Available online at www.sciencedirect.com
SCIENCE DIRECT®
Behavioural Brain Research 162 (2005) 279–288
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Expression of *egr-1* (*zif268*) mRNA in select fear-related brain regions following exposure to a predator

Jeffrey B. Rosen [a,*], Robert E. Adamec [b], Barbara L. Thompson [a,1]

[a] *Department of Psychology, University of Delaware, 108 Wolf HAll, Newark, DE 19716, USA*
[b] *Department of Psychology, Memorial University Newfoundland, St. John's, Nfld, Canada*

Received 8 October 2004; received in revised form 31 March 2005; accepted 2 April 2005
Available online 4 May 2005

Abstract

Research has demonstrated that immediate-early genes/inducible transcriptional factors (e.g., *c-fos*, *egr-1*) are increased in amygdala nuclei (lateral, basal and central nuclei) known to be involved in fear conditioning, footshock stress and novelty. Although these data suggest that expression of inducible transcriptional factors are involved in fear, other non-shock ethologically based paradigms (predator or predator odor exposure) do not appear to increase *c-fos* in the lateral and basal nuclei. While the lack of *c-fos* expression may indicate that predator stress does not engage the lateral and basal amygdala nuclei, it may be that *c-fos* in the amygdala is not responsive to predator exposure. Therefore, *egr-1*, which increases in the lateral nucleus following fear conditioning, footshock and novelty, was assessed to determine if its expression is induced in rats exposed to a cat. Five minutes of cat exposure did not increase expression of *egr-1* mRNA in the lateral nucleus of the amygdala. *egr-1* was increased in the paraventricular nucleus of the hypothalamus, indicating cat-induced stress, and visual cortex compared to rats that were either confined for 5 min or handled. In the lateral periaqueductal gray, handled rats displayed a left hemisphere dominance, which disappeared in both the cat-exposed and confined group, suggesting that immobility, induced by either cat-induced stress or unstressed confinement, increased right hemisphere *egr-1* expression. The results are discussed in a context of differences and similarities in neural circuitry for conditioned and unconditioned fear.

Keywords: *c-fos*; *egr-1*; Amygdala; Fear; Paraventricular nucleus; PVN; Periaqueductal gray; PAG; Predator; Stress; Sensory cortex

1. Introduction

The study of the neurobiology of fear has primarily relied on conditioned fear paradigms (e.g., conditioned emotional responses, Pavlovian fear conditioning) and behavior in unfamiliar situations that are fear- or anxiety-provoking (e.g., elevated-plus maze, open field) in rodents. These paradigms exploit the rodent's normal behavior to threat or danger and have produced our best understanding of the neurobiology of fear. In the last several years, other paradigms have been designed that are arguably more ethological relevant (e.g., social defeat, predator or predator odor exposure) because they emphasize interaction with other conspecifics or predators and predator odors that are part of normal evolutionarily derived ecological niches (for example, [13]). In the laboratory, some of the paradigms are quite ecologically natural, such as cat or cat odor exposure in a rodent visual burrow environment, while others take the naturally fearful stimuli or derivatives of these stimuli (e.g., cat odors or synthetic predator odors) into more traditional experimental chambers. One rationale for the development of these paradigms is that these unconditioned stimuli are more "natural" than those typically employed in fear-conditioning paradigms (i.e., electrical shock) but are still aversive or threatening without necessarily being painful. Additionally, these ethologically based paradigms rely on the unconditioned or unlearned nature of the fear stimuli, whereas fear-conditioning paradigms

* Corresponding author. Tel.: +1 302 831 4209; fax: +1 302 831 3645.
E-mail address: jrosen@udel.edu (J.B. Rosen).
[1] Present address: Department of Pharmacology, Vanderbilt University, TN, USA.

0166-4328/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2005.04.001

explicitly study learning and memory of fear. (The term ethologically based does not suggest that learned fear is not ethologically important nor evolutionarily relevant, but simply that some ethologically based stimuli have a specific unconditioned quality derived from survival pressures in the ecological niches animal species evolved.) Both conditioned and unconditioned paradigms measure similar defensive behaviors, so differences are in the nature of the eliciting stimuli and not the behavioral responses. Therefore, unconditioned, ethologically based paradigms may contribute to our understanding of the neurobiology of fear in unique ways that differ from conditioning paradigms.

