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Temporal dynamics of *Arc* gene induction in hippocampus: Relationship to context memory formation

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ABSTRACT

Past studies have proposed a role for the hippocampus in the rapid encoding of context memories. Despite this, there is little data regarding the molecular processes underlying the stable formation of a context representation that occurs in the time window established through such behavioral studies. One task that is useful for investigating the rapid encoding of context is contextual fear conditioning (CFC). Behavioral studies demonstrate that animals require approximately 30 s of exploration prior to a footshock to form a contextual representation supporting CFC. Thus, any potential molecular process required for the stabilization of the cellular representation for context must be activated within this narrow and behaviorally defined time window. Detection of the immediate-early gene *Arc* presents an ideal method to assess the activation of specific neuronal ensembles, given past studies showing the context specific expression of *Arc* in CA3 and CA1 subfields and the role of *Arc* in hippocampal long-term synaptic plasticity. Therefore, we examined the temporal dynamics of *Arc* induction within the hippocampus after brief context exposure to determine whether experience-dependent *Arc* expression could be involved in the rapid encoding of incidental context memories. We found that the duration of context exposure differentially activated *Arc* expression in hippocampal subfields, with CA3 showing rapid engagement within as little as 3 s of exposure. By contrast, *Arc* induction in CA1 required 30 s of context exposure to reach maximal levels. A parallel behavioral experiment revealed that 30 s, but not 3 s, exposure to a context resulted in strong conditioned freezing 24 h later, consistent with past studies from other laboratories. The current study is the first to examine the rapid temporal dynamics of *Arc* induction in hippocampus in a well-defined context memory paradigm. These studies demonstrate within 30 s of context exposure *Arc* is fully activated in CA3 and CA1, suggesting that the engagement of plastic processes requiring *Arc* function (such as long-term potentiation) occurs within the same temporal domain as that required for behavioral conditioning.

1. Introduction

Extensive evidence suggests that the dorsal hippocampus of rodents is critically involved in the processing of an environmental context (Fanselow, 2000; Hirsh, 1974; Maren, 2001; Rudy, Huff, & Matus-Amat, 2004). For example, lesions and inactivations of the dorsal hippocampus severely impair contextual conditioning, but not conditioning to a discrete stimulus (Kim & Fanselow, 1992; O'Keefe et al., 1975; Sutherland, Kolb, & Whishaw, 1982). In complement to behavioral studies, principal cells in CA1 and CA3 of the hippocampus show preferential firing to discrete locations within an environment (Lee, Yoganarasimha, Rao, & Knierim, 2004b; Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; O'Keefe, 1976; O'Keefe and Dostrovsky, 1971). The collective firing of such "place cells" is posited to code for a spatial map of the environment (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971). These findings suggest that the dorsal hippocampus is specifically involved in the processing of an environmental context.

It is further proposed that the hippocampus is able to rapidly form such contextual representations (Frank, Stanley, & Brown, 2004; Morris & Frey, 1997; Wilson & McNaughton, 1993). Contextual fear conditioning (CFC) experiments provide strong evidence for behaviorally relevant rapid encoding of context. Studies have revealed that the time of contextual exposure prior to a shock, termed the placement-to-shock interval (PSI), predicts an animal's ability to condition to a context. Specifically, if the PSI is extended to over 20–30 s, then an animal will display robust context conditioning during a retention test as measured with freezing (Fanselow, 1986; Wiltgen, Sanders, Behne, & Fanselow, 2001). In

Abbreviations: FISH, fluorescence *in situ* hybridization; *Arc*, activity regulated cytoskeleton-associated protein; PSI, placement-to-shock interval; CFC, contextual fear conditioning; IEG, immediate-early gene; ISD, immediate shock deficit.

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contrast, if animals are immediately shocked upon exposure to a context, then they exhibit a diminished fear response to that context during testing (Blanchard, Fukunaga, & Blanchard, 1976; Fanselow, 1986). This "immediate shock deficit" (ISD) at intervals of less than 20 s is hypothesized to result from the lack of an established contextual representation in the hippocampus (Fanselow, 1990; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006). Lengthening the PSI is thought to allow sufficient time for the formation/stabilization of a hippocampus-dependent contextual representation that can be associated with the shock and result in behavioral expression of the fear memory (Fanselow, 1990; Rudy et al., 2004; Westbrook, Good, & Kiernan, 1994). Thus, a minimum amount of time is needed for the animal to form a behaviorally relevant contextual representation. This interval (~30 s) behaviorally defines the time window in which any potential molecular process required for context representation must be activated. Given this, CFC, and the associated ISD at short exposures, provides a powerful means to uncover the neural substrates of context memory.

