# Exploring Neural Patterns: Rat Brain Activation During a Transitive Inference Task

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## Abstract

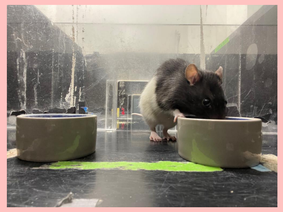
Humans and some animal's species construct mental hierarchies that aid in decision-making. Transitive inference (TI) a form of deductive reasoning that involves the use of explicitly learned relationships (A>B>C) to make inferences about implicitly related stimuli (A>C). Research on TI in rodents can provide insight on the evolutionary origins of TI as a cognitive process. In our previous study, ten male Long-Evans rats were trained on a TI behavioral task. Here we examine patterns of neural activation in the rats' brains during these tasks. We examine immediate early genes (IEG) expression in four brain regions: CA1 and CA3 in the hippocampus, as well as the parietal and prefrontal cortices. Neurobiological data were collected using double Compartmental Analysis of Temporal activation with a Fluorescence *in situ* hybridization technique known as RNAScope, allowing for the identification of neuron activity-dependent Arc/Homer1a double IEG expression. This study presents a comprehensive investigation into the neural mechanisms underlying TI in rodents, shedding light on its evolutionary origins and cognitive underpinnings.

## Introduction

During the first epoch, rats received training on the (A>B>C>D>E) list to ensure that they had retained the premise pairs. In the second epoch, the rats had to use transitive inference to infer the hierarchy of the probe pairs (A>C, B>D, C>E). Euthanasia and brain extraction occurred immediately after the conclusion of the second epoch. Neural activation patterns were examined to determine which parts of the brain were activated during the TI task.

**Epoch 1:**
A+B-, B+C,
C+D-, D+E-

**Epoch 2:**
A+C-, B+D-,
C+E-



## Methods

Brain tissue was kept at -80°C to preserve the mRNA until a cryostat was used to slice it into 20-µm sagittal slices, which were then mounted into microscope slides. The RNAScope procedure was performed as indicated by the kit instructions. We used a Keyence florescent microscope BZ-X series to image the brains and create a neural map. Then, we used a confocal fluorescence microscope to examine IEG expression in four brain regions: CA1 and CA3 in the hippocampus, as well as the parietal and prefrontal cortices. Using the Imaris software, pictures of the different fluorescent markers were taken to discern the location of the Arc and Homer1a dots. We will quantify neurons that were active at baseline, epoch 1 only, epoch 2 only, and both epochs' using IEG cellular distribution. For instance, Homer1a's transcription kinetics are different from Arc's. Arc mRNA moves to the cytoplasm 20–30 minutes after neuronal activity, when it is visible as a halo surrounding the nucleus. Homer1a takes a longer to moves to the cytoplasm, specifically about 60 minutes following neuronal activation (Guzowski 1999; Marrone et al., 2008). Given the differences, we can leverage these differences to parse out individual variations in neural activity patterns across brain regions. Furthermore, by examining the differential activation of specific brain regions, such as the hippocampus, parietal cortex, and prefrontal cortex, we can elucidate the neural circuitry underlying TI-related cognitive processes.

## Results

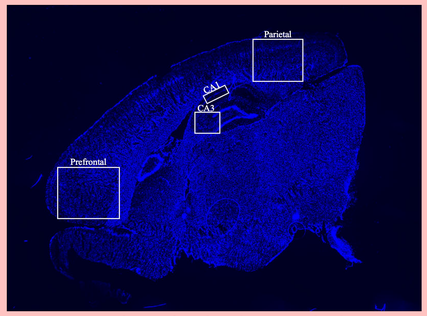



**Figure 1.** A sagittal view of a rodent's brain stained with DAPI using a Keyence florescent microscope BZ-X series. DAPI is a fluorescence dye that bins to the DNA within the nuclei and emits a blue fluorescence when illuminated with ultraviolet (UV) light. This helps visualize cell nuclei and study the structure of the brain.

