Arc mRNA expression pattern in the CA1 subregion of rat hippocampus following spatial behavior

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Introduction

Aging poses significant challenges to cognitive function, particularly in the domain of episodic memory, which relies on the integrity of the hippocampus CA1. Previous studies (Henriksen et al., 2010; Hartzell et al., 2013; Beer et al., 2018; Soltesz and Losonczy, 2018) have addressed functional specialization along the transversal axis of CA1, corresponding to input from the entorhinal cortex. Specifically, the distal CA1 receives projections from the lateral entorhinal cortex (LEC), the proximal CA1 receives projections from the medial entorhinal cortex (MEC), and the medial CA1 receives projections from a combination of LEC and MEC. These projections play a crucial role in processing distinct components of memory along the transversal axis of CA1, resulting in intricate neural representations and diverse behavioral performance. To enhance our understanding of chronological changes within the hippocampus neural network and the impact on cognitive competence, we conducted a comprehensive study investigating the cellular distribution of *Arc* mRNA in the distal, middle, and proximal subregions of the hippocampus CA1 in three distinct age groups (6 months, 15 months, and 23 months) of male Fisher-344 rats. Additionally, within each age group, rats were categorized into three different cognitive performance levels based on their behavior in the spatial version of the Morris watermaze. Our hypothesis was that an effect of aging and level of cognition within age groups would be evident in the cellular distribution of *Arc* mRNA within CA1 subregions.

Methods

Behaviorally induced *Arc* mRNA expression was investigated by allowing the rats to explore the same environment twice for 5 minutes each, with a 20-minute rest period in their home cage (Fig 1). To visualize the transient *Arc* mRNA expression pattern, in situ hybridization (catFISH) was performed as previously described (Guzowski et al., 1999). Each brain slide contained three behavioral brains and two control brains. Imaging was conducted using a Zeiss LSM 800 confocal microscope with 40x oil objective (Fig 2). Our behavioral strategy enabled the observation of cells with *Arc* mRNA expression in the nucleus, cytoplasm, or both compartments (Fig 3), which were subsequently counted using ImageJ software.

Fig 1. Schematic of exploratory behavioral task

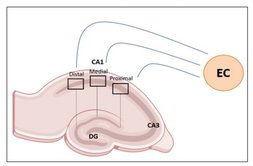


Fig 2. Diagram of hippocampal CA1 and its connection with entorhinal cortex (EC)
Black boxes represent where the confocal images were taken, distal CA1, medial CA1, and proximal CA1 are located by the features of DG. Each CA1 region receives projection from different parts of entorhinal cortex (EC).

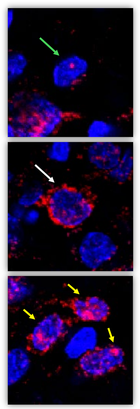
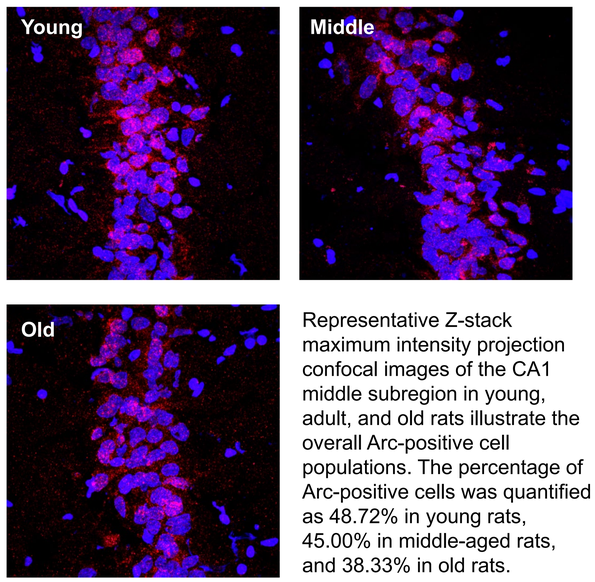


Fig 3. Subcellular distribution of *Arc* showing different staining profiles under confocal microscopy.
Cell nuclei are represented in blue, and *Arc* mRNA staining is shown in red. The patterns of *Arc* expression are indicated by colored arrows: the green arrow indicates intranuclear foci only, the white arrow indicates cytoplasmic labeling only, and the yellow arrow indicates both intranuclear foci and cytoplasmic signal.

