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Research report

Variants of contextual fear conditioning induce differential patterns of *Egr-1* activity within the young adult prefrontal cortex

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HIGHLIGHTS

- Preexposure to the immediate shock context facilitates fear conditioned freezing.
- sCFC increases *Egr*-1 in the AC, IL and OFC, but not in the PL.
- CPFE conditioning increases *Egr*-1 in the AC, PL, IL and OFC.
- sCFC and CPFE conditioning may differ in the recruitment of different PFC regions.

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ABSTRACT

Contextual fear conditioning is a form of associative learning where animals must experience a context before they can associate it with an aversive stimulus. Single-trial contextual fear conditioning (sCFC) and the context preexposure facilitation effect (CPFE) are two variants of CFC where learning about the context is temporally contiguous (sCFC) with or separated (CPFE) from receiving a footshock in that context. Neural activity within CA1 of the dorsal hippocampus (CA1), amygdala (LA), and prefrontal cortex (PFC) may play a critical role when animals learn to associate a context with a footshock (i.e., training). Previous studies from our lab have found that early-growth-response gene 1 (*Egr*-1), an immediate early gene, exhibits unique patterns of activity within regions of the PFC following training in sCFC and the CPFE of juvenile rats. In the present study, we extended our studies by examining *Egr*-1 expression in young adult rats to determine (1) if our previous work reflected changes unique to development or extend into adulthood and (2) to contrast expression profiles between sCFC and the CPFE. Rats that learned context fear with sCFC showed increased *Egr*-1 in the anterior cingulate, orbitofrontal and infralimbic cortices relative to non-associative controls following training, but expression in prelimbic cortex did not differ between fear conditioned and non-associative controls. In contrast, rats trained in the CPFE also showed increased *Egr*-1 in all the prefrontal cortex regions, including prelimbic cortex. These findings replicate our previous findings in juveniles and suggest that *Egr*-1 in specific PFC subregions may be uniquely involved in learning context-fear in the CPFE compared to sCFC.

1. Introduction

A major strength of single-trial contextual fear conditioning (sCFC) paradigms is that animals rapidly acquire and express long-term fear to the training context [1–4]. Animals are typically given a few minutes to experience the context and then one or more footshocks occur. During the test for long-term memory of the learned fear, the animals are brought back to the context later, usually 24 h, and the amount of time spent freezing is measured as the strength of conditioned fear [5]. However, given that all learning about the context and context-shock association occurs within the same trial in sCFC, it is difficult to distinguish which brain regions are uniquely recruited during different phases of learning, and uniquely contribute to learning about the context vs. the context-shock association. To overcome this limitation, the context preexposure facilitation effect (CPFE) paradigm has been used to temporally dissociate learning about the context from learning the context-shock association [6].

The CPFE paradigm relies on the immediate shock deficit – a phenomenon in which animals fail to show fear conditioned freezing to a context during a retention test if they do not have enough time to form a representation of the training context before receiving a foot-shock [2,7]. However, a preexposure trial in the training

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context on the day prior to receiving an immediate shock overcomes this deficit, allowing animals to exhibit fear-conditioned freezing [2]. By temporally separating context learning from context-shock learning, the unique contributions of different brain regions during different phases of acquisition may be dissociated [8–10].

Although the behavioral characteristics of fear learning are well understood, our understanding of the neural activity within specific brain regions during discrete phases of learning is continually evolving [11–13]. Recent work has used immediate early gene (IEG) activity to measure the changes occurring within specific nuclei during different phases of contextual fear learning and expression [14–20]. Of particular interest, early-growth-response gene 1 (*Egr-1,* also known as zif268, krox24, and NGFI-A), a transcription factor associated with synaptic plasticity and learning and memory, is increased in structures necessary for contextual fear learning, such as the hippocampus and amygdala [1,21,22]. Using *in situ* hybridization and antisense knockdown strategies, work from our lab and others has shown that *Egr-1* mRNA in the dorsolateral division of the lateral nucleus of the amygdala (LA) and the dorsal hippocampus (dHC) is necessary for acquiring the context-shock association in sCFC and the CPFE paradigm [1,14,23,24]. However, *Egr-1* mRNA is also increased following exposure to unpredictable situations and exposure to novel contexts – suggesting its role in learning contextual information may be quite complex [15,16,25–27]. While a number of studies have examined the functional role and molecular changes occurring within the hippocampus and amygdala during different phases of learning in sCFC and the CPFE, recent evidence has begun to point to a role for the prefrontal cortex (PFC) in contextual fear learning [10,19,20,28,29]. Although the neuroanatomical pathways between the PFC, hippocampus, and amygdala are fairly well characterized, the function of the PFC in contextual fear conditioning is just starting to be explored [30–33].