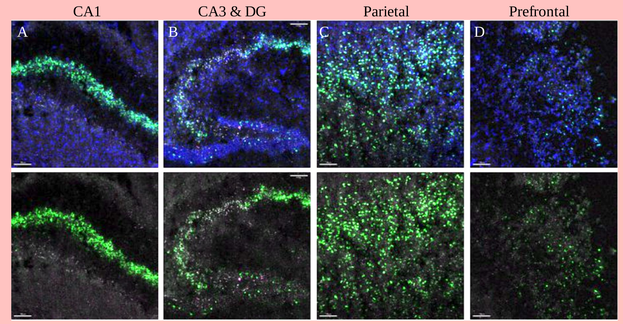



**Figure 2**. Arc and Homer1a neural patterns using a confocal microscope at a magnification of 10X. A) Neural pattern of Arc and Homer1a RNA expression in the CA1 region of the hippocampus. The CA1 region is involved in memory formation and spatial navigation. B) Neural patterns of Arc and Homer1a RNA expression in the CA3 region and the dentate gyrus (DG) of the hippocampus. The CA3 region is also implicated in memory and is interconnected with other parts of the hippocampus, while the DG plays a role in memory formation and pattern separation. C) Neural patterns of Arc and Homer1a RNA expression in the region of the parietal cortex. The parietal cortex is involved in sensory processing, spatial reasoning, and attention. D) Neural patterns of Arc and Homer1a proteins in the region of the prefrontal cortex. The prefrontal cortex is associated with executive functions such as decision-making, planning, and impulse control.

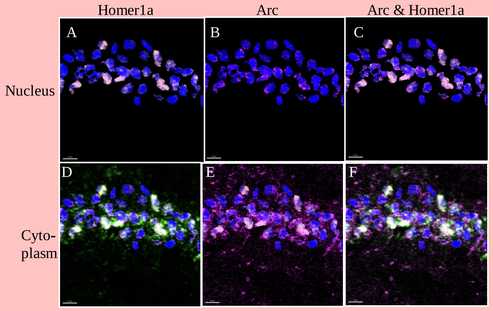



**Figure 4**. Imaging of Davis' CA1.

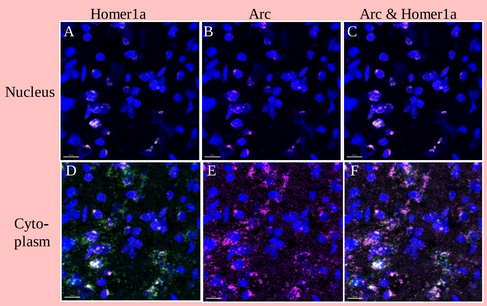



**Figure 5**. Imaging of Davis' CA3.

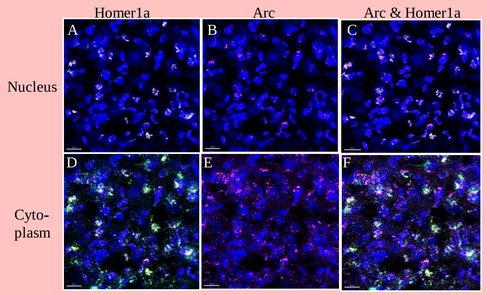



**Figure 6**. Imaging of Lazareva's parietal cortex.

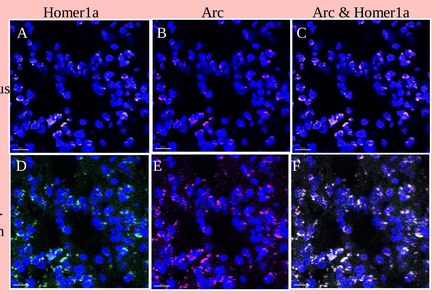



**Figure 7**. Imaging of Lazareva's prefrontal cortex.

**Figure 4-7 Legend:** A) Arc mRNA expression inside of the nucleus, color was changed to yellow to differ from the cytosolic mRNA. B) Homer1a expression in the nucleus. C) Arc and Homer 1a in the nucleus. D) Arc expression in the cytoplasm. E) Homer1a expression in the cytoplasm. F) Arc and Homer1a in the both the nucleus and cytoplasm.

