Neurobiology of Aging 70 (2018) 217–232

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Dissociable effects of advanced age on prefrontal cortical and medial temporal lobe ensemble activity

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ARTICLE INFO

Article history:
Received 20 October 2017
Received in revised form 20 June 2018
Accepted 21 June 2018
Available online 30 June 2018

Keywords:
Arc
CA1
Cognition
Infralimbic cortex
Prelimbic cortex

ABSTRACT

The link between age-related cellular changes within brain regions and larger scale neuronal ensemble dynamics critical for cognition has not been fully elucidated. The present study measured neuron activity within medial prefrontal cortex (PFC), perirhinal cortex (PER), and hippocampal subregion CA1 of young and aged rats by labeling expression of the immediate-early gene *Arc*. The proportion of cells expressing *Arc* was quantified at baseline and after a behavior that requires these regions. In addition, PER and CA1 projection neurons to PFC were identified with retrograde labeling. Within CA1, no age-related differences in neuronal activity were observed in the entire neuron population or within CA1 pyramidal cells that project to PFC. Although behavior was comparable across age groups, behaviorally driven *Arc* expression was higher in the deep layers of both PER and PFC and lower in the superficial layers of these regions. Moreover, age-related changes in activity levels were most evident within PER cells that project to PFC. These data suggest that the PER-PFC circuit is particularly vulnerable in advanced age.

1. Introduction

Cognitive decline associated with advanced age can reduce an individual's quality of life. As no single neurobiological deficit can account for the wide spectrum of behavioral impairments observed in old age, it is critical to develop an understanding of how interactions among different brain regions change over the lifespan. Rats have been used to model age-related cognitive decline, showing age-related impairments in the ability to perform a range of behavioral tasks, including the hippocampal-dependent spatial Morris water maze (Gallagher et al., 1993), prefrontal cortical (PFC)–dependent working memory and set-shifting tasks (Beas et al., 2013, 2017; Bizon et al., 2012) and perirhinal cortical (PER)–dependent object recognition tasks (Burke et al., 2010, 2011; Johnson et al., 2017). In fact, the PFC and medial temporal lobe (MTL) are among the first regions vulnerable to the effects of advancing age (Morrison and Baxter, 2012; Peters, 2006; Samson and Barnes, 2013). Interestingly, when aged rats are tested on an object-place paired association (OPPA) task (see Methods section and Figure 1), which requires PFC-MTL communication (Hernandez et al., 2017; Jo and Lee, 2010a; Lee and Solivan, 2008), deficits are observed before detectable hippocampal-dependent water maze impairments (Hernandez et al., 2015). Specifically, the OPPA task tests cognitive flexibility and associative learning, as such it requires the hippocampus, PFC, and PER, as well as functional connectivity between these structures (Hernandez et al., 2017; Jo and Lee, 2010a; Lee and Solivan, 2008). Together, these data suggest that tasks requiring both PFC and MTL structures are particularly sensitive to detecting decline in advanced age.

Although it is known that there are age-related impairments on behaviors that rely on the PFC and MTL, there is no neuronal cell loss within the infralimbic cortex (IL) and prelimbic cortex (PL) of the medial PFC (Stranahan et al., 2012), hippocampus (Rapp and Gallagher, 1996), or PER (Rapp et al., 2002). Rather than loss of neurons, biochemical analyses (Bañuelos et al., 2014; Beas et al., 2017; Liu et al., 2008; McQuail et al., 2015), in vivo neurophysiology (Thomé et al., 2016; Wang et al., 2011), and human imaging data (Cabeza et al., 2002; Grady, 2012; Ryan et al., 2012; Yassa et al., 2011, 2011; Zarahn et al., 2007) have suggested that age-related

Conflicts of interest: none to report.

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https://doi.org/10.1016/j.neurobiolaging.2018.06.028

