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A fine balance: Regulation of hippocampal Arc/Arg3.1 transcription, translation and degradation in a rat model of normal cognitive aging

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ABSTRACT

Memory decline is a common feature of aging. Expression of the immediate-early gene *Arc* is necessary for normal long-term memory, and although experience dependent *Arc* transcription is reportedly reduced in the aged rat hippocampus, it has not been clear whether this effect is an invariant consequence of growing older, or a finding linked specifically to age-related memory impairment. Here we show that experience dependent *Arc* mRNA expression in the hippocampus fails selectively among aged rats with spatial memory deficits. While these findings are consistent with the possibility that blunted *Arc* transcription contributes to cognitive aging, we also found increased basal ARC protein levels in the CA1 field of the hippocampus in aged rats with memory impairment, together with a loss of the experience dependent increase observed in young and unimpaired aged rats. Follow-up analysis revealed that increased basal translation and blunted ubiquitin mediated degradation may contribute to increased basal ARC protein levels noted in memory impaired aged rats. These findings indicate that *Arc* expression is regulated at multiple levels, and that several of these mechanisms are altered in cognitively impaired aged rats. Defining the influence of these alterations on the spatial and temporal fidelity of synapse specific, memory-related plasticity in the aged hippocampus is an important challenge.

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1. Introduction

Many individuals develop cognitive deficits during normal aging, prominently involving memory, while others at the same chronological age perform on par with younger adults. In humans and animal models, this loss of function can occur in the context of largely preserved neuron and synapse numbers throughout the hippocampal memory system, and impairment is thought to arise instead from subtle alterations in the plasticity and connectivity required to form and maintain memories (Burke & Barnes, 2006; Fletcher, 2012).

Expression of the immediate early gene *Arc* (activity-regulated cytoskeleton-associated protein; also termed Arg3.1) is necessary for multiple forms of synaptic plasticity, and is induced under a variety of behavioral and experimental conditions, including LTP (Lyford et al., 1995; Steward, Wallace, Lyford, & Worley, 1998), LTD, novel environmental exploration (Guzowski, McNaughton, Barnes, & Worley, 1999; Pinaud, Penner, Robertson, & Currie, 2001; Vazdarjanova, 2004), and learning (Fletcher, 2006; Guzowski, Setlow, Wagner, & McGaugh, 2001; Pinaud et al., 2001). Blocking *Arc* protein expression by anti-sense oligonucleotide injection impairs LTP maintenance and memory consolidation while sparing short-term memory, suggesting a potentially critical role in stabilizing enduring synaptic modifications (Guzowski et al., 2000). ARC protein also regulates synaptic strength and homeostatic scaling by promoting the internalization of AMPARs (Chowdhury et al., 2006; Rial Verde, Lee-Osbourne, Worley, Malinow, & Cline, 2006; Shepherd et al., 2006). This background suggests the possibility that disrupted *Arc* induction or processing might give rise to impairment in memory-related hippocampal plasticity associated with aging. Recent studies have reported data consistent with that proposal, prompting the conclusion that changes in *Arc* transcription, mediated in part by epigenetic regulation, may contribute to impoverished consolidation and poor memory retrieval in aging (Burke, Ryan, & Barnes, 2012; Marrone, Satvat, Shaner, Worley, & Barnes, 2012; Ménard & Quirion, 2012; Penner et al., 2011; Blalock et al., 2003).

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The transcription, translation, trafficking and turnover of *Arc* mRNA and protein are tightly regulated and vary according to cell and stimulus type. Within minutes of NMDA receptor (NMDAR) or voltage-gated calcium channel activation, a burst of *Arc* transcription is initiated in all principal fields of the hippocampus (Adams, Robinson, Hudgins, Wissink, & Dudek, 2009), lasting for hours in the dentate gyrus (Lyford et al., 1995), and subsiding more rapidly in pyramidal neurons (Guzowski et al., 1999). Resulting mRNA is trafficked and targeted to specific synapses in an NMDAR-dependent manner (Farris, Lewandowski, Cox, & Steward, 2014; Moga et al., 2004; Steward et al., 1998), and although it has been widely thought to target recently activated synapses, current evidence indicates that locally translated ARC preferentially accumulates at relatively less active synaptic sites (Okuno et al., 2012). This account suggests that *Arc* mediates an 'inverse tagging' mechanism, distinguishing potentiated synapses by inhibiting enhancement at weak or inactive sites through AMPA receptor endocytosis. Temporal regulation of *Arc* expression is achieved via a network of activating and repressive factors that control transcription and translation (Bramham et al., 2009). Regulation of mRNA stability by translation dependent decay (TDD) pathways provides an additional means of controlling the dendritic localization of *Arc* mRNA and fine tuning Arc protein levels (Farris et al., 2014; Giorgi et al., 2007). Finally, ARC protein turnover is regulated by ubiquitin proteasome dependent degradation (Greer et al., 2010; Kuehnle, Mothes, Matentzoglu, & Scheffner, 2013). Disrupted ARC protein regulation at the synapse is suspected to play a role in a variety of disorders in which cognitive function is prominently affected, including Angelman Syndrome (Greer et al., 2010), Huntington's disease (Maheshwari, Samanta, Godavarthi, Mukherjee, & Jana, 2012) and fragile-X syndrome (Niere, Wilkerson, & Huber, 2012).

