THE BRAIN

- Enriched environments (EEs), or sensory-rich settings, enhance cognitive and motor function in both animals and humans (Harati et al., 2012; Laver et al., 2017).
- In rodents, lifelong EE exposure improves spatial memory, reduces anxiety, and promotes hippocampal neuroplasticity (Harati et al., 2012; Sampedro-Piquero et al., 2013).
- Virtual reality (VR) has emerged as a non-invasive tool to simulate EEs for rehabilitation in humans (Dahdah et al., 2017; Mathews et al., 2016).
- To our knowledge, VR in rodents has been limited to study spatial navigation and hippocampal wave activity (Aronov & Tank, 2014).

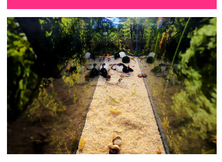
Made with ChatGPT

- **Hypothesis:** VR rats will exhibit enhanced spatial learning in the Dry Land Maze (DLM) and improved escape responses in the Swim Challenge Task compared to physically enriched rats (ENR) due to limited haptic input, but higher performance than controls (CTRL) from enhanced visual engagement. ENR will also have higher active GFAP and DCX in the dentate gyrus (DG) and visual cortex (V1) than VR, but VR will have higher levels than CTRL.

## ANIMALS

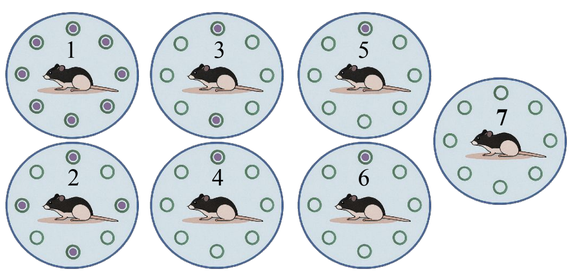
- 18 female Long-Evans rats, one month old.
- Housed in groups of three in ventilated (47 * 25 * 21 cm) cages on a 12-hour light cycle with Aspen bedding, a hide, and weekly nesting material (paper towel).
- Divided into three experimental groups (n=6 each).

## AMBIENT EXPOSURE

| VIRTUAL ENVIRONMENT (VR) | PHYSICAL ENVIRONMENT (ENR) | STANDARD ENVIRONMENT (CTRL) |
|---|---|---|
|  |  |  |
| 30 min. each weekday in 55-gallon aquarium. Animated 5–7 naturalistic objects replaced weekly on dynamic natural background. | 30 min. each weekday in 55-gallon aquarium. Same 5–7 naturalistic objects and static naturalistic background as VR. | Stayed in cages with no daily enrichment exposure. As depicted, the control environments were larger than typically used for standard rodent housing. |

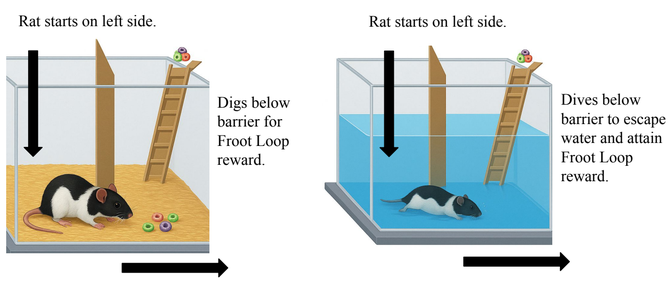
### DRY LAND MAZE

- Task with three stages—habituation, acquisition/ testing, and probe—intended to assess exploration, learning, and search strategy, respectively.
- **Days 1-3:** Habituation
- **Days 4-5:** Acquisition
- **Day 6:** Testing
- **Day 7:** Probe



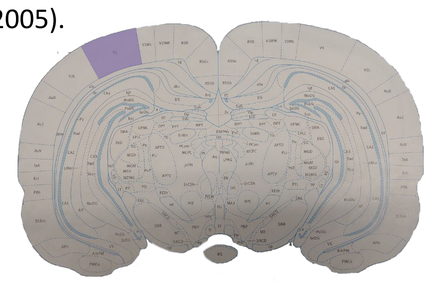

### SWIM CHALLENGE TASK

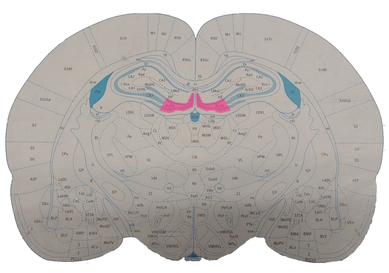
- Problem-solving task to assess exploration and transfer of learning.