## Expected Distribution of Arc & Homer1a Expression

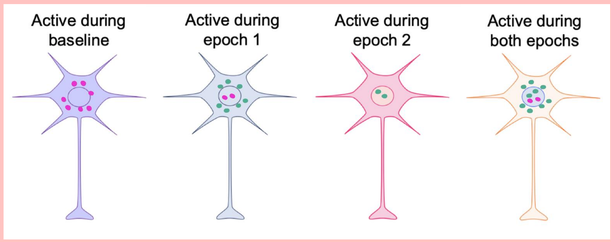



**Figure 3.** The green fluorescent dots are Arc and the magenta ones are Homer1a. For instance, if neuron was active at baseline 60 minutes prior to euthanasia, it will exhibit Homer 1A in the cytoplasm. If the neuron was active during epoch one, 30 minutes prior to euthanasia, it will exhibit Arc in the cytoplasm and Homer1a in the nucleus. Moreover, if the neuron was active during the second epoch, 5 minutes before euthanasia, it will exhibit Arc in the nucleus. Lastly, if the neuron was active during both epochs, it will exhibit both Arc and Homer1a in the nucleus, and only Arc in the cytoplasm. To determine the total number of neurons and the percentage of neurons that were positive for Arc and Homer1a mRNA, picture must be manually quantified. This approach enables us to investigate individual differences in neural responses to TI and discern brain patterns of activation associated with successful task performance.

## Behavioral Analysis

**Table 1.** Epoch 1 Performance

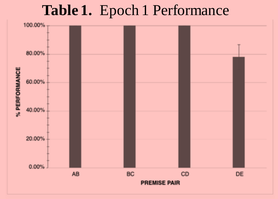



**Table 2.** Epoch 2 Performance

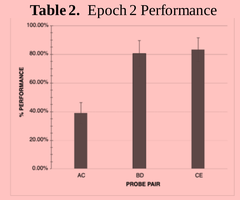



**Table 3.** Results of Epoch 2, probe pairs testing (N=9).

| Rat ID | AC | BD | CE |
|---|---|---|---|
| Gazes | 0.5 | 0.75 | 0.5 |
| Lazareva | 0.5* | 1 | 1 |
| Jensen | 0.5 | 0.5 | 0.5 |
| Eichenbaum | 0.5* | 1 | 1 |
| Davis | 0 | 1 | 1 |
| Zentall | 0 | 0.75 | 1 |
| Terrace | 0.5* | 1 | 1 |
| Wynne | 0.5 | 0.25 | 1* |
| Treichler | 0.5* | 1* | 0.5 |

* Significant on a one sample t-test ($p<0.05$).

## Discussion & Conclusion

The total number of neurons and the number of neurons active during each behavioral task will be quantified using ImageJ. By quantifying the distribution of Arc fluorescence (green dots) and Homer1a (magenta dots) fluorescence within specific brain regions, we can discern the timing and extent of neuronal activation in response to TI task demands. By investigating immediate early gene (IEG) expression in key brain regions associated with memory and decision-making, such as the hippocampus, parietal cortex, and prefrontal cortex, the study will provide valuable insights into the neural activity underlying TI behavior. The findings of this study contribute to our understanding of how rodents utilize deductive reasoning processes to infer relationships between stimuli and construct mental hierarchies. Furthermore, examining individual differences in TI performance among rodents could uncover important variables influencing TI behavior and its neural correlates. In conclusion, while this study represents a significant step forward in unraveling the neural basis of TI in rodents, there remains ample opportunity for future research to deepen our understanding of this intriguing cognitive phenomenon and its implications for both neuroscience and evolutionary biology.

## References

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