Recently, we have begun examining activation of the PFC during contextual fear learning using the CPFE paradigm in developing rats [29,34]. We have found rats that learn the CPFE via preexposure to the training context (relative to rats preexposed to an alternate context) show increased *Egr-1* mRNA levels in the prelimbic and infralimbic regions of the PFC following the context-shock learning phase. However, there is no difference in PFC *Egr-1* expression between rats that learn the CPFE and those that do not during the context preexposure phase. This suggests that neural activation, as measured by *Egr-1* mRNA expression, in the PFC is related to the novelty of experiencing a new environment, but also in learning to associate an immediate foot shock with a previously acquired representation of the training context. However, given the dynamic nature of the PFC during development, it is unclear if these findings in developing rats would also be present in adulthood when the PFC is more mature [35,36]. It is also unclear how *Egr-1* activity in the PFC differs between learning sCFC and the CPFE in adult rats.

In the present study we investigated changes in *Egr-1* expression during discrete phases of contextual fear learning in sCFC and the CPFE of young adult rats. Our primary aim was to determine whether *Egr-1* mRNA expression patterns in the PFC, dorsal hippocampus, and lateral amygdala correspond to contextual fear learning in young adult rats in a manner similar to our previously reported findings in developing rats [29,34].

2. Methods

2.1. Subjects

Procedures for animal husbandry were identical to our previous reports (e.g., [37]). Rats were approximately 60 days of age at the start of the behavioral experiments. Subjects were 34 male Long Evans rats derived from 8 time-bred dams in the University of Delaware breeding colony and 181 male Long Evans rats purchased from Harlan Breeders (Indianapolis, IN). In-house bred and vendor bought rats did not differ on their fear conditioning behavior or gene expression, so their data were pooled for all analyses (data not shown). Out of a total of 215 animals, 113 were assigned to sCFC and 102 were assigned to the CPFE. For the sCFC study, 50 animals were assigned to the gene expression assays and 63 were assigned to the behavior assays. For the CPFE study, 68 animals were assigned to the gene expression assays and 34 were assigned to the behavioral assays.

Animals were pair-housed in opaque polypropylene cages ($45 \times 24 \times 21$ cm) five days prior to the start of the experiment. Animals were given *ad libidum* access to food and water throughout the experiment. All procedures were approved and subjects were treated in accordance with the Institutional Animal Care and Use Committee at the University of Delaware.

2.2. Apparatus and stimuli

Animals were fear conditioned in two distinct chambers distinguished as "Context A" and "Context B." Context A consisted of a Plexiglas and metal chamber measuring $25 \times 31 \times 32$ cm. The chamber floor was made of 19 stainless steel bars (0.5 cm diameter placed 1.25 cm apart) connected to a shock scrambler that delivered a 1s 1.5 mA alternating current footshock (Med Associates, Georgia, VT ENV-414S). Two walls were made of clear Plexiglas, allowing the animal to view a unique pattern of black and white stripes and circles specific to Context A. The other two walls consisted of opaque Plexiglas and metal panels to prevent animals from seeing other subjects in adjacent chambers. Context B was similar to Context A in overall chamber size but differed in its lack of the visual pattern of stripes and circles found in Context A. In addition, Context B contained a mesh insert which altered the spatial configurations and tactile cues of one wall. Both contexts were placed in a room on a metal rack in which background noise and light were controlled. All chambers were cleaned with a 5% ammonium hydroxide solution prior to the start of each trial. Cameras affixed to the roof of each chamber recorded animal activity and transmitted data to a nearby computer running FreezeFrame software (Coulbourn Instruments, Whitehall, PA).

FreezeFrame software was used to analyze all animal behavior. Activity thresholds were adjusted on an individual basis to exclude small movements from being calculated as part of an animal's total amount of freezing. Bouts of freezing were defined as $\geq$1.00 s without changes in pixel luminance. Animals were transported to and from each training session in their homecage. Animals in the sCFC condition were only trained in Context A. Animals in the CPFE experiments were preexposed in either Context A or Context B and then trained and tested in Context A.

2.3. Design and procedures

2.3.1. Handling

Prior to experimentation, all animals were transported to a holding room in the behavioral lab and handled for five minutes a day for five consecutive days by the same experimenter, similar to Malkani, et al. and Hamilton et al. [15,38].

2.3.2. Single-trial contextual fear conditioning (sCFC)

The general training procedure used was identical to Malkani and Rosen [1]. Four experimental conditions (abbreviated sCFC, ImmShck, NoShck, and HC) were run over a period of two days. The sCFC condition, consisted of animals that were exposed to the context for 3 min, given a 1s, 1.5 mA footshock, and left in