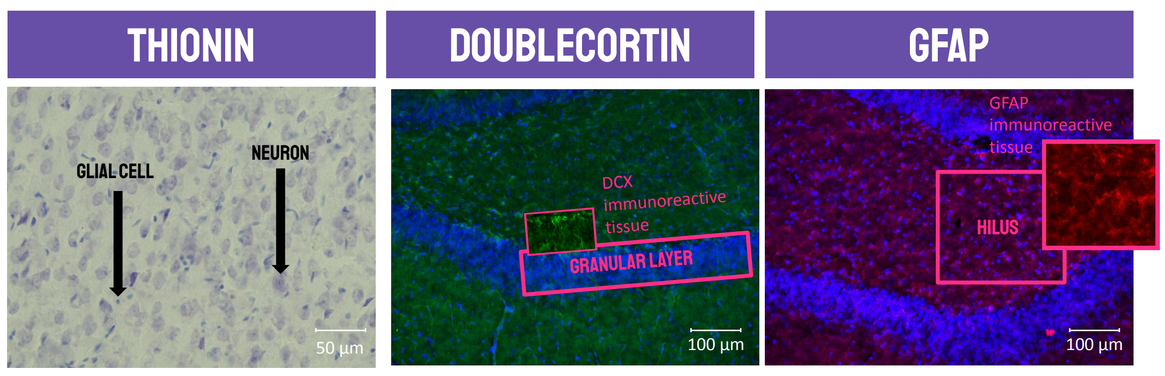

## HISTOLOGY

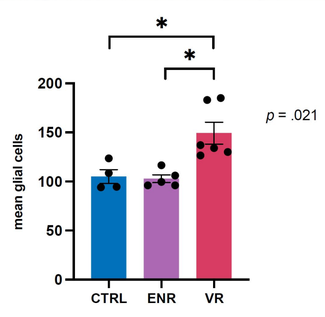
- Fresh brains were post-fixed and sectioned at 40 µm through the primary visual cortex and dorsal hippocampus dentate gyrus areas and processed for immunoreactivity of 1:200 GFAP, an astrocyte marker; 1:100 doublecortin (DCX) for marking new neuronal cells; and 1:1000 Thionin to mark glial and neuronal cell bodies. Images were taken at 20x with a Keyence BZ-X800 confocal microscope in the DG and 40x in V1.
- **Visual Cortex:** Chosen for high plasticity in previous enriched environment studies (Bibollet-Bahena et al., 2023) and vulnerability to neurodegeneration. Layers 2 and 3 analyzed for high density and low transiency of spines (Holtmaat et al., 2005).
- **Dorsal Hippocampus:** Dentate gyrus selected due to its critical role in spatial memory and learning, high neurogenic potential, and sensitivity to both enrichment and neurodegeneration (Bartsch & Wulff, 2015).

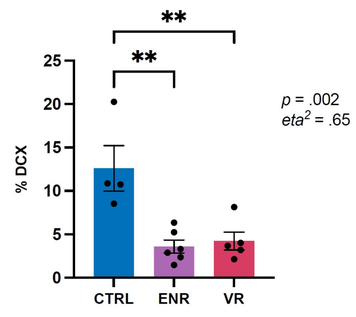


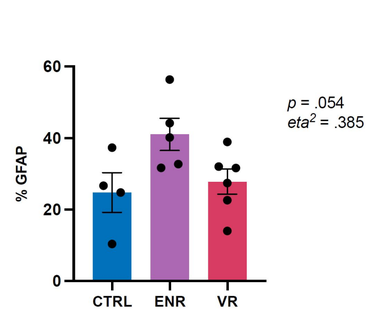


## GLIAL AND NEURAL ACTIVATION



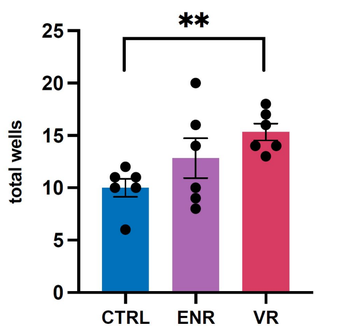



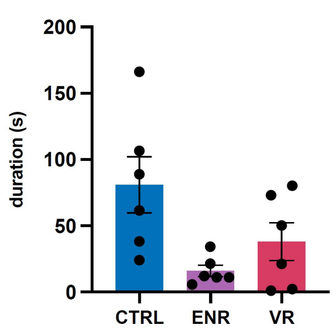

## COGNITIVE PERFORMANCE

- Behaviors assessed in DLM included time spent in the center of the arena, the number of wells visited, the proportion of treats consumed, latency to approach the previously baited well, latency to consume all treats, and the number of rears and grooms