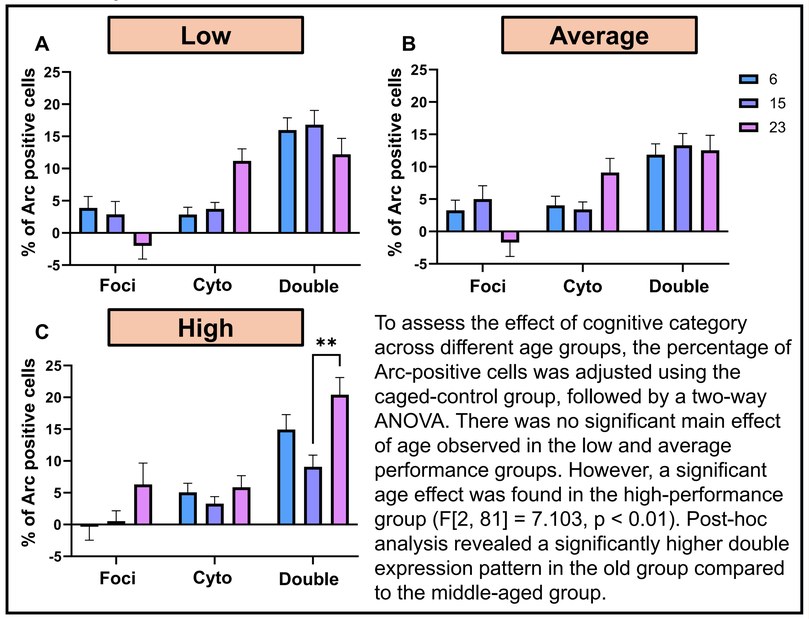
Results

Figure 4. Representative confocal images of CA1 middle subregion across age groups under average performance



Representative Z-stack maximum intensity projection confocal images of the CA1 middle subregion in young, adult, and old rats illustrate the overall Arc-positive cell populations. The percentage of Arc-positive cells was quantified as 48.72% in young rats, 45.00% in middle-aged rats, and 38.33% in old rats.

Figure 5. Arc expression patterns in CA1 middle region across age groups under different cognitive performance levels (Low, Average, High cognitive levels.)



To assess the effect of cognitive category across different age groups, the percentage of Arc-positive cells was adjusted using the caged-control group, followed by a two-way ANOVA. There was no significant main effect of age observed in the low and average performance groups. However, a significant age effect was found in the high-performance group ($F[2, 81] = 7.103$, $p < 0.01$). Post-hoc analysis revealed a significantly higher double expression pattern in the old group compared to the middle-aged group.

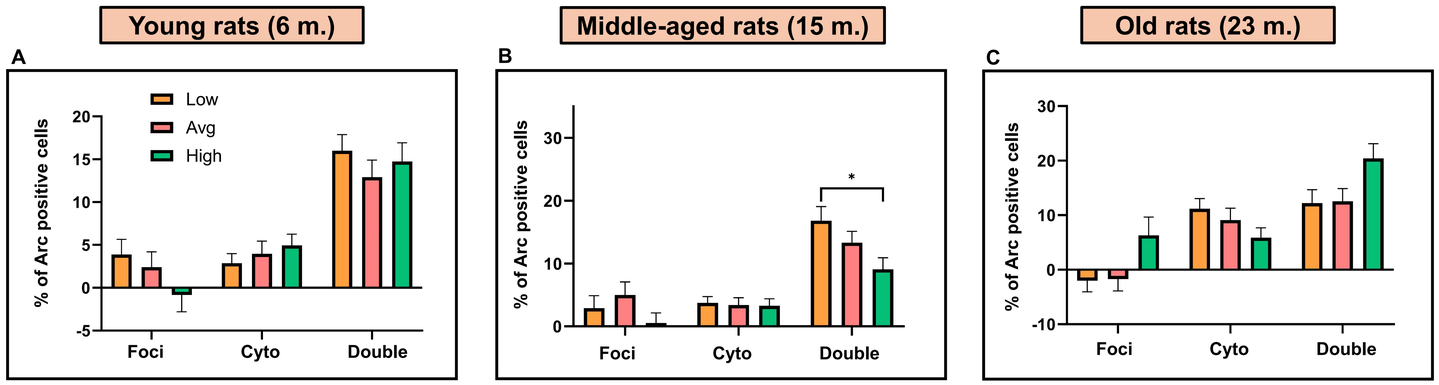


Figure 6. Arc expression patterns in CA1 middle region across performance groups within different age groups (Young, Middle-aged, Old Rats)

A two-way ANOVA was conducted to assess the effect of different cognitive performance levels in each of the three age groups, adjusted by the caged-control group. A significant main effect of cognitive level was observed only in the middle-aged rats ($F[2, 102] = 3.169$, $p < 0.05$). Post-hoc comparisons were performed using Tukey's multiple comparison test to adjust for multiple comparisons. Pattern of Arc expression in young rats in consistent with previous Arc catFISH experiments, and was not impacted by cognitive status across cognitive category. In the middle-aged rats, however, there was a paradoxical decrease in the number of double-labeled cells in the high-performing rats. For the older rats, consist with Figure 5C, the high-performing old rats showed more double-labeled cells, suggesting more network stability in this cognitive category.

Conclusions & Future directions

- Overall, the expected decrease in network stability in the low performing cognitive category did not occur within any age group.
- There was also no decrease in network stability overall when comparing young to middle age, and middle age to old as was predicted.
- Surprisingly, the old high-performing rats showed greater network stability than other subgroups tested. This potentially suggests a mechanism that could help explain the spatial memory superiority of this older group.

Because middle CA1 region that was examined here receives both MEC and LEC inputs, fuutre analysis will focus on the proximal and distal portions of CA1, as these are the regions that receive inputs exclusively from MEC and LEC respectively, to determine if a different pattern of results might arise. In particular, we predict that the older rats will have a less stable network in the proximal region that carries predominantly spatial information from MEC.

References

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