A possible molecular step needed for formation of long-term context memory is the activation of immediate-early gene (IEG) transcription. IEGs have been studied extensively for their role in synaptic plasticity and the structural changes that are hypothesized to accompany the formation of long-term memories (Flavell & Greenberg, 2008; Guzowski, 2002; Loebrich & Nedivi, 2009). One such gene, the activity regulated cytoskeleton-associated protein (*Arc*, also known as *Arg3.1*) has been shown to be required for specific neuroplastic processes critical for memory function (Bramham et al., 2010; Link et al., 1995; Lyford et al., 1995; Miyashita, Kubik, Lewandowski, & Guzowski, 2008). *Arc* plays specific roles in long-term potentiation, long-term depression, and synaptic scaling (Guzowski et al., 2000; Messaoudi et al., 2007; Park et al., 2008; Plath et al., 2006; Shepherd et al., 2006; Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008). Moreover, knockdown or knockout of *Arc* function impairs long-term memory without affecting task acquisition or short-term memory (Czerniawski et al., 2011; Guzowski et al., 2000; Plath et al., 2006).

In addition to helping define the molecular mechanisms of long-term memory, IEGs have been useful for mapping task-specific neuronal ensembles in behaving animals (Guzowski, 2002; Kubik, Miyashita, & Guzowski, 2007). Exploiting the temporal signature provided by the transcription and subsequent translocation of *Arc* mRNA from nucleus to cytoplasm, cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) demonstrated that *Arc* is induced in CA1 neuronal ensembles in a context-specific manner (Guzowski, McNaughton, Barnes, & Worley, 1999), as predicted from electrophysiological studies (Muller & Kubie, 1987). Further, IEG imaging demonstrated that transcriptional activation of *Arc* is differentially engaged within hippocampal ensembles in CA3 and CA1 subfields in response to discrete perturbations within an experimental context (Vazdarjanova & Guzowski, 2004). This finding is consistent with electrophysiological recording studies, lesion work, and theoretical models that posit distinct roles for CA3 and CA1subfields in context encoding (Guzowski, Knierim, & Moser, 2004; Kesner, 2007; Kubik et al., 2007; Lee & Kesner, 2002; Lee et al., 2004b; Leutgeb & Leutgeb, 2007; Leutgeb et al., 2004). Broadly speaking, CA3 is thought to encode a general and rapid representation of context, while CA1 is responsible for representing detailed context information (Guzowski et al., 2004; Leutgeb & Leutgeb, 2007). Taken together, these IEG imaging findings substantiate that *Arc* is a useful and reliable marker of context-specific neural ensemble activity within the hippocampus.

In contrast to the substantial evidence available for the role of the hippocampus in contextual processing, the temporal dynamics of the molecular mechanisms activated by brief context exposure are poorly defined. One study by McHugh and Tonegawa (2009) demonstrated that CFC is impaired in mice with localized knockout of the CA3 NMDA receptor only when the PSIs were brief (<60 s). This implies that formation of a contextual representation during brief exposures is critically dependent on synaptic plasticity in CA3. In a different experimental design, Miyashita, Kubik, Haghighi, Steward, and Guzowski (2009) showed that a single lap on a novel closed track was sufficient to activate *Arc* transcription in the full CA3 ensemble, but not in CA1. From these data, it was proposed that CA3 *Arc* activation could provide a substrate necessary for rapid, one-trial learning, but behavioral memory was not tested in that study. Here, we asked whether CA3 and CA1 exhibited different temporal requirements for *Arc* activation upon exposure to a novel context by measuring *Arc* transcription in rats exposed to a conditioning chamber for different periods of time. Next, we assessed the impact of exposure times used in the *Arc* imaging study to behavioral performance in CFC, which provided a behavioral readout of the incidentally encoded context memory. The reported findings not only further our understanding of the behavioral regulation of the IEG *Arc* but also provide unique insight into the temporal dynamics of the molecular processes engaged in the hippocampus associated with successful context memory formation.

2. Materials and methods

2.1. Animals

Subjects were male Sprague–Dawley rats (weighing 250–275 g on arrival) ordered from Charles River Laboratories (Wilmington, MA). All animals were individually housed in a temperature-controlled vivarium maintained on a 12 h light/dark cycle. Access to food and water was *ad libitum* throughout the length of the experiment. All rats were handled in the holding room for 5 days before the start of the experiment. On each day prior to and during training all animals were transported on a wheeled rack from the vivarium to a holding room and allowed to sit for 2 h undisturbed. On the day of training the rat's home cage was covered and individually carried from the holding room to the experimental room. All procedures complied with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

2.2. Apparatus

The conditioning chamber (30.5 × 25.4 × 30.5 cm) was housed within a sound-attenuating chamber. The floor of the conditioning chamber consisted of 18 steel rods (1.2 cm apart) wired to a shock generator (Coulbourn Instruments) for delivery of footshock. The chamber contained a constantly lit house light, a 63 dB fan and was cleaned with 10% ethanol between each animal. No other background odor was used in the conditioning chamber. All behavior was filmed with a digital Panasonic camera (model WV-BP334) and recorded using the FreezeFrame software (Coulbourn Instruments).

2.3. Transcriptional activation of Arc

To assess transcriptional activation of *Arc* during incidental encoding of a context, different groups of rats were given 3 s ($n = 6$), 30 s ($n = 6$) or 300 s ($n = 5$) of free exploration in the conditioning chamber. No footshock was administered during the exploration. Once the allotted time in the chamber expired, animals were placed back into their home cage and the top of the cage was covered with a towel. All subjects were sacrificed 6 min after being placed into the chamber. Thus, animals in the 3 s group were left undisturbed in their covered home cage for 5:57, the 30 s