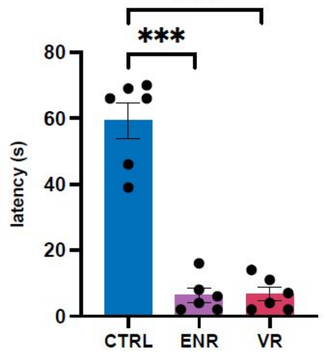
**DLM HABITUATION DAY I: TOTAL WELLS VISITED**
(p=.003 (post-hoc) VR > CTRL; F(1, 9.35)=9.64, p=.005)



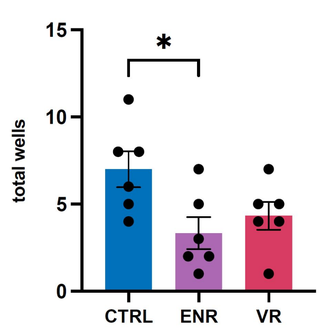

**DLM HABITUATION DAY 3: DURATION IN PERIMETER**
(p=.06 (post-hoc) ENR < CTRL; F(2, 7.43)=4.97, p=.043)



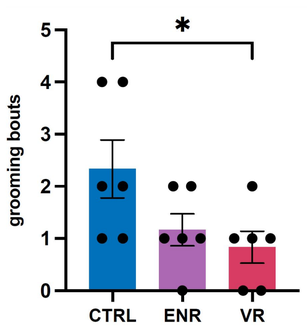

**DLM ACQUISITION DAY 2: LATENCY TO FIRST WELL**
(p<.001 (post-hoc) CTRL > VR and CTRL > ENR; F(2, 15)=72.8, p<.001)




**DLM ACQUISITION DAY 2: TOTAL WELLS VISITED**
(p=.033 (post-hoc) CTRL > ENR; F(2,15)=4.22, p=.035)




**DLM PROBE: NUMBER OF GROOMS**
(p=.05 (post-hoc) CTRL > VR; F(2, 15)=3.72, p=.049)




## CONCLUSIONS

### BEHAVIORAL DATA

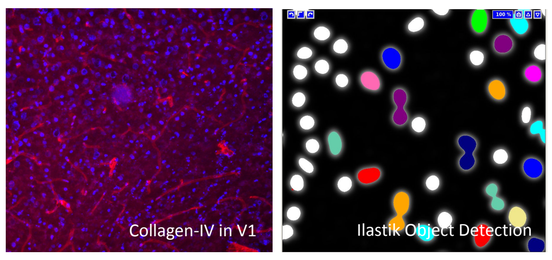
- VR and ENR rats were **more exploratory** than CTRL, with VR visiting more wells and ENR spending less time in the perimeter (e.g., thigmotaxic responses) during habituation than CTRL.
- During acquisition, CTRL took significantly longer to find the first well than VR and ENR and visited more wells than ENR, **suggesting slower learning**.
- In the probe phase, CTRL exhibited increased grooming than VR, **indicating potential anxiety.**
- **No significant differences were observed in the swim task/sawdust digging (e.g., latency to dive, etc.).**
- **Overall, contrary to the hypothesis, the VR rats were comparable to ENR rats.**

### HISTOLOGICAL DATA

- Increased density of glial cells in V1 for VR rats compared to ENR and CTRL (p=.004 VR and p=.003 ENR; F(2, 12) = 9.25, p=.021) suggests **higher visual novelty than physically enriched environments**, aligning with previous findings of environmental complexity and glia (Diamond et al., 1988).
- Nonsignificant trend of increased GFAP expression in the dentate gyrus for ENR compared to CTRL (p=.07; F(2,12) = 3.76, p=.054) supports previous enrichment literature (Sampedro-Piquero et al., 2014), alongside observations of better spatial memory performance in DLM.
- Higher DCX expression in granular layer of the dentate gyrus in CTRL compared to VR and ENR potentially from transition to more enriched environment during behavioral training (p=.002 ENR and p=.005 VR; F(2, 12) = 11.2, p=.002).

## FUTURE DIRECTIONS

- **Glial cells in V1:** Use object detection to categorize glial cells in V1 from thionin stain.
- **Additional metrics of neuroplasticity:** BDNF for demarcating neurogenesis, Collagen-IV to mark mature blood vessels, and Nestin to indicate new vasculature growth.




- **Larger sample with both sexes.**
- **Preventative/Interventional potential:** As glial, neuronal, and behavioral data from healthy animals in this study suggest that VR environments may support neuroplasticity, evaluate neural markers and behavioral outcomes following experimentally induced neurodegeneration (e.g., kainic acid model).
- **More interactive VR environment:** Add contingency to the virtual environments to parallel physical interactions with stimuli in physical enriched environment. For example, when the rat moves forward, the stimuli would move forward.

## ACKNOWLEDGEMENTS

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