One of the most widely studied of these paradigms is exposure to a predator or predator odor. Large lesions of the amygdala in rats block defensive behavior in response to a cat [12,30]. Smaller lesions or chemical inactivation of specific amygdaloid nuclei have shown that the medial nucleus and the associated bed nucleus of the stria terminalis reduce defensive freezing to cat or fox odor [28,39], but lesions or inactivation of the basal, lateral or central nuclei of the amygdala have little effect on freezing to these predator odors [28,39,56,63]. Interestingly, while not producing major effects on unconditioned freezing, the lesions of the basal, lateral or central nuclei of the amygdala severely disrupt fear conditioned responses [39,61,63]. These results are corroborated by a lack of an effect of MK-801, an NMDA antagonist, in the amygdala on unconditioned passive and escape responses to a live cat [8], but a lasting reduction of fearful sensitization induced by cat exposure [1,9,21]. Both the lesion and pharmacological studies suggest that conditioned and unconditioned fear responses may rely on different amygdala circuitry.

Another way of addressing the neurobiology and neurocircuitry of fear is to map activation patterns during or just following exposure to a fearful stimulus. Expression of genes that are rapidly transcribed and translated, particularly inducible transcription factors and immediate-early genes, has been used as neuronal markers of activity (e.g., [34]). One of these, *c-fos* and its protein product Fos, is activated in a number of amygdala nuclei and periaqueductal gray following fear conditioning and retention tests of fear learning [15,19,35,49,52,54,57–59]. However, *c-fos* expression is not increased in the lateral and basal nuclei of the amygdala with exposure to a live cat or predator odors [24,27,29,47], whereas it is in the periaqueductal gray [18,24,27,47]. While these data suggest that the lateral and basal nuclei of the amygdala are involved in fear conditioning but not unconditioned fear to a predator or predator odor, *c-fos* expression may not be an appropriate activity marker for the lateral and basal amygdala nuclei during fear. While some have found *c-fos* mRNA and Fos protein increased in the lateral and basal amygdala nuclei following fear conditioning or presentation of a conditioned fear stimulus [11,49,58], others have not [52,57,59].

Another inducible transcription factor/immediate-early gene, early-growth response 1 gene (*egr-1*, also called *zif268*, *ngfi-a*, *krox 24*, *tis-8*), does increase in the lateral nucleus of the amygdala with fear [32,33,40–42,57]. Some research suggests that *egr-1* increases in the lateral nucleus of the amygdala shortly following fear conditioning in a fear-conditioning specific manner [41,57], whereas others suggest that its increase is involved in the stress of unconditional fear or novelty and not specifically to its learning ([32]; for discussion of this issue, see [37]). In any case, *egr-1* expression may indicate that the lateral nucleus of the amygdala is activated during unconditioned fear to a predator.

Expression of *egr-1*, as opposed to *c-fos*, has not been examined with exposure to a predator or predator odor. While expression of *egr-1* in the lateral nucleus of the amygdala is of particular interest, whether increased *egr-1* is also found in other regions that display *c-fos* expression during stress and predator exposure is also not known. Thus, we have investigated the expression of *egr-1* mRNA by in situ hybridization in the lateral nucleus of the amygdala, the paraventricular nucleus of the hypothalamus, periaqueductal gray, and sensory cortex.

2. Methods

2.1. Animals

Thirty naïve male Long-Evans rats, about 60 days old, were purchased from Harlan. The rats were housed individually with a 12 h light:12 h dark (Memorial University Newfoundland) cycle and ad libitum access to food and water. All behavioral experiments were conducted at Memorial University Newfoundland. The Animal Care and Use Committee of Memorial University Newfoundland approved experimental protocols. Brains were shipped to the University of Delaware for in situ hybridization.

2.2. Apparatus

2.2.1. Cat exposure chamber

The chamber was a 1.52 m × 1.83 m room without separate cat and rat compartments and not cleaned of cat odors from previous experiments. The floor of the testing environment was divided into 0.1 m^2 with masking tape.

2.2.2. Confinement chamber

The chamber used to confine rats was Plexiglas cylinder (8.6 cm diameter, 20 cm long) from a commercial startle chamber (SR-Lab animal enclosure, San Diego Instruments, San Diego, CA).

3. Procedure

3.1. Cat exposure

For 3 days prior to treatments, all rats were handled for 1 min each day. Rats were randomly assigned to three groups of 10 rats each: (1) handled rats were handled for 1 min on the day of cat exposure; (2) confined rats were first acclimated to