Identification of transcriptional patterns in hippocampus CA1 subregion associated with differential cognitive aptitude across the entire lifespan of rats

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Background & Methods

•Each hippocampus primary cell type has a unique transcriptomic composition. Therefore, it is possible that CA1 and CA3 pyramidal cells or DG granule cells may have distinct age-sensitive trajectories. Additionally, these trajectories of cognitive decline may depend on the cognitive status of individual rats. Here we utilize the immediate early gene *Arc* to assess the transcription pattern in cognitively categorized rats. Male Fisher-344 rats (6 mo, 15 mo, and 23 mo old), were given a battery of cognitive tests and were categorized into three groups - low, average, or high performers depending on their performance on the spatial version of the Morris watermaze. Rats were given two-5 min exploratory sessions separated by a 20 min rest in the home cage and brains from behavior as well as two additional controls (caged, a negative control, and maximal electroconvulsive shock-treated, a positive control) were quickly extracted, hemisected and rapidly frozen until sectioning and processing for in situ hybridization. In situ hybridization was performed as described previously (Guzowski et al., 1999), and slides were imaged using an SP5 Leica or a Zeiss LSM 880 confocal microscope. Three different areas of CA1 were imaged: distal CA1, which receives input primarily from the lateral entorhinal cortex; proximal CA1, which receives inputs primarily from the medial entorhinal cortex; and middle CA1, which receives a mixture of entorhinal inputs. Cells with *Arc* mRNA expression in the nucleus, cytoplasm or both compartments were counted using Image J software.

•1. Exploratory behavior task

•F344 rats were divided into groups according to their ages and cognitive performance and given two 5 min exploration in the same environment separated by 20 min rest in their home cages.

•2. Fluorescence *In Situ* Hybridization

Fluorescence *in situ* hybridization was performed as described previously (Guzowski et al., 1999). This method enables visualization of activated neurons by two separate behavioral episodes in the same animal (Fig 1). Each brain tissue slide containing three behavioral brains and two control brains, were imaged using SP5 Leica or a Zeiss LSM 880 confocal microscope. Fig 2 represents where the confocal images were taken in CA1 region.

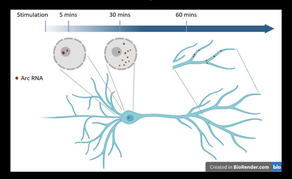


Fig 1. Timeline of *Arc* mRNA translocation to dendrites after stimulation. *Arc* mRNA form intranuclear foci within 5 mins after neural activation. By 30 mins, the labeled *Arc* mRNA is dominantly distributed in the cytoplasm and transported to the dendrites by 45 min.

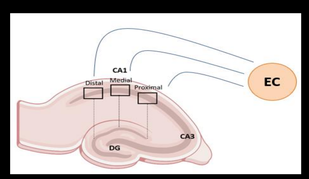
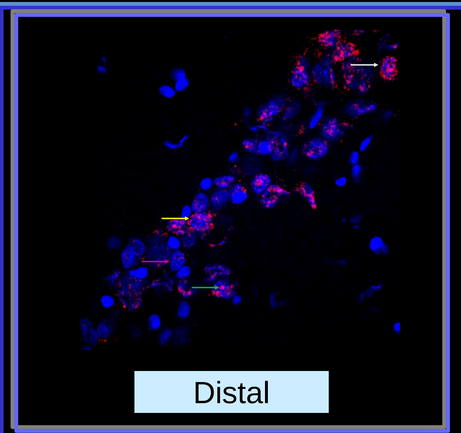
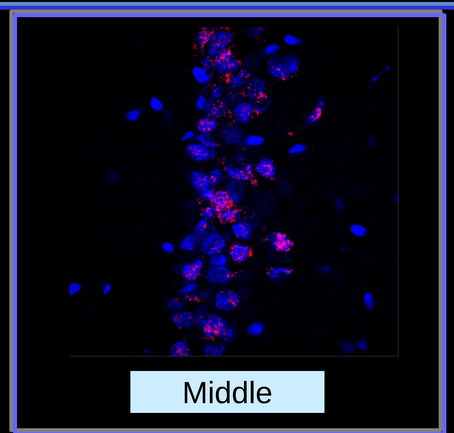
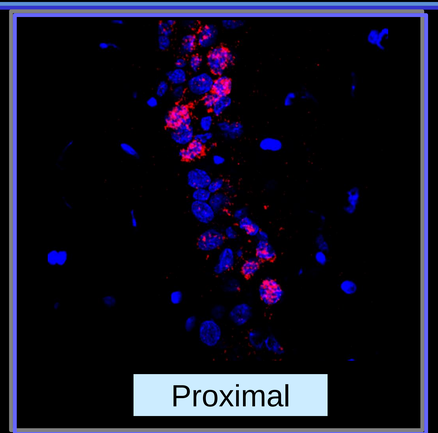