Current evidence indicates that experience dependent Arc transcription is blunted in the aged rat hippocampus, consistent with a potential contribution to the memory impairment that frequently accompanies aging (Penner, Chawla, Roth, Sweatt, & Barnes, 2010). On the basis of available studies, however, it has been difficult to disentangle whether reduced *Arc* transcription is an obligatory consequence of chronological aging, or a finding more specifically tied to the effects of aging on memory supported by the hippocampus. A previous study in Fischer 344 rats, for example, reported that aged subjects exhibit both memory impairment and decreased *Arc* expression in the CA1 field of the hippocampus relative to young controls, but no correlation between the mRNA and cognitive results (Blalock et al., 2003). In addition, earlier work on *Arc* in the context of neurocognitive aging has concentrated predominantly on mRNA expression, and how the complex regulatory network responsible for ARC protein translation might be affected has not been examined. Independent of neurocognitive aging, limited attention has been directed at testing the possibility that, like *Arc* mRNA induction, the regulatory factors that mediate *Arc* translational control may also be dynamically modulated in response to recent behavioral training (Barker-Haliski, Pastuzyn, & Keefe, 2012). As a starting point in filling these gaps, here we quantified experience dependent *Arc* transcription and translation in a rat model that reveals substantial individual differences in cognitive aging, from aged subjects that display significant deficits in spatial learning and memory supported by the hippocampus, to age-matched rats that perform as well as young adults. Overall, the results indicate that changes in the dynamic regulation of hippocampal *Arc* in relation to age-related memory impairment extend beyond transcription, involving multiple levels of control, including both translation and degradation.

2. Methods

2.1. Water maze training

2.1.1. Background behavioral characterization

Young ($n = 36$; 6 months of age) and aged ($n = 71$; approximately 24 months of age) male Long-Evans rats (Charles River Laboratories) were housed singly in a climate-controlled vivarium on a 12:12 h light:dark cycle with food and water provided *ad libitum*. Spatial learning and memory were assessed using a hippocampus dependent, 'place' version of the Morris water maze, following an established protocol identical to many previous studies (Gallagher, Burwell, & Burchinal, 1993). Briefly, key features of the protocol include sparse training (3 trials/day for 8 consecutive days), and the use of multiple, interpolated probe trials (last trial every other day) to document the development of spatial bias for the escape location. Individual differences in learning and memory were assessed according to a learning index score validated in earlier studies (Gallagher et al., 1993), reflecting average proximity to the hidden escape platform over the course of training. By this measure, low scores reflect relatively greater search accuracy focused on the escape location. Aged animals with learning index scores approximating the range of young animals were classified as unimpaired (AU), and those that scored above that range were classified as impaired (AI). These animals were subsequently used to analyze *Arc* transcription and translation, as described in the following sections.

2.1.2. Behavioral induction of Arc transcription

To induce *Arc* transcription rats were trained on a redundant place–cue (RPC) task in a new testing environment (young, $n = 11$; AU, $n = 9$; AI, $n = 9$), two weeks after background behavioral characterization. Training consisted of five days of testing (two, 2-trial blocks per day, 25 min between blocks) throughout which a visible platform was maintained in a constant location. During the last trial on the final day the platform was lowered to the bottom of the tank for the first 30 s of the trial, making it unavailable for escape, after which it was raised and visible. During this probe, young and aged-unimpaired rats demonstrate a significant bias for the former, cued escape location, whereas aged–impaired rats exhibit no evidence of spatial learning. Accordingly, the multi-day redundant place/cue procedure provided a setting sensitive to age-related memory impairment while minimizing nonspecific performance differences across groups that might influence *Arc*. Animals were killed five minutes after the last trial (30 min after the first trial) and tissue was processed for *Arc* in situ hybridization. This time point was chosen on the basis of the well documented time-course of *Arc* mRNA transcription (Guzowski et al., 1999), in order to maximize *Arc* mRNA levels in the CA1 and CA3 regions of the hippocampus. To assess baseline levels of *Arc* mRNA, rats held in the same room as animals that underwent RPC training were killed directly from their home cage (young, $n = 5$; AU, $n = 4$; AI, $n = 5$).

2.1.3. Behavioral induction of Arc translation

In order to examine behaviorally induced *Arc* translation, two weeks after background behavioral characterization, rats (young, $n = 10$; AU, $n = 12$; AI, $n = 10$) were tested on a single session RPC task that consisted of 15 trials with a 15 s inter-trial interval. For trials 1 through 9, and 11, 13, and 15, the escape platform was visible. On the remaining three, interleaved trials (10, 12, and 14), the platform was slightly submerged and hidden from view, but available for escape. The platform remained in a constant location throughout training, and the start location was varied pseudo-randomly across trials.

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