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Age-related changes in *Arc* transcription and DNA methylation within the hippocampus

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Abstract

The transcription of genes that support memory processes are likely to be impacted by the normal aging process. Because *Arc* is necessary for memory consolidation and enduring synaptic plasticity, we examined *Arc* transcription within the aged hippocampus. Here, we report that *Arc* transcription is reduced within the aged hippocampus compared to the adult hippocampus during both "off line" periods of rest, and following spatial behavior. This reduction is observed within ensembles of CA1 "place cells", which make less mRNA per cell, and in the dentate gyrus (DG) where fewer granule cells are activated by behavior. In addition, we present data suggesting that aberrant changes in methylation of the Arc gene may be responsible for age-related decreases in *Arc* transcription within CA1 and the DG. Given that *Arc* is necessary for normal memory function, these subregion-specific epigenetic and transcriptional changes may result in less efficient memory storage and retrieval during aging.

Keywords: Immediate-early gene; Epigenetics; Normal aging; Hippocampus

1. Introduction

Normal aging inevitably involves changes in memory function (Park and Reuter-Lorenz, 2009). While these changes occur with minimal gross brain pathology (Coleman and Flood, 1987; West, 1993), a number of subtle neural alterations do occur (Burke and Barnes, 2006).

The formation and maintenance of memories relies on rapid and sustainable synaptic modification, and these processes require new gene expression. Several studies have reported age-associated changes in the expression of immediate-early genes (IEGs) within the hippocampus (Yau et al., 1996; Desjardins et al., 1997; Small et al., 2004), a brain region vulnerable to the aging process (Burke and Barnes, 2006). Among these genes, *Arc* (activity regulated cytoplasmic gene (Lyford et al., 1995), also known as Arg3.1 (Link et al., 1995) is necessary for memory consolidation (Guzowski et al., 2000; Plath et al., 2006), and is induced selectively in the principal cells of the hippocampus and other brain regions by neural activity specifically associated with active information processing (Miyashita et al., 2008). Thus, *Arc* has been used in the development of a method (catFISH; cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization, Guzowski et al., 1999) that allows precise identification of cells active in networks engaged by discrete behaviors. Although catFISH represents a powerful way to identify specific cells that comprise circuits supporting behavior, in isolation it does not provide a quantitative indication of the magnitude of gene transcription in the activated cells. Methods such as RT-PCR and gene microarrays, offer alternative approaches to determine

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whether the amount of a particular mRNA species changes with age (Blalock et al., 2003; Verbitsky et al., 2004; Rowe et al., 2007) but lack consideration for the identity of the cells that transcribe the mRNA of interest. Therefore, these approaches cannot address questions concerning the precise composition of behaviorally relevant circuits. Using catFISH, the initial development of a neural ensemble engaged as a result of brief exploratory activity was assessed, as well as the short-term stability of these ensembles within the dorsal hippocampus. Using RT-PCR within the same brain, the relative amount of *Arc* mRNA transcribed could also be determined. The combination of these methods revealed region-specific, age-related alterations in *Arc* transcription under both resting conditions, and following exploratory behavior.

To understand the mechanisms that may be responsible for attenuated *Arc* transcription, we measured DNA methylation of the Arc gene. DNA methylation involves the addition of methyl groups across CG-rich gene regulatory regions or at specific CG sites within those regions. These CpG sites or "islands" are generally found near or within the promoter region of mammalian genes, with about 40% of genes containing a CpG island. DNA methylation has been extensively studied in development, and until very recently, was thought to be a static process (Kangaspeska et al., 2008; Metivier et al., 2008; Ma et al., 2009). Recent work, however, suggests that DNA methylation remains an active process in the mature CNS, and can be rapidly and dynamically regulated by learning and memory processes. Further, interfering with this process can disrupt long-term memory consolidation (Miller and Sweatt, 2007; Lubin et al., 2008). Here, using bisulfite sequencing of the Arc gene, we demonstrate significant subregion-specific age-associated changes in the regulation of Arc DNA methylation.

2. Methods

2.1. Behavioral procedures

Experiments were performed in accordance with NIH guidelines for the care and use of animals and the Institutional Animal Care and Use Committee at the University of Arizona. Adult (9–12 months, $n = 29$) and aged (24–32 months, $n = 29$) male Fischer-344 rats (National Institute on Aging at Harlan Sprague Dawley) were used in these experiments. Prior to the beginning of experiments, the spatial and visual discrimination abilities of each rat were assessed using the Morris swim task as described in detail by Barnes et al. (1997). Performance on the the Morris swim task was analyzed using the corrected integrated path length (CIPL; Gallagher et al., 1993).

Experiments took place at least 2 hours after the house lights in the colony room were shut off. Animals were divided into 3 groups: (1) caged control (CC) animals that were sacrificed directly from their home cages; (2) rats that explored the environment once for 5 min, and were sacrificed immediately (A-5′); (3) animals that explored the same environment twice, with an intervening rest period of 20 min (A/A). Animals used for DNA methylation analysis explored the environment for 5 min, then returned to their home cages for 25 min prior to tissue collection. The procedure for exploration is described elsewhere (Guzowski et al., 1999). To ensure that the amount of exploratory activity between age groups could not explain differences in *Arc* transcription we verified that adult and aged rats showed similar exploratory behavior (see Supplementary Fig. S4).

2.2. Tissue harvesting and fluorescence in situ hybridization

After decapitation, brains were removed and hemisected, and half of the brain (used for *in situ* hybridization) was quick-frozen in isopentane. The remaining half (used for RT-PCR or DNA methylation analysis) was dissected into the CA region and the DG. These samples were flash frozen and stored at −70 °C. Hemisections containing the dorsal hippocampus from 8 rats were molded in a block with Tissue-Tek OCT compound, so that all experimental conditions were represented in each block for each time point. Use of tissue blocks helps control for slide-to-slide variation in signal detection. Brains were sectioned at 20 μm coronally through the dorsal hippocampus (−3.2 to −3.8 mm from bregma; Paxinos and Watson, 1998), thaw-mounted on slides, and stored at −70 °C. Fluorescence *in situ* hybridization was performed as described in detail elsewhere (Guzowski et al., 1999; Vazdarjanova and Guzowski, 2004).

2.3. Image acquisition and analysis

Images were collected using a Zeiss 510 Metaseries laser scanning confocal microscope. Photomultiplier tube assignment, pinhole size and contrast values were held constant for each brain region on a slide. The areas of analysis were z-sectioned in 1.0 μm optical sections. Similar methods were used to acquire images of the DG, except that the whole structure was imaged. Image analysis was conducted as described earlier (Guzowski et al., 1999; Vazdarjanova et al., 2002) using MetaMorph imaging software (Universal Imaging). *Arc* is not present in hippocampal glia or interneurons under these experimental conditions (Vazdarjanova et al., 2006). Only whole neuron-like cells found in the middle 20% of each stack were included in the analyses. Neurons were classified as: (1) foci$^+$ which had one or two intense intranuclear foci present in at least three planes; (2) cytoplasmic$^+$ which had perinuclear or cytoplasmic staining surrounding at least 60% of the cell and visible in at least three plains; (3) double$^+$ which fulfilled both criteria 1 and 2, or (4) negative which did not have any detectable staining. An assessment of intranuclear foci fluorescent intensity in CA1 was conducted on tissue from rats that explored the environment for one 5 min session using a method similar to that described by Guzowski et al. (2006)