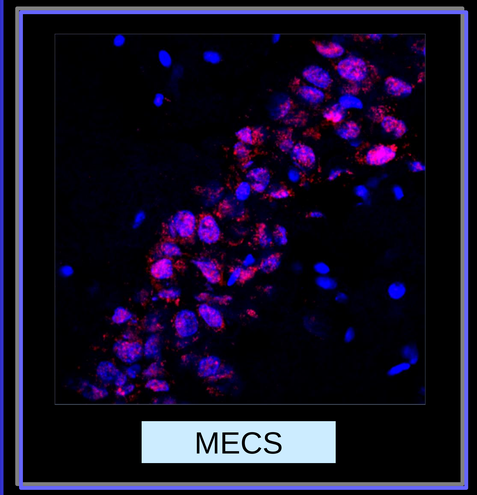
Fig 2. Diagram of hippocampal CA1 and its connection with entorhinal cortex (EC) Black boxes represent where the confocal images were taken, distal CA1, medial CA1, and proximal CA1 are located by the features of DG. Each CA1 region receives projection from different parts of entorhinal cortex (EC).

Figure 3: Representative confocal images from CA1 distal, middle and proximal subregion.

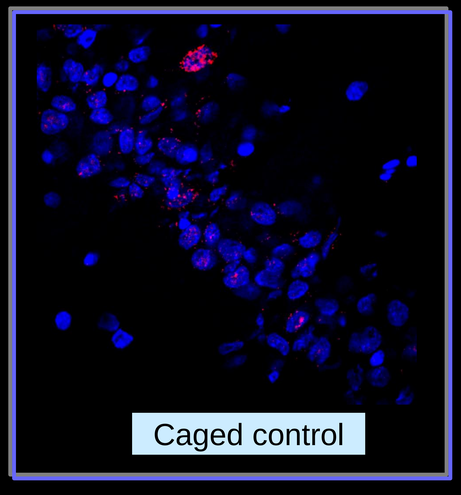








Arc mRNA positive cells can be seen in red and DAPI stained nuclei in blue. Green arrow indicates *Arc* foci, yellow arrow indicates cytoplasmic *Arc*, white arrow indicates both cytoplasmic and foci *Arc* and pink arrow indicates no *Arc* staining.



Experimenters are blinded to age and cognitive status of animals as imaging and cell counting is still ongoing. We can only report that Arc expression is robust in MECS group and low in caged animals with behavior animals showing levels that are intermediate.

Prediction and future directions

Using compartment analysis of temporal activity with FISH (catFISH) we can determine the reliability of cell firing in brain networks by two experiences in the same environment separated by 20 minutes. In this manner, we can assess the circuit stability of these specific brain regions, across age and across different cognitive competences.

We can predict that older poor performers would have a less stable network which may be evident with less double labeled *Arc* mRNA positive neurons (suggesting that neuronal ensembles in old CA1 exhibit a higher degree of remapping). On the other hand, young good performers may have higher double labeled population.

Future studies will include CA3 and DG subregions of the hippocampus and entorhinal cortex.

The overall goal is to identify the circuit characteristics associated with successful cognitive outcomes during normal aging.

References

1. Guzowski JF, McNaughton BL, Barnes CA, Worley PF. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. Nat Neurosci. 1999 Dec;2(12):1120-4

2. Hartzell AL, Burke SN, Hoang LT, Lister JP, Rodriguez CN, Barnes CA. Transcription of the immediate-early gene Arc in CA1 of the hippocampus reveals activity differences along the proximodistal axis that are attenuated by advanced age. J Neurosci. 2013 Feb 20;33(8):3424-33.

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