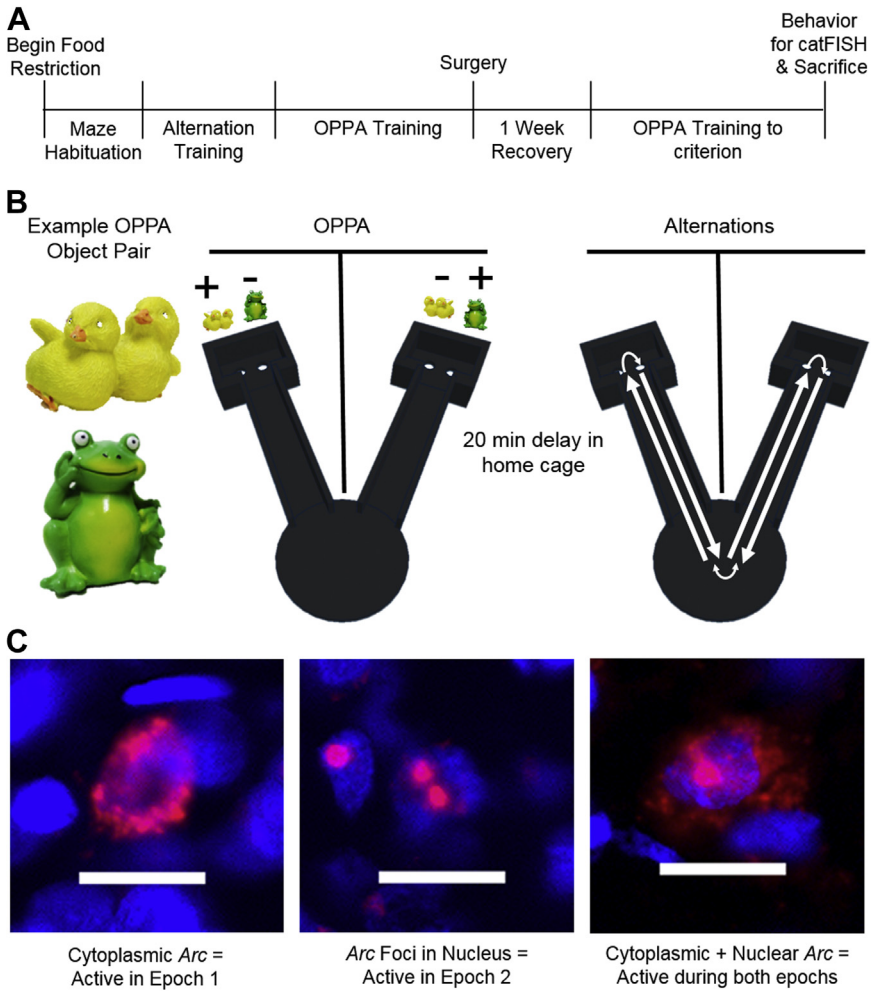

Fig. 1. Behavioral paradigm on final testing day. (A) Experimental timeline. (B) Objects used during the OPPA task are shown. During the OPPA task, rats traversed back and forth between the two arms of the testing apparatus, choosing an object in each arm. In the left arm, the chicks object was correct, but in the right arm the frog object was correct. Following a 20-minute rest in their home cages, animals then performed an alteration task in which they traversed back and forth between the two start platforms for reward, but did not perform the object discrimination component. Order of testing was counter balanced across rats. (C) Representative images of subcellular distribution of *Arc* within neurons. Subcellular location was used to infer which behavioral epoch a neuron was active in. Scale bar is 20 μm.

cognitive impairments are the result of changes in neural activity patterns that show distinct disruptions between different brain regions. In many of these studies, however, the young and older study participants were either performing differently on the behavioral tasks during which neural activity was examined or were performing a task that did not depend on the region of interest. Thus, the extent to which age-related alterations in neural activation across the hippocampus, PER, and PFC persist when subjects are performing comparably on behavioral tasks that require those regions has not been well defined.

In the present study, young and aged rats were sacrificed from their home cages, and the IL, PL, PER, and hippocampal subregion CA1 were examined for baseline expression of the activity-dependent immediate-early gene *Arc*. Another group of young and aged rats received the retrograde tracer, cholera toxin subunit b, into the PL and IL of medial PFC to label neurons in PER and CA1 of hippocampus that project to the PFC. These animals were trained to criterion performance of >81% correct on 2 consecutive days of testing on the OPPA task, which requires animals to use a biconditional object-in-place rule to learn the correct choice from an object pair. On the final day, rats completed two 5-minute epochs of behavior separated by a 20-minute rest. In one epoch, rats performed OPPA task, and in the other, they alternated between the 2 arms of the same maze without doing the biconditional object discrimination. Tissue was then processed for cellular compartment analysis of temporal activity with fluorescence in situ hybridization (catFISH) by labeling expression of the neuron activity-dependent immediate-early gene *Arc* in hippocampal subregion CA1, PER, and medial PFC regions of IL and PL. The subcellular localization of *Arc* can be used to determine which neural ensembles across the brain were active during 2 distinct episodes of behavior. *Arc* is first transcribed within the nucleus of neurons 1–2 minutes after cell firing. Importantly, *Arc* mRNA translocate to the cytoplasm approximately 15–20 minutes after cell firing, which allows for cellular activity during 2 epochs of behavior, separated by a 20-minute rest to be represented within a single neural population (Guzowski et al., 1999). Combining this catFISH approach with retrograde labeling allowed for the examination of neural activity in anatomically defined cellular populations. Importantly, aged rats in this study were trained on the OPPA task until they performed at the same level of accuracy as young rats. Therefore, alterations in neural activity could not be attributed to overt behavioral differences.